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ABSTRACT BOOK

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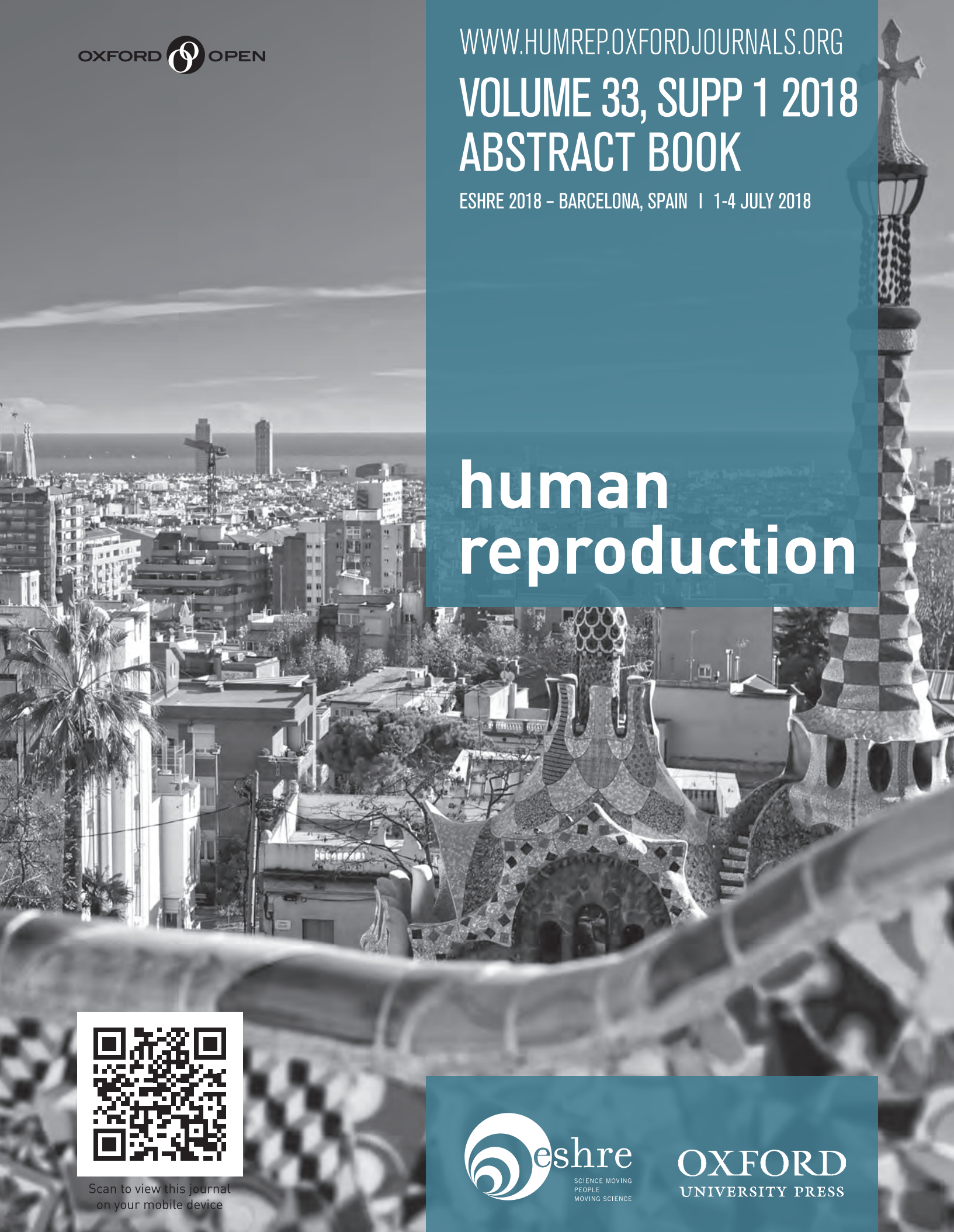

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Abstracts of the 34th Annual Meeting of the European Society of Human Reproduction and Embryology

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Abstracts

34th Annual Meeting of the
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ORAL PRESENTATIONS

Monday 02 July 2018

(*continued overleaf*)

Tuesday 3 July 2018

Wednesday 4 July 2018

POSTERS PRESENTATIONS

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human reproduction

Oral presentations

INVITED SESSION

SESSION 01: KEYNOTE SESSION

Monday 2 July 2018 Forum (Auditorium) 08:30–09:30

O-001 Human Reproduction Keynote Lecture - Semen quality of young adult ICSI offspring: The first results

F. Belva[1], M. Bonduelle[1], M. Roelants[2], D. Michielsen[3], A. Van Steirteghem[4], G. Verheyen[4], H. Tournaye[4]

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[2]KU Leuven, Environment and Health/Youth health Care- Department of Public health and Primary Care, Leuven, Belgium
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Background: Up till now, the health of children conceived by ART has been described from infancy up to pubertal age, but not beyond. Also, adverse cardiometabolic and vascular outcomes have been described in both IVF and ICSI offspring, but the impact of ART on the reproductive health of the offspring remained unknown. Whether ICSI- conceived men born to fathers with impaired spermatogenesis are at risk of inheriting deficient spermatogenesis from their fathers, could not be answered due to their young age. Since the oldest ICSI offspring cohort worldwide has recently reached adulthood, their reproductive health can now be investigated. Moreover, since these children were conceived by ICSI because of severe male-factor infertility, there is reasonable concern that male offspring have inherited the deficient spermatogenesis from their fathers. Previously normal pubertal development and adequate Sertoli and Leydig cell function has been described in pubertal ICSI boys, however, no information on their sperm quality is currently available. Therefore, we investigated the semen quality of young adult men that were conceived 18-22 years ago by ICSI for male infertility.

Material and Methods: The present study was conducted at UZ Brussel between March 2013 and April 2016 and is part of a large follow-up project focussing on reproductive and metabolic health of young adults, between 18 and 22 years and conceived after ICSI with ejaculated sperm. Results of both a physical examination and semen analysis were compared between young ICSI men being part of a longitudinally followed cohort and spontaneously conceived controls who were recruited cross-sectionally.

Results of a single semen sample in 54 young adult ICSI men and 57 spontaneously conceived males are reported. All young adults were individually assessed and the results of their physical examination were completed by questionnaires. Data were analysed by multiple linear and logistic regression, adjusted for covariates. In addition, semen parameters of the ICSI fathers dating back from their ICSI treatment application were analysed for correlations.

Results: Young ICSI adults had a lower median sperm concentration (17.7 million/ml), lower median total sperm count (31.9 million) and lower median total motile sperm count (12.7 million) in comparison to spontaneously conceived peers (37.0 million/ml; 86.8 million; 38.6 million). The median percentage progressive and total motility, median percentage normal morphology and median semen volume were not significantly different between these groups. After adjustment for confounders (age, BMI, genital malformations, time from ejaculation to analysis, abstinence period), the statistically significant differences between ICSI men and spontaneously conceived peers remained: an almost doubled sperm concentration in spontaneously conceived peers in comparison to ICSI men (ratio 1.9, 95% CI 1.1-3.2) and a two-fold lower total sperm count (ratio 2.3, 95% CI 1.3-4.1) and total motile count (ratio 2.1, 95% CI 1.2-3.6) in ICSI men compared to controls were found. Furthermore, compared to males born after spontaneous conception, ICSI men were nearly three times more likely to have sperm concentrations below the WHO reference value of 15 million/ml (AOR 2.7; 95% CI 1.1–6.7) and four times more likely to have total sperm counts below 39 million (AOR 4.3; 95% CI 1.7-11.3). In this small group of 54 father-son pairs, a weak negative correlation between total sperm count in fathers and their sons was found.

Conclusion: Although these first results in a small group indicate a lower semen quantity and quality in young adults born after ICSI for male infertility in their fathers, results cannot be generalised to all ICSI offspring because the indications for ICSI have nowadays been extended and ICSI is also being performed with non-ejaculated sperm and reported differences may thus either decrease or increase.

O-002 Oocytes from stem cells and back

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Faculty of Medical Sciences- Kyushu University, Developmental Stem Cell Biology, Fukuoka, Japan

Abstrat text

The female germline undergoes a unique sequence of differentiation processes that finally endows the eggs with totipotency. The reconstitution *in vitro* of oogenesis using pluripotent stem cells, which eventually produces functional oocytes, has long been sought in reproductive biology and developmental biology, since it would contribute not only to a better understanding of the basic mechanisms underlying totipotency, but also to an alternative source of gametes for reproduction.

We recently developed a culture systemthat reconstitutes the entire process of oogenesis from mouse pluripotent stem cells, yielding *in vitro*-generated eggs that gave rise to healthy offspring. In the culture system, primordialgerm cells (PGCs), origin of eggs and sperm, are induced from from ESCs/iPSCs, and then are aggregated with somatic cells from fetal ovaries. The aggregates, named reconstituted ovaries, pass through three different culture stages: *in vitro* differentiation (IVDi), *in vitro* growth (IVG) and *in vitro* maturation (IVM), which in total take approximately 5 weeks. After these stages, a number of mature oocytes are produced in the reconstituted ovaries.

The culture system is extremely useful, as genetic manipulation can be easily done in pluripotent stem cells, and outcome can be seen quickly in culture. In the symposium, I will introduce recent advances in oocyte production from pluripotent stem cells and updatecurrent experiments to address molecular mechanisms underlying oocyte differentiation.

SELECTED ORAL COMMUNICATIONS

SESSION 02: GENOMIC AND MITOCHONDRIAL INTEGRITY DURING DEVELOPMENT

Monday 2 July 2018 Forum (Auditorium) 10:00–11:30

O-003 Oocyte DNA repair capacity of controlled sperm DNA damage is affected by female age

F. Horta[1], S. Catt[1], B. Vollenhoven[1,2], P. Temple-Smith[1]

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[2]*Monash IVF, Reproductive medice, Melbourne, Australia*

Study question: Does female aging have a negative effect on oocyte DNA repair capacity of controlled DNA damage levels introduced by spermatozoa?

Summary answer: Oocytes from older females have reduced capacity to repair damaged DNA introduced by spermatozoa, compared to oocytes from younger females.

What is known already: The reproductive lifespan in women declines with age predominantly due to poor oocyte quality. This leads to decreased reproductive outcomes for older women undergoing assisted reproductive technology (ART) treatments compared to young women. Aging and oocyte quality have been clearly associated with aneuploidy, but factors determining oocyte quality remain unclear.

DNA repair activity prior to embryonic genomic activation is considered of maternal origin, with maternal transcripts and proteins controlling DNA integrity. With increasing maternal/oocyte age, stored mRNAs decrease, which could result in diminished efficiency of DNA repair and/or negative effects on embryo development, especially considering the presence of DNA damage.

Study design, size, duration: Oocytes from two groups of 30 superovulated female mice (Young: 5-8 weeks old, n = 15; Old: 42-45 weeks old, n = 15) were inseminated with sperm from 5 males with three different controlled DNA damage levels; control:≤10%, 1GY:11-30% and 30GY:>30%. Inseminated oocytes (Young:125, Old:78) were assessed for the percentage zygotes (per oocyte) and blastocysts (per zygote). Five replicates of 5 GVs and 5 MII oocytes from each age group were analyzed for gene expression.

Participants/materials, setting, methods: Using the C57BL6 mouse strain, swim-up epididymal sperm samples were divided into: control (no irradiation); 1GY and 30 GY. Treated spermatozoa were irradiated at 1 Grey and 30 Grey respectively using a linear accelerator Varian 21iX. Following irradiation, samples were removed for DNA damage assessment (Halomax) and for insemination. Presumed zygotes were cultured in a time-lapse incubator (MIRI, ESCO). Gene expression of 91 DNA repair genes was assessed using the Fluidigm Biomark HD system.

Main results and the role of chance: Average sperm DNA damage for the 3 groups was statistically different (Control: 6.1%, 1GY: 16.1%, 30GY: 53.1%, $p < 0.0001$), but following IVF showed no significant differences in fertilization rates within or between age groups [(Young; Control: 86.79%, 1GY: 82.75%, 30GY: 76.74%) (Old; Control: 93.1%, 1GY: 70.37%, 30GY: 68.18%) *Fishers exact*]. However, blastocyst rates were significantly different ($p < 0.0001$) among groups [(Young; Control: 86.95%, 1GY: 33.33%, 30GY: 0.0%) (Old; Control: 70.37%, 1GY: 0.0%, 30GY: 0.0%)]. Between age groups, 1GY samples showed a significant decrease in the blastocyst rate in old females compared to young females (Young; 1GY: 33.33%, Old; 1GY: 0.0, $p = 0.0166$). Gene expression analysis revealed a decreased relative expression of 21 DNA repair genes in old GV oocytes compared to young GV oocytes ($p < 0.05$) and similarly, old MII oocytes showed 23 down-regulated genes compared to young MII oocytes ($p < 0.05$). The number of down-regulated genes in older GV and MII oocytes significantly affected pathways such as Double Strand Break (GV: 5; MII: 6), Nucleotide excision repair (GV: 8; MII: 5), and DNA Damage Response (GV: 4; MII: 8). The down-regulation in DNA repair gene expression of oocytes from older females suggests a decrease in DNA repair capacity during IVF.

Limitations, reasons for caution: Ionizing radiation was used only for experimental purposes, aiming for controlled levels of sperm DNA damage, however it can also damage spermatozoa proteins. The female age groups selected in mice were intended to model effects in young and old women, but clinical studies are required to demonstrate a similar effect.

Wider implications of the findings: Fertilisation can occur with sperm populations with medium and high DNA damage but subsequent embryo growth is affected, and to a greater extent with aging females, supporting the theory that oocyte DNA repair capacity decreases with age. The assessment of oocyte DNA repair capacity could be a useful diagnostic tool.

Trial registration number: N/A.

O-004 Pronuclear transfer in zygotes from diet-induced obese mice suggests a cytoplasmic origin of transgenerational transmission of mitochondrial dysfunction leading to cardiac dysfunction in offspring

K. Moley[1], K. Latham[2], J. Ferey[3], M. Reid[3]

[1]*Washington University School of Medicine, Obstetrics and Gynecology, St. Louis, U.S.A.*
[2]*Michigan State University, Animal Science, East Lansing, U.S.A.*
[3]*Washington University, Obstetrics and Gynecology, St. Louis, U.S.A.*

Study question: Is the epigenetic inheritance of mitochondrial dysfunction in offspring from obese mothers of nuclear or mitochondrial DNA origin?

Summary answer: The cytoplasmic fraction of the zygote from obese mice and not the pronucleus (PN) appears responsible for the abnormal cardiac phenotype in the subsequent generations.

What is known already: Maternal obesity impairs offspring health. We have previously shown that maternal programming of metabolic disease in offspring can be passed through the maternal oocyte. Specifically, offspring from the obese mice demonstrated significant mitochondrial dysfunction and morphologic changes in the heart leading to profound changes in cardiac function. We showed by IVF with oocytes from the obese vs control mice that this phenotype was replicated in the IVF conceived pups, confirming an oocyte origin. In this study we use pronuclear transfer to distinguish nuclear vs mitochondrial DNA contribution to the phenotype.

Study design, size, duration: C57Bl/6 females were fed high fat/high sugar (HF/HS) diet vs control (CD) diet for 6-8 weeks and mated. One-cell zygotes were recovered from both groups and four cohorts were created by control PN transfer into enucleated HF/HS zygotes and visa versa. The manipulated zygotes were transferred into pseudopregnant ICR females mice on a control diet and weaned to CD. Cardiac function and heart tissues were obtained at 8 weeks.

Participants/materials, setting, methods: Four treatment groups were created: 1) PN from HF/HS zygote transferred into enucleated zygote (EZ) from CD; 2) PN from CD zygote into EZ from HF/HS; 3) PN from CD zygote into EZ from CD; and 4) PN from HF/HS zygote into EZ from HF/HS. Cardiac function was assessed by echocardiography (ECHO) and high resolution respirometry was used to measure tissue oxygen consumption (Oxygraph). Tissue morphology was analyzed by electron microscopy.

Main results and the role of chance: Offspring from zygotes created from HF/HS cytoplasm and CD pronucleus demonstrated significant alterations in cardiac muscle morphology, and cardiac function as determined by Oxygraph and ECHO. These changes were not seen in offspring from zygotes created from CD cytoplasm and HF/HS pronucleus. The controls responded as predicted with no changes seen in the CD PN/CD cyto and significant changes seen in the HF/HS PN//HF/HS cyto. These findings suggest that the mitochondrial contribution, and most likely the mitochondrial DNA (mtDNA) from the HF/HS zygotes in the cytoplasm, is responsible for the epigenetic changes regulating the mitochondrial dysfunction phenotype in these mice. We have shown previously that this change is seen for 3 generations, suggesting a transgenerational inheritance.

Limitations, reasons for caution: Although this is a mouse study, the same changes to mitochondrial function are also seen in human oocytes from obese women. With pronuclear transfer it is difficult to remove the PN without some mitochondrial contamination. This could explain increased variability in this study.

Wider implications of the findings: Epigenetic modifications to oocyte mtDNA may occur as a result of maternal overnutrition and obesity. These modifications affect mitochondrial function leading to cardiac dysfunction that persists for three generations. These findings in mice may explain the epidemiologic data suggesting a strong correlation between maternal BMI and offspring cardiovascular disease risk.

Trial registration number: N/A.

O-005 Tripolar mitosis underlies direct unequal cleavages in human embryos and is associated with maternal genotype at a quantitative trait locus spanning the centrosomal regulator PLK4

N. Zaninovic[1], R.C. McCoy[2], Q. Zhan[1], E.R. Hoffmann[3], L.J. Newnham[4], C.S. Ottolini[5], Z.P. Demko[6], Z. Rosenwaks[1], A.H. Handyside[5]

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Study question: What causes blastomeres in diploid embryos to cleave into three or more daughter cells during preimplantation embryo development, and what is the underlying mechanism?

Summary answer: Direct unequal cleavages (DUCs) are induced by multipolar mitosis and are associated with common maternal genetic variants spanning *PLK4*, the master regulator of centrosome duplication.

What is known already: Multipolar mitosis (including tripolar mitosis, or DUCs) has recently been documented in a substantial proportion (26%) of diploid cleaving embryos based on time-lapse microscopy (TLM) and karyomapping. DUCs exhibit reduced rates of blastocyst formation and implantation with increased rates of complex aneuploidies. The gene Polo-like kinase 4 (*PLK4*) plays an essential role as a master regulator of centrosome duplication. Altered expression of *PLK4* is associated with centrosome amplification, generating multipolar spindles. Chromosome segregation on multipolar spindles may in turn induce complex aneuploidies. Common maternal genetic variants spanning *PLK4* have previously been associated with complex aneuploidies via an unknown molecular mechanism.

Study design, size, duration: IVF cases that had previously undergone TLM as well as SNP genotyping for PGT-A were included. A total of 58 IVF patients with 742 embryos (77 biopsied on day 3 and 665 biopsied on day 5/6 embryos) were analyzed.

Participants/materials, setting, methods: DUC embryos were identified by TLM. DUC was defined as cleavage of a single blastomere into 3+ daughter blastomeres. Blastomeres (day 3) or trophectoderm cells (day 5/6) were used for genotyping by SNP microarray. Copy number and parental origin of all chromosomes were inferred from the SNP data using the Parental Support algorithm. Binomial linear regression was used to test for an association between the diploid tripolar pattern and maternal genotypes at rs2305957.

Main results and the role of chance: The diploid tripolar PGT-A signature was significantly enriched among blastomeres from embryos documented by TLM to have undergone one or more tripolar mitosis (i.e., DUC) during the first three cleavage divisions compared to embryos that underwent normal cleavage (i.e., Non-DUC; Fisher's exact test: $OR = 6.64$ [95% CI: 1.34–37.4]; $P = 0.0087$). The signature was most prevalent (up to 50%) among embryos undergoing tripolar cleavage during the first division (DUC-1) or undergoing multiple tripolar cleavages (DUC-Plus), indicating that these cleavage phenotypes may confer a wider distribution of abnormal cells at the time of biopsy. For direct validation of the association with the maternal *PLK4* genotype, data from cases undergoing day-5 blastocyst biopsy were also incorporated, as the availability of time-lapse data from these cases does not depend on embryo survival to the blastocyst stage. The minor allele of rs2305957 ("A" allele) was positively associated with time-lapse–based incidence of tripolar mitosis (i.e., DUC; quasi-binomial GLM: $OR = 1.53$ [95% CI: 1.01–2.31]; $P = 0.047$).

Limitations, reasons for caution: Retrospective analysis with relatively few genotyped patients and DUC embryos. *PLK4* has yet to be validated as the causal gene, and the causal variant remains to be identified.

Wider implications of the findings: Our data support DUC/tripolar mitosis as a key mechanism generating complex aneuploidies in cleavage-stage embryos and implicate the maternal genotype at a quantitative trait locus spanning *PLK4* as a factor influencing its occurrence. Our DUC data also elucidate the molecular origins of chaotic aneuploidies affecting embryo development and IVF success.

Trial registration number: none.

O-006 Factors associated with mitochondrial DNA (mtDNA) levels in human blastocyst

J. Ortiz[1], B. Lledo[1], R. Morales[1], A. Turienzo[1], J. Ten[2], J. Llacer[3], R. Bernabeu[3]

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[3]Instituto Bernabeu, Medicina Reproductiva, Alicante, Spain

Study question: Are embryo and maternal factors related with the mtDNA content in human euploid blastocyst?

Summary answer: Maternal age, the day where the embryo biopsy is performed and the embryo quality are associated with mtDNA levels in trophoectoderm biopsies.

What is known already: The main function of mitochrondria is to provide energy by ATP production. The role of mitochondrial in the developing human embryo is thought to be critical to a successful delivery. Although initial reports supported the hypothesis that high levels of mtDNA in trophoectoderm biopsies are associated with low implantation potential, other publications were unable to confirm previous results. It is possible that unidentified counfounding factors may influence the mtDNA copy number in euploid embryos. The aim of this study is identified the main factors that could increase the mtDNA in euploid blastocyst.

Study design, size, duration: A retrospective study was performed. We included 159 blastocysts biopsies from 142 couples who attended to Instituto Bernabeu for PGT-A from 2016 to 2017. The aneuploid testing was performed by NGS using Veriseq® kit and the BlueFuse Multi software (Illumina). All blastocysts were diagnosed as euploid non-mosaic. The mtDNA analysis were performed once the diagnosis was known. We evaluated the relationship between several maternal and embryo factors and trophoectoderm mtDNA levels.

Participants/materials, setting, methods: Sequencing reads mapping to the mtDNA genome were extracted from bam files to identify copy number. The relative measure of mtDNA copy number was calculated by diving the mtDNA reads by the nuclearDNA value to normalize for technical variants and the number of cells collected at the biopsy. All the results were subjected to correction factor according to the embryo genome. The association between variables and mtDNA was evaluated by logistic regression and chi-square (Rv3.4).

Main results and the role of chance: Quantification of mtDNA was successful in all cases. The mean average of mtDNA was 0.0016 + 0.0012. Concerning to maternal factors, there are not statistically significant differences according to the oocyte origin, donated or own oocyte (0.0017 vs 0.0017; $p = 0.423$). However, mtDNA was negatively correlated with female age when we compare with different female age ranges (ranges 25y-30y: 0.0017, 30y-35y: 0.0012, 35y-40y: 0.0016, older 40 y: 0.0024, $p = 0.044$). At last, no significance differences were reported according to the indication for PGT-A recurrent implantation failure (0.0016 vs 0.0015; $p = 0.365$) or repeated miscarriage (0.0016 vs 0.0017; $p = 0.523$). With regard to embryo factors, embryos biopsied on day 5 were more likely to have higher quantities of mtDNA compared with those biopsied on day 6 (0.0017 vs 0.0009; $p = 0.0014$). Embryo quality is not associated with mtDNA (A:0.0017, B:0.0015, C: 0.0006; $p = 0.304$). Finally, we also examined the possible relationship between the sex of the embryo and mtDNA copy number. We did not observe any significant

differences in mtDNA levels between male and female embryos (0.0016 vs 0.0016; p = 0.851).

Limitations, reasons for caution: Limitation may be due to the size of the sample and the high-troughput sequencing technology. More research in the mitochondrial function are needed. Evaluating the entire cohort of embryos from each patient could detect patient variability.

Wider implications of the findings: Our data suggest that blastocyst from older women had higher mtDNA, thus oocyte ageing could affect ooplasmic factors. Moreover, our results show that the mtDNA in a given cell of an embryo is dependent upon the cell divisions that preceded biopsy because higher mtDNA were found in D5 biopsied embryos.

Trial registration number: Not applicable.

O-007 Estimation of age-dependent decrease in blastocyst euploidy in in vitro fertilization/intracytoplasmic sperm injection cycles

S. Esteves[1,3], P. Humaidan[1,2], J. Carvalho[4], M. Ciro[5], A. Dias[54], C. Alviggi[6], A. Conforti[6], R. Fischer[7], C. Andersen[8], K. Bühler[9], F. Ubaldi[10]

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Study question: Is it possible to estimate the effect size of female age-dependent decrease in the probability of a blastocyst being euploid?

Summary answer: The decrease in blastocyst euploidy is age-dependent, progressing with every year of female age, from 1.2% to 24.5% in 28-44 years-old women.

What is known already: Array comparative genomic hybridization (CGH) data show that female age is the most significant determinant of embryo genetic status. Aneuploidy affects more than one-half of human embryos and is the main reason for the decrease in live birth rate among aging women undergoing Assisted Reproductive Technology (ART) cycles. However, estimation of the age-dependent probability of a blastocyst being euploid and the effect size of the decrease in this probability with every year of female age using next-generation sequencing (NGS) analysis has not been performed yet.

Study design, size, duration: Retrospective analysis of 1,220 trophectoderm biopsies from 436 infertile couples undergoing intracytoplasmic sperm injection and preimplantation genetic testing for aneuploidy (PGT-A) between 2016 and 2017. PGT-A was used for reasons of advanced maternal age, severe male factor infertility, recurrent miscarriage, repeated implantation failure, as well as patients who were concerned about euploidy of their embryos.

Participants/materials, setting, methods: Biopsied trophectoderm cells were subjected to lysis, whole genome amplification, construction of libraries, and NGS using the Ion PGM™ platform. Logistic regression was fit to the dataset. The dependent and independent variables were embryo genetic status (euploid/aneuploid) and female age, respectively. The method of fitting was quadratic on age, and the model was validated by splitting the data (80% and 20% to train and test the model respectively). Computations were performed using JMP 13 (www.jmp.com).

Main results and the role of chance: The model is $\log[p(t)/(1-p(t))] = 7.575\text{-}0.21\cdot\text{age}\text{-}0.011\cdot(\text{age}\text{-}38.41)^2$, where p(t) is the probability of a blastocyst being euploid for age (t) in years. There was no statistical evidence against the fitted model ($p > 0.10$). The model is not biased as there is linearity in the relationship between the estimated probability distribution and the distribution of empirical data. The % decrease in probability from year (t) to year (t + 1) was defined as the ratio $p(t+1)/p(t)\times100$. There was a significant ($P < 0.0001$) decrease in the probability of a blastocyst being euploid with every year of female age. The geometric mean of the yearly variation was 13.6%. However, the decrease is progressive with every year of female age. At age 29 it was 1.2%, whereas at ages 30, 32, 35, 37, 39, 41 and 44, the decreases were 2.0%, 3.5%, 6.7%, 9.8%, 13.6%, 17.9%, and 24.5%, respectively.

Limitations, reasons for caution: The impact of other covariates that could affect embryo genetic status and effect of cycle number were not analyzed. Furthermore, the estimations cannot be generalized to IVF patients undergoing cleavage-stage embryo transfer.

Wider implications of the findings: The decrease in the probability of a blastocyst being euploid increases progressively with every year of female age, from 1.2% to 24.5% in 28-44 years-old women. This information will aid clinicians counsel patients concerning their individual chances of having euploid blastocysts for transfer.

Trial registration number: N/A.

O-008 Global DNA methylation profiling of mouse embryo inner cell mass following IVF

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Study question: Is preimplantation embryo culture (in vitro fertilization; IVF) associated with global DNA methylation changes in the mouse inner cell mass (ICM)?

Summary answer: Preimplantation embryo manipulation results in widespread DNA methylation changes in ICM.

What is known already: Numerous imprinting disorders are correlated with assisted reproductive technologies (ARTs) and animal models indicate that offspring generated by IVF have increased metabolic alterations in adulthood. Given that the preimplantation embryos undergo dramatic changes in DNA methylation, there is a need to understand the global DNA methylation changes occurring in the embryo following embryo culture. Since the ICM will give rise to the fetus and adult individual, limiting the analysis to this tissue will provide valuable information regarding candidate gene alterations.

Study design, size, duration: Mouse blastocysts were obtained from CF-1 females crossed with B6D2F1 males and utilized for in vitro fertilization or flushed out of the uterus (FB = flushed blastocyst = control). IVF was performed using KSOM with amino acids and 5% O_2.

Participants/materials, setting, methods: Blastocysts were collected (IVF and in vivo) and immuno-surgery performed isolating ICM. Pooled ICM of IVF or FB (n = 15/group, 2 replicates/group) were utilized for whole genome bisulfite sequencing (WGBS). Methyl-MaxiSeq was used for WGBS and sequenced on the Illumina HiSeq4000. Reads were mapped to the mouse genomes (mm9) using Bismark and differentially methylated regions (DMR) identified using BSSEQ Bioconductor package in R. DMR were functionally characterized using Genomic Regions Enrichment of Annotations Tool (GREAT).

Main results and the role of chance: The low amount of starting material allowed for an excellent quality but relatively low mapping efficiency (~ 12%). As expected, only 20% of CpG were methylated, confirming the low level of DNA methylation in the blastocyst. There were 3195 differentially CpG methylated regions (DMR: 1661 hypomethylated and 1534 hyper-methylated) between IVF and control ICMs. The majority of DMR were located between 5 and 500 nucleotides from the transcription start site (TSS) and approximately 50% were located in the body of the genes.

The hypomethylated regions corresponded to genes whose function was related to endosomal transport, regulation of neurotransmitter secretion, protein O-linked glycosylation, cytokinesis, ventricular development and adipose tissue development,

The hypermethylated regions were in proximity of genes whose function was involved in negative regulation of gliogenesis, cell junction assembly, gluconeogenesis, cellular response to peptide hormone stimulus, cell junction organization, positive regulation of Rho GTPase activity.

Limitations, reasons for caution: The low amount of starting material (15 pooled ICM, corresponding to a total of approximately 200 cells) could introduce amplification errors.

Wider implications of the findings: This is the first report of global DNA methylation changes in isolated ICMs of IVF or spontaneously conceived blastocysts. The candidate gene identified as being differentially methylated in ICM of IVF embryo can elucidate the phenotypic alterations noted in adult animal conceived by IVF.

Trial registration number: not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 03: ENDOMETRIOSIS AND ENDOMETRIUM: DISCOVERY SCIENCE

Monday 2 July 2018 Room 211 + 212 10:00–11:30

O-009 Discovery of novel loci for endometriosis in genome-wide association analysis of 63 K cases and 700 K controls

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[2]*Liverpool University, Blostatistics Department, Liverpool, United Kingdom*

Study question: What common genetic variants and corresponding functional mechanisms underlie endometriosis, and its surgical sub-types (ASRM-stages), clinical sub-types (Infertile-endometriosis) and symptom-based sub-types (Severe pelvic-pain with endometriosis)?

Summary answer: With the largest sample size to date for endometriosis genome-wide-association-study (GWAS) meta-analysis, we expect to identify many novel-susceptibility-loci for this debilitating condition and its sub-types.

What is known already: Endometriosis has an estimated heritability of ~ 50%, with ~26% estimated to be related to common genetic variation. To date, meta-analysis of endometriosis GWAS (17 K cases and 191 K controls) have robustly identified 19 variants explaining 5.2% of disease variance. Previous GWAS analyses also highlighted that the signals for most of the genome-wide significant loci are driven by ASRM stage III/IV disease, highlighting the need for further phenotype-stratified analyses.

Study design, size, duration: IEGC consists of 25-centres contributing in total, 63 K endometriosis cases, 7800 stage III/IV, and 700 K controls to the GWAS meta-analysis. Some of these are population-based cohorts such as the UK Biobank, and also clinical-investigations such as ENDOX, which is a prospective study recruiting women undergoing laparoscopy, that has dense clinical-phenotype data. Some of the centres have expression and eQTL-datasets for endometrium, fat and ectopic-disease-tissue, allowing for evaluation of functional mechanisms of the identified genetic variants.

Participants/materials, setting, methods: Each GWAS was imputed up to reference panels from the Haplotype Reference Consortium or 1000 Genomes Project. Association testing by each centre was conducted in a regression framework under an additive genetic model, with adjustment for confounding due to population structure. Association testing was conducted for overall, stage I/II, stage III/IV and infertile endometriosis groups. Association summary statistics were aggregated across GWAS via fixed-effects meta-analysis.

Main results and the role of chance: The GWAS meta-analysis is on-going, and results will be presented at the meeting. Preliminary results from the UK Biobank (n = 8,190 cases) identified two novel associations at near genome-wide significance ($p < 5 \times 10\text{-}8$) that map outside previously reported loci. The first maps to IGF2BP3 (rs550554633, $p = 4.4 \times 10^{-8}$), which is involved in regulation of metabolism and adiposity, supporting the genetic link between endometriosis and fat distribution. The second maps to GDAP1 (rs554964149, $p = 7.2 \times 10^{-8}$), which is involved in neuronal development that may play a role in endometriosis-associated pain, and previously has been associated with pain severity in dysmenorrhea. Moreover, a further 18 loci with nominal significance ($p < 5 \times 10^{-6}$) were identified, many of which are expected to increase in association strength in the complete IEGC meta-analysis.

Limitations, reasons for caution: For phenotype-stratified analysis we are limited by the datasets with dense phenotypic data (i.e. infertility, pelvic pain severity).

Wider implications of the findings: The identified genetic variants shed light on the underlying pathogenesis of endometriosis and may point to novel therapeutic interventions.

Trial registration number: N/A.

O-010 Understanding endometriosis heterogeneity—sub-phenotype analysis of genetic associations

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[2]*University of Oxford, Wellcome Centre for Human Genetics, Oxford, United Kingdom*

Study question: The aim of this study is to investigate whether sub-phenotype analysis of established genetic variants associated with endometriosis reveals differential mechanisms underlying surgical and symptomatic sub-types of the disease.

Summary answer: Preliminary results from a reduced dataset showed suggestive differential effects for genome-wide association study (GWAS) loci across surgical sub-phenotypes.

What is known already: Most of the known 14 genetic loci have been reported based on association with overall endometriosis, with sub-phenotypic investigations limited to ASRM stages I/II vs. III/IV. Studies using the World Endometriosis Research Foundation-Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) standardized data collection protocols now afford the investigation of these loci for detailed surgical and symptomatic phenotypes.

Study design, size, duration: Cross-sectional study in which genotype information from 358 women in the Oxford ENDOX study (294 cases, 64 controls) undergoing laparoscopic surgery was analysed for association with EPHect-compliant surgical and symptomatic phenotype data.

Participants/materials, setting, methods: Preliminary analysis of 108 endometriosis cases included 51% with an endometrioma (not exclusive to other surgical subtypes), 68% with peritoneal disease and 31% with deep endometriosis; 40%, 14%, 19% and 27% of women were diagnosed with ASRM Stage I to IV respectively.

All 14 known GWAS loci for endometriosis were tested for association with sub-phenotypes using Pearson's chi-squared and linear-by-linear association test.

Main results and the role of chance: The GWAS locus on 2q35 (rs1250241, located in *FN1*), with genotype T/A, showed a borderline association ($p = 0.035$) with peritoneal endometriosis (odds ratio (OR) of TT vs. TA = 0.08, 95% confidence interval (CI): 0.01-0.78; OR of TT vs. AA = 0.13, 95% CI: 0.01-1.25; OR of TA vs. AA = 1.68, 95% CI:0.69-4.07), but not with endometrioma ($p = 0.856$) or deep endometriosis ($p = 0.307$). Analysis on additional genotype data (n = 250) with more sub-phenotypes including infertility and pain status are underway and results will be presented at the meeting.

Limitations, reasons for caution: Sample size was small in the preliminary analysis, with limited power to discover differential association pattern.

Wider implications of the findings: Insight into aetiological heterogeneity of endometriosis elucidated by genetics is an important avenue to ultimately develop targeted non-invasive markers of disease and novel treatment strategies.

Trial registration number: Not applicable.

O-011 Exosomes as biomarkers in endometriosis

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[3]*Nuffield Department of Medicine, Target Discovery Institute, University of Oxford*

Study question: Could exosomes be considered as biomarkers of endometriosis?

Summary answer: Potentially – we found exosomes in peritoneal fluid of endometriosis and control samples. The differences observed in number and size likely translates into qualitative differences.

What is known already: Endometriosis, the presence of endometrial lesions outside the uterus, is a common condition affecting up to 10% of women of reproductive age. Patients suffer from pain and infertility. The current diagnostic model relies on non-specific symptoms, the (inconsistent) application of endometriosis risk factors, and invasive diagnostic laparoscopy as the gold standard. There is no clinically relevant biomarker for endometriosis yet.Recently, nano-sized extracellular vesicles – exosomes – produced by virtually every cell, have been described in diseases such as cancer, diabetes and pre-eclampsia. Exosomes could similarly be important in endometriosis and serve as biomarkers of the disease.

Study design, size, duration: Peritoneal fluid (PF) samples were obtained from participants in the ENDOX study at the Endometriosis CaRe Centre, Nuffield Department of Women's and Reproductive Health, University of Oxford (REC ref. 09/H0604/58) under WERF EPHect standards. Women between 18-49 years of age (n = 28) who had undergone diagnostic laparoscopy for abdominal or pelvic pain and/or subfertility investigation were classified according to menstrual cycle phase (proliferative or secretory) and severity of endometriosis (ASRM stages I + II or stages III + IV).

Participants/materials, setting, methods: Groups, control proliferative, n = 4; control secretory, n = 2; StI+II proliferative, n = 9; StI+II secretory, n = 7; StIII+IV proliferative, n = 3; StIII+IV secretory, n = 3. Exclusion criteria: Hormonal treatment, malignancy, pregnancy, breastfeeding and inability to understand the consent form. 1 mL PF was centrifuged to remove cells, debris and microvesicles. Exosomes were enriched by size-exclusion chromatography and confirmed by nanoparticle tracking analysis (NTA), immunoblotting and transmission electron microscopy (TEM). The study of exosome cargo by mass spectroscopy and RNA-sequencing is ongoing.

Main results and the role of chance: We confirmed the presence of exosomes in PF from women at different stages of endometriosis and from disease-free patients at different menstrual cycle phases by TEM (n = 6 pooled group samples) and NTA (n = 6 pooled group samples). Enriched exosomes were positive for CD9 and Syntenin. The mean size of PF particles from women with endometriosis was 217 ± 11.2 nm (n = 4 pooled group samples), whereas in non-endometriotic women it was 182.9 ± 9.11 nm (n = 2 pooled group samples). We observed a higher concentration of PF particles in stage I-II endometriosis compared to controls in both proliferative and secretory phases ($p < 0.0001$) (n = 2 pooled group samples). In stage III-IV endometriosis, the opposite was seen in the proliferative phase ($p < 0.0001$) (n = 2 pooled group samples).

Limitations, reasons for caution: Due to the very limited material obtained, PF samples had to be pooled according to menstrual cycle phases and ARSM staging for analyses.

Wider implications of the findings: The difference in concentration and size of exosomes in the patient groups is likely to translate into qualitative differences in exosome populations given genetic and phenotypic differences in endometriosis of different stages. This will be shown by the pending proteomics and RNA-seq results.

Trial registration number: Not applicable.

O-012 The baboon miRNAome: small RNA sequencing of 25 baboon tissues

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[4]UGent, Center for Medical Genetics, Ghent, Belgium

Study question: Can we identify orthologous miRNAs between baboons (papio Anubis) and humans through deep sequencing of baboon RNA and annotation to the human genome.

Summary answer: Mapping of the small RNA sequencing reads from a pooled baboon sample to the human genome identified 941 orthologous miRNAs between humans and baboons.

What is known already: Baboons (papio Anubis) are a very good model for reproductive research because of the small phylogenetic gap and the very high similarity in reproductive physiology and anatomy. They have been used to study endometriosis, embryo-implantation, uterine transplantation and in the development of contraceptives. MicroRNAs are important gene expression regulators and their role in the pathophysiology of several reproductive diseases has been implicated. The use of baboons in miRNA research is currently limited because only a few miRNA genes have been described and annotated.

Study design, size, duration: One baboon was euthanized and during post-mortem examination biopsies of 25 tissues were collected and snap frozen (ovary, endometrium, myometrium, fallopian tube, cervix, heart, aorta, lung, kidney, liver, pancreas, bladder, peritoneum, lymph nodes, muscle, spleen, skin, tongue mucosa, rib cartilage, stomach mucosa, thyroid glands, pituitary glands, intestine, brain cortex, plasma). Prior to RNA extraction tissue samples were subsampled using a microtome (10-12 scrolls of 55 µm thickness/samples) and stored in Qiazol reagent (Qiagen).

Participants/materials, setting, methods: RNA was isolated using the miRNeasy serum/plasma kit (Qiagen) and the miRNeasy mini kit (Qiagen). RNA of the 25 samples was pooled and processed as a single sample pool using the TruSeq small RNA library preparation kit (Illumina). Single end sequencing was performed (140 million reads, read length 75 nt) on a NextSeq 500 sequencer (Illumina). After filtering and adapter trimming, the reads were collapsed, mapped and annotated to the human reference genome.

Main results and the role of chance: Quality of the extracted RNA was assessed by determining RNA concentration (Qubit 2.0 fluorometer, Thermo Fisher Scientific) and RNA integrity (Fragment Analyzer automated capillary electrophoresis system, Advanced Analytical Technologies). On average, an RNA concentration of 783 ng/µl and an RNA Quality Number (RQN) value of 8.1 was obtained. In total, 143 million sequencing reads were generated. Read length distribution and annotation was evaluated to ensure enrichment of miRNAs in the 20-24 nucleotide read fraction. Mapping the baboon sequencing reads to the human reference genome, allowing no mismatches, detected 708 mature canonical miRNAs with identical nucleotide sequences in baboons (papio Anubis) and humans. In a second step, the unmapped fraction of sequencing reads was remapped allowing 1 and 2 mismatches, respectively. This resulted in the detection of 233 additional miRNAs. In total, 941 unique miRNAs were detected (708 with 0 mismatches, 749 with 1 mismatch, 607 with 2 mismatches). Because of the very high sequence homology between baboons and humans these 941 miRNAs can be considered as orthologous between humans and baboons.

Limitations, reasons for caution: In this study we analyzed 25 tissue samples from one baboon. Validation in more animals is required. Analysis of other tissues than those included or deep sequencing of the individual tissues included in this study could identify other miRNAs with tissue specific or very low levels of expression.

Wider implications of the findings: The identification of orthologous miRNAs in baboons is a first step in establishing the baboon miRNAome and will facilitate miRNA research in baboons. All our data will be made open access and online available so they can be used by other researchers to develop primers for their miRNAs of interest.

Trial registration number: Not applicable.

O-013 Human endometrium demonstrate a dynamic TERRA expression and hormone regulation: implications in endometrial proliferative conditions

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Study question: Does healthy endometrium express TERRAs and is their expression altered with endometrial proliferative conditions and by ovarian steroid hormones?

Summary answer: Healthy human endometrium expressed TERRAs with an individual TERRA-specific dynamic thus hormonally regulated expression pattern and an altered expression observed in endometrial pathologies.

What is known already: Telomeres are transcribed as long non coding RNAs, called TERRAs (Telomeric repeat containing RNA) that are proposed to participate in a variety of cellular regulatory functions and DNA damage response activation. Endometrial telomere transcription has not yet been studied. Telomere length and telomerase activity change according to ovarian hormones in the human endometrium and high telomerase is associated with endometrial proliferative conditions, such as endometriosis and endometrial cancer. The effect of ovarian steroid hormones on telomere transcription in human endometrium is not known.

Study design, size, duration: A prospective observational study, included endometrial biopsies of 66 women was carried out in the academic labs of University of Liverpool/ Liverpool Women's Hospital.

Participants/materials, setting, methods: Endometrial samples were collected from women with (n = 10) or without endometriosis (n = 16), women using mirena (n = 9), healthy postmenopausal (PM) women (n = 7) and women with endometrial cancer (EC, n = 24). Ethical approval was obtained from Liverpool Adult Local Research Ethics Committee (LREC) and informed written consent was obtained from all patients. Endometrial samples analysed for TERRA (chromosome 1, 16, 20) and hTERC levels by qPCR, TRAP assay, ki67 proliferative index and steroid receptor expression by immunohistochemistry.

Main results and the role of chance: All three TERRAs from chromosomes 1, 16, and 20, were expressed in the human endometrium. Pre-menopausal endometrial TERRAs did not change according to cycle phase but Ch-20 TERRA levels were significantly increased in the PM samples compared with premenopausal proliferative phase ($p = 0.017$). There were no significant differences in TERRA levels in the secretory phase endometrium of women with endometriosis compared with the healthy women without endometriosis ($p > 0.05$), however the hTERC mRNA levels were significantly upregulated in secretory endometrium of women with endometriosis ($p = 0.02$). EC samples had significantly reduced levels of Ch-16 and Ch-20 TERRA ($p = 0.001$; $p = 0.000$ respectively) compared to PM endometrium. Ch-16 TERRA levels positively correlated with hTERC but none of the TERRA levels correlated with telomerase activity (TRAP assay). Ch-16 TERRA levels negatively correlated with progesterone receptor and both Ch-16 and Ch-20 TERRA levels negatively correlated with the proliferative marker Ki67 ($p = 0.03$; $p = 0.009$ respectively). Treatment of local progesterone, Mirena did not significantly changed TERRA levels compared with untreated proliferative endometrium. TERRA expression was found to be downregulated with *in vitro* treatment with progesterone and also combined oestrogen and progesterone treatment for 72 hrs ($p = 0.02$ and $p = 0.04$ respectively).

Limitations, reasons for caution: This is an observational study including a modest samples number including only three different TERRAs. However, corresponding Telomerase and hTERC levels, proliferative indices of the samples are included to examine TERRA levels in the context of cellular proliferation and telomerase biology.

Wider implications of the findings: The negative correlation of TERRA with cellular proliferation, significant reduction in endometrial cancer and their hormonal regulation indicate a regulatory role for TERRA in the human endometrium. Further studies are warranted to explore TERRAs as therapeutic targets in endometrial pathologies.

Trial registration number: Not applicable.

O-014 CAS as a novel interacting protein of CD147 is involved in the epithelial-to-mesenchymal transition in human endometriosis

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Study question: Does the elevated CD147 regulate the epithelial-to-mesenchymal transition (EMT) during endometriosis progression?

Summary answer: A novel CD147-interacting protein, CAS was identified. The upregulation of CD147 led to the disruption of CAS/E-cadherin/β-catenin complex, which promoting EMT of endometriotic cells.

What is known already: Our previous studies have demonstrated elevated CD147 in the ovarian endometriosis with enhanced cell survival.

Study design, size, duration: Experimental clinical study and laboratory-based investigation. The endometriotic lesions samples were collected from 47 ovarian endometriosis women, and the normal endometrial tissues were collected from 12 women without endometriosis and endometritis. CD147 and CAS overexpression or knockdown in human endometrial Ishikawa cells (ISK) were designed to examine the effect on cell migration and EMT marker and related signaling.

Participants/materials, setting, methods: Expression levels of CAS were evaluated in endometriotic tissues by quantitative real-time polymerase chain reaction (qPCR) and western blot. The interactions of CD147, CAS and E-cadherin were confirmed by co-immunoprecipitation. ISK cells were transfected with the CD147-overexpressing plasmid or infected with lentivirus packaged human CAS. CAS-knockdown was achieved by transfection of CAS siRNA in ISK cells. Gene-manipulated ISK cells were used to do cell migration and invasion assay or explore the signaling pathway.

Main results and the role of chance: In human endometriotic tissues, both mRNA and protein expression levels of CAS were significantly decreased in endometriosis lesions with elevated expression of CD147. We next determined a complex formed with CD147/CAS/E-cadherin, which stabilized the interaction between E-cadherin and β-catenin. Furthermore, overexpression of CD147 disrupted the interaction between CAS and E-cadherin, resulting in the EMT process via the release of β-catenin from CAS/E-cadherin/β-catenin complex. Of note, overexpression of CD147 or knockdown CAS led to the nuclear translocation of β-catenin and further activated its downstream EMT signaling such as EMT-promoting gene Snail. In addition, overexpression of CAS was able to inhibit the migration and invasion capacity in CAS-overexpressing ISK cells.

Limitations, reasons for caution: In vivo study is lacking to further confirm the function of CD147 and CAS in animal models.

Wider implications of the findings: The demonstrated increase of cell migration and invasion through CD147-CAS-E-cadherin interactions suggest the involvement of the CD147-regulated signaling in the progression of human endometriosis and provide potential targets for endometriosis treatment.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 04: RECURRENT IMPLANTATION FAILURE AND UNEXPLAINED INFERTILITY

Monday 2 July 2018 Room 111 + 112 10:00–11:30

O-015 Androgen receptor (AR) mRNA expression is positively associated with live birth in women undergoing in-vitro fertilization independently of the type of ovarian response

C. Vaitsopoulou[1], E. Kolibianakis[1], E. Neofytou[1], A. Makedos[1], M. Malandri[1], A. Kalpatsanidis[1], J. Bosdou[1], D. Sabbaidou[1], K. Chatzimeletiou[1], S. Lymperi[1], A. Lambropoulos[2], G. Grimbizis[1], B. Tarlatzis[1]

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Study question: Is androgen receptor (AR) mRNA expression associated with live birth in normal and poor responders undergoing in-vitro fertilization (IVF)?

Summary answer: AR mRNA expression is positively associated with live birth in women undergoing in-vitro fertilization independently of the type of ovarian response.

What is known already: AR is a steroid hormone receptor that regulates various genes' expression and affects cellular proliferation and differentiation. Androgens interact with AR, exert their paracrine action on granulosa cells and regulate gonadotropin synthesis, follicle development, oocyte maturation and corpus luteum function. Estrogens increase AR mRNA and testosterone binding site in uterine endometrium and leiomyoma. In human cancer cells, low estradiol increases AR levels, suggesting the estrogen/androgen ratio as a predictor of sex steroid response. AR mRNA expression is, also, regulated by progesterone. Although AR mRNA is expressed in the female reproductive tissues, its expression has never been studied in peripheral blood.

Study design, size, duration: A prospective clinical trial was performed from 2014 to 2017, in 40 normal responders and 27 poor responders according to Bologna criteria, undergoing IVF/ICSI. Ovarian stimulation was performed with 200 IU recombinant follicle stimulating hormone (rFSH) and gonadotrophin releasing hormone (GnRH) antagonists. Triggering of final oocyte maturation was performed by recombinant human chorionic gonadotrophic (rhCG).

Participants/materials, setting, methods: Vaginal ultrasound evaluation of follicular development, peripheral blood collection for RNA extraction, c-DNA synthesis, mRNA expression and hormonal assessments were performed on days 1, 6 and 10 of stimulation. Serum FSH, luteinizing hormone (LH), estradiol and progesterone were measured by ELISA. AR mRNA expression was evaluated by Real-time PCR and analyzed by the ΔΔCT method. Statistical analysis was performed using multivariable analysis.

Main results and the role of chance: On day 6 of stimulation, AR mRNA expression was negatively associated with LH level ($p = 0.027$), adjusting for patient type. On day 10 of stimulation, AR mRNA expression was negatively associated with estradiol ($p = 0.010$) and progesterone ($p < 0.001$) levels, adjusting for patient type. Concomitantly, AR mRNA expression was positively associated with the number of developing follicles (11-17 mm) ($p = 0.010$), adjusting for patient type. Achievement of live birth was positively associated with AR mRNA expression prior to initiation of stimulation ($p < 0.001$), adjusting for female age and number of embryos transferred.

Limitations, reasons for caution: This is the first study of AR mRNA expression in peripheral blood mononuclear cells during ovarian stimulation for IVF. However, it is performed on a limited number of normal and poor responders.

Wider implications of the findings: If the association of AR mRNA expression with live birth is confirmed in further studies, its assessment prior to initiation of ovarian stimulation, could be used as a novel prognostic factor for the achievement of pregnancy regardless of the type of ovarian response.

Trial registration number: NCT01984286

O-016 Endometrial receptivity analysis (ERA) using a next generation sequencing (NGS) predictor improves reproductive outcome in recurrent implantation failure (RIF) patients when compared to ERA arrays

M. Clemente-Císcar[1], M. Ruiz-Alonso[2], D. Blesa[3], J. Jimenez-Almazan[1], M. Bahceci[4], M. Banker[5], I. Vladimirov[6], S. Mackens[7], C. Miller[8], D. Valbuena[2], C. Simon[9,10,11,12]

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Study question: Does next generation sequencing (NGS) based endometrial receptivity analysis (ERA) predictor improve reproductive outcome in recurrent implantation failure (RIF) patients when compared to arrays based ERA predictor?

Summary answer: Reproductive outcome in RIF patients increases significantly when performing personalized embryo transfer (pET) based on ERA NGS predictor compared to previous ERA arrays predictor.

What is known already: ERA identifies the window of implantation (WOI) of a given patient based on the transcriptomic signature of 236 genes in an endometrial biopsy. Subsequently, it allows pET according to this ERA result. Several groups have reported that pET increases pregnancy rates (PR) and implantation rates (IR) in RIF patients (Ruiz-Alonso 2013; Mahajan 2015; Hashimoto 2017; Tan 2018). To improve the diagnostic accuracy and reproductive outcome in RIF patients of endometrial origin, a NGS ERA predictor based on our experience with more than 20,000 endometrial samples, has recently been developed.

Study design, size, duration: This study comprises NGS predictor development and clinical follow-up to validate its diagnostic accuracy after pET. The NGS predictor was developed by sequencing 411 endometrial biopsies previously diagnosed by arrays. Then, 1,432 endometrial biopsies (716 patients with two endometrial samples per patient) were used for prediction analysis. Clinical follow-up of the NGS predictor was performed in 261 patients collected from 5 different clinics. These clinical results were compared with previously known outcome using ERA arrays.

Participants/materials, setting, methods: Endometrial biopsies were collected using a pipelle catheter during the expected WOI in natural cycles (at LH + 7) or hormone replacement therapy cycles (at P + 5). RNA was extracted and sequenced using the ERA gene panel. Several machine learning methods were compared to select the one with better performance. A statistical proportion test was used for reproductive outcome comparison between samples diagnosed with NGS ERA predictor and arrays ERA predictor.

Main results and the role of chance: The machine learning method with best performance was Random Forest, with a global accuracy of 0.88, sensitivity 0.90 and specificity 0.97. Reproductive outcome for RIF patients performing arrays based ERA and subsequent pET was obtained based on results from 352 patients (age: 37.4 ± 4.2) previously published (Ruiz-Alonso 2013; Ruiz-Alonso 2014; Mahajan 2015; Hashimoto 2017). PR was 56.5%, IR 39.9% and ongoing pregnancy per transfer (OT) of 43.2%. The clinical follow-up after pET based on NGS ERA predictor in 261 patients (age: 37.3 ± 5.1), however, was 70.9% PR, 55.7% IR and 59.4% OT. The reproductive outcome comparison thus shows a significant increase in PR (p-value = 0.000390), IR (p-value = 0.000003) and OT (p-value = 0.000101) in case of NGS ERA predictor.

Limitations, reasons for caution: The failure of an embryo to implant is a complex process that not only involves endometrial receptivity but also embryo quality. ERA only focuses on the endometrial origin or contribution of RIF.

Wider implications of the findings: These results demonstrate that the ERA NGS predictor enhances the clinical effectiveness of endometrial diagnosis in RIF patients when compared to ERA arrays predictor. This more accurate predictor tool, based on a wide reference set of samples with known clinical result, has led to significantly better reproductive outcome following pET.

Trial registration number: Not apply.

O-017 Interventions for unexplained infertility: a systematic review and network meta-analysis

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Study question: Which is the most effective and safe intervention in couples with unexplained infertility, expectant management, ovarian stimulation (OS), intrauterine insemination (IUI), OS-IUI or IVF?

Summary answer: IVF and OS-IUI were the most effective but least safe interventions, while expectant management and IUI were the least effective but safest interventions.

What is known already: Existing systematic reviews have conducted head to head comparison of interventions using pairwise meta-analyses to evaluate the effectiveness of interventions in couples with unexplained infertility. As this approach only allows the comparison of two interventions at a time and is dependent on the availability of appropriate primary evaluative studies, it is difficult to identify the best intervention in terms of effectiveness and safety. Network meta-analysis compares multiple treatments in a single statistical model by using both direct and indirect evidence and provides a hierarchy of these treatments which can better inform clinical decision making.

Study design, size, duration: We performed a systematic review and network meta-analysis of relevant randomised controlled trials (RCTs). We searched electronic databases including MEDLINE, Embase, PsycINFO, CINAHL, the Cochrane Gynaecology and Fertility Group specialised register of controlled trials, the Cochrane Central Register of Studies Online, as well as reference lists to identify eligible studies. We also searched trial registers for ongoing trials.

Participants/materials, setting, methods: We included RCTs comparing at least two of following interventions in couples with unexplained infertility: 1) expectant management (including timed intercourse); 2) ovarian stimulation using gonadotropins, aromatase inhibitors or anti-estrogens; 3) IUI alone; 4) OS-IUI; and 5) in vitro fertilisation (IVF) or combined with intracytoplasmic injection (ICSI). The primary effectiveness outcome is a composite of cumulative live birth or ongoing pregnancy and the primary safety outcome is multiple pregnancy.

Main results and the role of chance: Of 906 titles and abstracts initially identified, we included 29 trials reporting on 4514 couples with unexplained infertility. The most frequent direct comparisons were OS versus OS-IUI (7 studies, 743 couples), OS-IUI versus IVF (6 studies, 1101 couples), and IUI versus OS-IUI (6 studies, 895 couples). Thirteen studies reported data on live birth or ongoing pregnancy in 3181 couples and 15 studies reported data on multiple pregnancy in 2834 couples.

Network meta-analysis showed that IVF resulted in more live births/ongoing pregnancies than expectant management (OR 2.87, 95%CI 1.31-6.29) and OS (OR 2.63, 95%CI 1.12-6.17); and it may also result in more live birth/ongoing pregnancies than IUI alone (OR 1.98, 95%CI 0.99-3.97) and OS-IUI (OR 1.58, 95%CI 0.96-2.59). OS-IUI may lead to higher live birth/ongoing pregnancy rate compared to expectant management (OR 1.82, 95%CI 0.98-3.39). The ranking showed that IVF was the most effective intervention, followed by OS-IUI, IUI alone and OS alone, with expectant management as the least effective intervention. Both IVF and OS-IUI resulted in more multiple pregnancies compared to IUI alone (OR 5.08, 95%CI 1.15-22.51; OR 5.55, 95%CI 1.34-23.06). There were no significant differences in multiple pregnancy between other interventions.

Limitations, reasons for caution: The included studies were heterogeneous in study population, treatment protocol and duration of follow-up. IVF in earlier studies was not always applied with single embryo transfer. Also long-term safety was not considered. As multiple pregnancy was not reported in many early publications, our findings should be interpreted with caution.

Wider implications of the findings: In couples with unexplained infertility, IVF provides the fastest way to have a baby while OS-IUI is also a competitive intervention. Both long-term safety of the offspring and financial costs should be considered in the timing of IUI and IVF in the context of shared decision making.

Trial registration number: not applicable.

O-018 Infertility diagnosis in female adolescent and young adult survivors of non-gynecological cancers: a population-based cohort study

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Study question: Do female adolescent and young adults (AYAs) survivors of selected non-gynecological cancers have a higher risk of subsequent infertility diagnosis than AYAs without cancer?

Summary answer: Female AYAs survivors of certain non-gynecological cancers have a higher risk of subsequent infertility diagnosis. The risk is influenced by previous parity.

What is known already: Cancer therapies have improved substantially, leading to dramatic increases in survival. As survival improves, there is increasing emphasis on optimizing quality of life among survivors. Many cancer therapies increase the risk of infertility. At this time, no population-based studies have been conducted to determine the risk of subsequent infertility diagnosis in female AYAs with non-gynecological cancers. The literature is limited to epidemiological studies comparing birth rates after cancer, or small studies using markers of the ovarian reserve as a proxy of infertility.

Study design, size, duration: A population-based cohort study using universal health care databases in the province of Ontario, Canada. All women 15-39 years of age diagnosed with brain, breast, hematological, head/neck, thyroid, melanoma, colorectal, or urological cancer from 1992-2011 who lived at least 5 years recurrence-free were identified (Exposed, n = 15107). Each cancer survivor was matched by age, census subdivision, and parity to 5 randomly selected cancer-free women (Unexposed, n = 64314), and followed subjects until December 31, 2016.

Participants/materials, setting, methods: Infertility diagnosis after one year of cancer was identified using information on physician billing claims through the Ontario Health Insurance Plan database (ICD-9 code). Women with infertility, tubal ligation, bilateral oophorectomy, or hysterectomy previous to cancer diagnosis were excluded. Log-binomial regression models were used to calculate the risk of infertility diagnosis adjusted for sociodemographic factors (adjusted Relative Risk -aRR). Adjusted models were further stratified by parity at the time of cancer diagnosis (nulliparous and parous).

Main results and the role of chance: Mean age at cancer diagnosis was 31.2 years (SD 6.3). The median follow-up time for cancer survivors was 13.9 years and for unexposed women 14.3 years ($p < 0.001$). Overall, the frequency of infertility diagnosis was higher in cancer survivors compared to unexposed women (12.0% vs. 9.4%, $p < 0.001$). Mean age of infertility diagnosis was 34.5 years (SD 5.7) in survivors and 34.9 years (SD 5.5) in unexposed women ($p < 0.001$). Differences between AYAs survivors and unexposed women varied by type of cancer, notably survivors of breast (aRR 1.38; 95% CI 1.23–1.55), hematological (aRR 1.42; 95% CI 1.28-1.59), thyroid (aRR 1.17; 95% CI 1.08–1.27), and melanoma (aRR 1.13; 95% CI 0.99-1.30), had a higher risk of infertility diagnosis than women without cancer. After stratification by parity, the association remained strong in nulliparous survivors of breast and hematological cancers, while it disappeared in parous women. Parity did not modify the effect on infertility in survivors of thyroid cancer, and the association remained significant in both groups. In survivors of melanoma and urological cancer a significant association was observed only in nulliparous women. Survivors of brain, head/neck, and colorectal cancers did not have a higher risk of infertility diagnosis compared to women without cancer.

Limitations, reasons for caution: The accuracy of infertility diagnosis using ICD-9 codes in administrative datasets has not been validated. Non-biologic factors that may influence the likelihood of seeking a fertility assessment may not be captured in administrative databases. Information regarding cancer stage or treatment was not available for this analysis.

Wider implications of the findings: Reproductive health surveillance in female AYAs with cancer is a priority, especially those with breast and hematological cancer diagnoses. Our finding of a potential effect of thyroid cancer (subject to over-diagnosis) and melanoma needs to be further studied, and if any effect is confirmed, possible mechanisms elucidated.

Trial registration number: not applicable.

O-019 Differential proteomic analysis of endometrial fluid suggests increased inflammation and extracellular matrix remodeling in non-implantative IVF cycles

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Study question: Is there any difference in the protein composition of the endometrial fluid aspirate obtained the day of embryo transfer in cycles achieving and not achieving pregnancy?

Summary answer: We found 212 differentially expressed proteins when comparing 'implantative' and 'non-implantative' cycles, that is, those resulting in implantation success and failure.

What is known already: Endometrial fluid allows non-invasive characterization of the endometrium, and may contain important information on its receptivity when performing in vitro fertilization (IVF) cycles. Endometrial side of implantation has usually been studied with endometrial biopsy in a cycle prior to embryo transfer, focusing on "receptive/non-receptive" endometria, and with low-throughput proteomic techniques.

Study design, size, duration: We have compared the protein expression patterns in endometrial fluid aspirated from 38 women undergoing IVF, corresponding to 18 implantative and 20 non-implantative cycles using a high-throughput differential proteomic approach. The study period was 12 months.

Participants/materials, setting, methods: The population under study consisted of 38 women aged 18-40 years old, undergoing their first or second IVF/ intracytoplasmic sperm injection cycle, with normal uterus and endometrium, and 1-2 good quality embryos, and embryo transfer being performed on day 3. Endometrial fluid aspiration was performed immediately before the embryo transfer.

Filter-aided sample preparation was used for the in-solution tryptic digestion of the proteins present in the samples, followed by label-free mass spectrometry analysis.

Main results and the role of chance: From the 716 proteins detected in the differential proteomics analysis, 212 significantly differed in abundance between the groups under analysis ($p < 0.05$). Bioinformatic analyses denoted the deregulation of important processes governing receptivity, such as extracellular matrix remodeling, proteolytic activity and inflammatory signaling, and antimicrobial activity within the set of differential proteins. The results suggest higher activity of cytokines, including TNF, OSM and IL6, as well as lower activity of progesterone in non-implantative cycles. In addition, a number of pregnancy-related differential proteins were pinpointed in the mechanistic association analysis.

Limitations, reasons for caution: Our results were obtained from patients with normal uterus and endometrium and with good quality embryos, who had fresh day-3 embryo transfer, in stimulated cycles. Therefore, our observations may not be applicable to poor prognosis cases or non-stimulated cycles.

Wider implications of the findings: This work provides insights into the molecular features of implantative IVF cycles using non-invasive methods. It reveals that endometrial fluid aspirate may reflect an increased inflammatory state in non-implantative endometrium. This knowledge opens a new avenue for improving embryo transfer strategies and increasing pregnancy rates.

Trial registration number: Not applicable.

O-020 Pilot study investigating concentration of colony-stimulating growth factor (CSF) in uterine flushing as prognostic criterion of IVF cycle outcome in patients with recurrent implantation failure

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Study question: Study question was if colony-stimulating growth factor can be used as reliable prognostic criterion of clinical pregnancy in "fresh" cycles of assisted reproductive technology.

Summary answer: Evaluation of CSF level in uterine flushing can be considered perspective prognostic criterion for IVF cycle outcome with sensitivity of 87.5% and specificity of 94.3%

What is known already: The concept of recurrent implantation failure (RIF) in assisted reproductive technology has been enlarged and the tactics for such patients have included searching prognostic criteria of IVF outcome with the aim to minimize the number of unsuccessful attempts and the risk of patient's drop-out. However the studies on this topic are limited as most suggested methods require endometrial biopsy and subsequently do not allow to perform fresh embryo transfer therefore there is still need for non-invasive model for prediction of IVF cycle outcome.

Study design, size, duration: Study type: Interventional.
Design: randomized controlled pilot study.
Intervention Model: Parallel Assignment.
Masking: open-label
Size: 83 patients.
Duration: 12 months.

Participants/materials, setting, methods: After obtaining board approval 83 women aged 22–39 years were recruited. Matching criteria: recurrent implantation failure, normal karyotype, absence of uterine factors of infertility. The patients were randomized into study group (N = 43) and control group (N = 40): no intervention. At the day of oocyte retrieval the uterine flushing was collected using insemination catheter (CCD). CSF concentration in uterine flushing was determined using ELISA with following calculation per gram of protein.

Main results and the role of chance: As a primary assessed outcome the clinical pregnancy rate was analysed: there was no significant difference comparing between groups ($X^2 = 0.018$, $p > 0.05$, CC = 0.015). Thus the method of collection of uterine flushing doesn't affect IVF cycle outcome and can be used routinely. The CSF concentration in uterine flushing was significantly higher in women with clinical pregnancy. The ROC-curve showed sensitivity of 87.5% and specificity of 94.3% with cut-off value of CSF being 0.151

Limitations, reasons for caution: As the study was considered as pilot the number of recruited participants is limited. Thus further investigation is needed.

Wider implications of the findings: These data prove the necessity for further research of CSF role in implantation process and need for consideration the lack of CSF as a possible cause of recurrent implantation failure.

Trial registration number: not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 05: EMERGING DATA IN CLINICAL ANDROLOGY

Monday 2 July 2018 Room 113 + 114 + 115 10:00–11:30

O-021 The seminiferous tubule caliber pattern as evaluated at high magnification during microdissection testicular sperm extraction predicts sperm retrieval in patients with non-obstructive azoospermia

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Study question: Is the seminiferous tubule caliber pattern as evaluated during microdissection testicular sperm extraction (microTESE) predictive of sperm retrieval success in patients with non-obstructive azoospermia (NOA)?

Summary answer: sperm retrieval was successful in 90% of cases when dilated tubules were found, but only in 7% of cases when only not-dilated tubules were retrieved.

What is known already: MicroTESE proved to be the gold standard surgical approach to patients with NOA. However, despite it was introduced in the clinic about 20 years ago, still a great variability in sperm retrieval rates is recorded among centers.Among the factors able to explain this issue, the pattern of semiferous tubules caliber found at high magnification has been suggested to be associated with the chance of retrieving sperm. Few studies in the past, most with small sample size, have dealt with this issue, but none provided diagnostic accuracy measures in support of the association between seminferous tubules pattern and sperm retrieval rates.

Study design, size, duration: The present cross-sectional study was conducted between January 2015 and July 2017 on 143 infertile men with non-obstructive azoospermia (NOA) undergoing unilateral (64) or bilateral (79) microTESE. Assessment of sperm retrieval was evaluated as per testis (N = 222). Stepwise binary logistic regression was run to identify predictive factors of successful sperm retrieval (SSR) and seminiferous tubules pattern among serum FSH, LH and testosterone level, mean testis volume, testis histology and seminiferous tubules pattern.

Participants/materials, setting, methods: During microTESE, if present, dilated tubules (DT) were retrieved, otherwise any tubule whose caliber was greater than that of the surroundings was taken (slightly dilated tubules – SDT). When no DT or SDT were found, not dilated tubules (NDT) were excised according to a sort of mapping. A fragment of one or more of the tubules of the same diameter (DT, SDT or NDT) was fixed in Bouin's solution and sent for histological analysis.

Main results and the role of chance: Sperm were retrieved in 95 out of 222 testes (42,8%); DTs were found in 90% of testes with successful sperm retrieval (SSR), SDTs in 47% and NDTs in only 7% ($p < 0.0001$). The median number of sperm retrieved was significantly higher in DTs compared to SDTs and NDTs (5.000, IQ range 9.000-300.000, 1.000, IQ range 500-450.000, 500, IQ range 500 respectively; $p < 0.0001$). Stepwise binary logistic regression for SSR prediction excluded FSH, LH, testosterone and testicular volume from the model; on the other hand the tubules caliber pattern allowed the correct classification of 82,4% of testes (chi square 133.731, $p < 0.0001$, intercept odds ratio [IOR] 0.84), while histology classified 72,7% of them (chi square 63.329, $p < 0.0001$, IOR 38.9), with a good (AUC 0.89) and fair (AUC 0.70) diagnostic accuracy respectively as demonstrated by ROC AUC estimate computed on the predictive probabilities. The combination of both variables, however, explained the results in terms of SSR in 86,7% of cases (chi square 156.749, $p < 0.0001$, IOR 28.4,) with an excellent diagnostic accuracy (0.93). Stepwise binary logistic regression for the prediction of seminiferous tubules pattern individuated testis histology as the only significant predictor (chi square 33.843, $p < 0.0001$), although with a poor diagnostic accuracy (AUC 0.67).

Limitations, reasons for caution: the seminiferous tubules patter was subjectively evaluated, but the skill and experience (about 900 microTESE procedures performed to date) of the surgeon can account for the reliability of the results.

Wider implications of the findings: the present study provides reliable accuracy measures in support of the relationship between seminiferous tubules caliber pattern and SSR in patients with NOA. The tubules pattern may represent an additional outcome measure of microTESE and, together with histology, provide additional information on the degree of spermatogenesis impairment in individual patients.

Trial registration number: N/A.

O-022 Characterization of spermatogenic function in NOA men aiming at predicting success of testicular sperm retrieval

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Study question: Can germ cell characterization of testicular biopsy samples provide information on seminiferous tubule function in NOA men and enhance our ability to identify spermatozoa?

Summary answer: Using germ cell stage-specific markers, we can predict the extent of spermatogenic dysfunction in NOA men and relate it to the eventual retrieval of spermatozoa.

What is known already: In men with non-obstructive azoospermia (NOA) that undergo micro-TESE, about 40-60% of cases fail to yield spermatozoa. In these patients, the array of spermatogenetic progress within the seminiferous tubule vary from hypospermatogenesis, to maturation arrest, or germ cell aplasia (sertoli-cell only syndrome). These features can be difficult to identify even during surgical sperm extraction and about half of the cases fail to provide injectable sperm cells. The utilization of immunofluorescence or the isolation of RNA targeting specific germ cell maturational stages can help to identify the progression of spermatogenesis and offer a more complete profile to the reproductive physician.

Study design, size, duration: Over the course of the past year, ten samples retrieved from NOA men were analyzed for germ cell stage identification. Additionally, two OA (obstructive azoospermia) samples served as control. Patients were then categorized as to their extent of spermatogenesis using immunofluorescence markers previously validated in the OA control. Gene expression analysis of samples ($n = 6$) confirmed the presence of specific germ cell stages and helped to map the extent of the spermatogenic process.

Participants/materials, setting, methods: A total of 12 men underwent micro-TESE to identify spermatozoa for use with ICSI. All specimens were fixed and processed for immunofluorescent analysis using germ cell markers, DAZL (all germ cell stages), BOULE (meiotic), and PNA (post-meiotic). To confirm immunofluorescent findings, RNA-seq assessing 60,448 genes was carried out on some specimens for corresponding gene expression of the markers.

Main results and the role of chance: OA samples were positive for all immunofluorescent germ cell markers tested and spermatogenic line presence was further validated via RNA-seq. Immunofluorescent analysis of testicular specimens (NOA) revealed a varying proportion of germ cells at intermediate stages of meiosis. Of these, two NOA men displayed signal for DAZL, BOULE, and PNA, suggesting retention of the spermatogenic line and indeed they yielded scant spermatozoa that were used for ICSI, although neither resulted in a pregnancy. NOA patients, in which no spermatozoa were retrieved, evidenced a scattered number of immature germ cells compared to the control, which appeared smaller in size and irregular in shape. No post-meiotic stages were identified using the acrosomal marker, PNA. Analysis of differential gene expression corroborated our findings, revealing a significant decrease in expression of spermatogenic markers for the germ cell stages of interest. All NOA patients had poor expression of DAZL (all germ cells), BOLL (meiotic marker), and ACRBP (post-meiotic marker) compared to the OA control, with the lowest expression in NOA patients where no spermatozoa were identified ($P < 0.001$). Correspondingly, NOA men with spermatozoa identified in the testicular biopsy portrayed an increased expression of DAZL as compared to NOA patients that failed to yield spermatozoa ($P < 0.01$).

Limitations, reasons for caution: A refined method for characterization of germ cells will further portray a detailed depiction of seminiferous tubule function. Due to sample availability, not all specimens were analyzed for both immunofluorescence and RNA-sequencing. Querying unfamiliar genes will help in gaining information on the etiology of this severe form of male infertility.

Wider implications of the findings: The utilization of a non-invasive method to query specific genes that predict seminiferous tubule function will help to identify the best candidates for TESE likely to yield spermatozoa, sparing patients costs and emotional distress. Careful interpretation of RNA transcripts will help to illuminate the etiology behind this severe condition.

Trial registration number: N/A.

O-023 MSOME I + II: a new cut-off value for male infertility and embryo development prediction on intracytoplasmic sperm injection cycles

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Study question: Can motile sperm organelle morphology examination (MSOME) improve semen analysis by better defining male infertility and providing better prediction for intracytoplasmic sperm injection (ICSI) outcomes?

Summary answer: MSOME I + II ≥ 5.5% is a predictor for severity of the male factor and for the rate of blastocysts.

What is known already: Human sperm morphology has been described as an essential parameter for the diagnosis of male infertility and a prognostic indicator of natural or assisted pregnancies. Nevertheless, standard morphological assessment remains a subjective analysis and its impact on ICSI is also of limited value. The direct selection of spermatozoa by MSOME has proved to lead to better ICSI outcomes, such as higher implantation, pregnancy and live birth rates. However, whether the proportion of normal spermatozoa by WHO parameters can be correlated with MSOME classification and whether this could be correlated with ICSI outcomes are questions that remain to be elucidated.

Study design, size, duration: This prospective-cohort study included data from 483 patients undergoing conventional seminal analysis from June/2015 to June/2017. The spermatozoa were graded as: MSOME I, normal form and no vacuoles; MSOME II, normal form and ≤2 small vacuoles; MSOME III, normal form >2 small vacuoles or one large vacuole; and MSOME IV, large vacuole and abnormal head shapes or other abnormalities. The sum of MSOME Grades I and II was used as a normal spermatozoa parameter.

Participants/materials, setting, methods: The study was performed in a private university-affiliated IVFncenter. The proportion of MSOME grades were correlated with semen volume, concentration, total sperm count, motility, morphology and total motile sperm count (TMSC) by linear regression models. The correlation of MSOME with ICSI outcomes (i.e. fertilization rate, high-quality embryos and blastocyst rate, implantation, cycle's cancelation, and pregnancy rates) were evaluated by linear and logistic regression models. The MSOME normality cutoff was defined by ROC curve.

Main results and the role of chance: MSOME I + II was positively correlated with semen concentration ($\beta = 0.281$ $p < 0.001$), total sperm count ($\beta = 0.224$ $p < 0.001$), motility ($\beta = 0.178$ $p < 0.001$), progressive motility ($\beta = 0.192$ $p < 0.001$), morphology ($\beta = 0.341$ $p < 0.001$), and TMSC ($\beta = 0.2010$ $p < 0.001$). The MSOME I + II was statistically different between WHO seminal classifications, with normozoospermia group having the highest percentage of MSOME Grades I + II (14.10%). We observed a decreased incidence of MSOME I + II with the increased severity of the seminal classification, and oligoasthenoteratozoospermia was the most affected group (MSOME I + II 3.95% $p < 0.001$). MSOME I + II was positively correlated with fertilization ($\beta = 0.197$ $p = 0.044$), high-quality embryos ($\beta = 0.306$ $p = 0.013$), and blastocyst($\beta = 0.248$ $p = 0.047$) rates. The highest area under the curve (AUC) to discriminate blastocyst rate <50% from that ≥ 50% was obtained with MSOME Grades I + II ($AUC_{ROC} = 0.66$), with cut-off value of ≥ 5.5%, in which a sensitivity of 0.72 and specificity of 0.41 were obtained. Using this parameter to define normality, a significant difference in the blastocyst rate between MSOME I + II < 5.5% and MSOME I + II ≥ 5.5% could be noted (28.53 ± 5.69 vs 50.14 ± 5.05 %, $p = 0.005$) even when adjusted for confounding variables such as: male and female ages, ejaculatory abstinence and number of retrieved oocytes.

Limitations, reasons for caution: The limitation of the present study is the small samples size and the lack of correlation between MSOME results and clinical outcomes.

Wider implications of the findings: MSOME I + II ≥ 5.5% is a useful cut-off for the diagnosis of infertility severity and for the prediction of ICSI outcomes. The future use of MSOME as a routine method may be reliable for assessing male infertility and its impact on ICSI.

Trial registration number: None.

O-024 Effect of reactive oxygen species on the methylation status of human sperm imprinting genes, seminal plasma metabolites, and semen parameters

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Study question: Is there an association between seminal plasma reactive oxygen species (ROS) levels and alterations of imprinting gene methylation patterns, seminal plasma metabolites, and semen parameters?

Summary answer: A positive association exists between ROS levels and alterations in H19-Igf2 methylation as well as metabolite concentration, with an overall detrimental effect on semen quality.

What is known already: Oxidative stress (OS) is associated with sperm dysfunction via distinct pathophysiology mechanisms. Recent studies suggest that ROS affect methylation of genes involved in male fertility. Furthermore, OS may alter the seminal plasma (SP) metabolite milieu and contribute to sperm dysfunction. However, it remains unknown as to what extent the effect of seminal ROS on gene methylation, seminal metabolites, and sperm quality is level-dependent. Also, a possible association between ROS-induced H19/Ig2 methylation and SP metabolites with semen quality is yet to be confirmed. Such studies may help unravel pathophysiologic pathways leading to unexplained male infertility and allow development of targeted therapies.

Study design, size, duration: Prospective study including fresh semen specimens of 150 men between April and May 2016. Each patient contributed one specimen which was analyzed for ROS by chemiluminescence, and classified according to seminal ROS levels [in relative light units (RLU)/sec/106 sperm]: Group 1 (n = 39): Low (ROS < 20 RLU), Group 2 (n = 38): Mild (20 RLU≤ROS < 40 RLU), Group 3 (n = 31): Moderate (40 RLU≤ROS < 60 RLU), and Group 4 (n = 43): High (ROS≥60 RLU).

Participants/materials, setting, methods: Participants were normozoospermic (WHO 2010) men attending an infertility clinic. Specimens were collected after 3-5 days abstinence. A comprehensive analysis of SP and sperm function parameters were carried out, including conventional semen characteristics (computer-assisted semen analysis; CASA), measurement of total antioxidant capacity (TAC), sperm DNA fragmentation (sperm chromatin dispersion test), chromatin maturation index (aniline blue), H19-Igf2 methylation status (methylation-specific polymerase chain reaction), and untargeted seminal metabolic profiling using nuclear magnetic resonance spectroscopy (1H-NMR).

Main results and the role of chance: The methylation status in promoter region of H19 and Igf2 genes was significantly different in specimens with high/moderate ROS levels than mild/low counterparts ($P < 0.001$). Metabolic fingerprinting by 1H-NMR revealed upregulation of trimethylamine N-oxide ($P < 0.001$) and downregulation of tryptophan ($P < 0.05$) as well as tyrosine/tyrosol ($P < 0.01$) in SP samples with high ROS levels. A significant reduction in total sperm motility ($P < 0.05$) and concentration ($P < 0.001$) was noted in

specimens with high ROS levels, whereas higher CMI and sperm DNA fragmentation index (DFI) were noted both in specimens with moderate ($P < 0.05$) and high ROS ($P < 0.005$). In contrast, TAC values were decreased in all specimens but those with low ROS levels ($P < 0.001$). Pearson's and Spearman correlations showed that ROS levels were positively correlated with Igf2 methylation ($r = 0.19$, $P < 0.05$), DFI ($r = 0.27$, $P < 0.001$), CMI ($r = 0.21$, $P < 0.01$), and trimethylamine N-oxide ($r = 0.45$, $P < 0.05$) and negatively correlated with H19 methylation ($r=-0.20$, $P < 0.05$), tryptophan ($r=-0.45$, $p < 0.05$), sperm motility ($r=-0.18$, $P < 0.05$), sperm vitality ($r=-0.35$, $P < 0.001$), sperm concentration ($r=-0.38$, $P < 0.001$).

Limitations, reasons for caution: Although none of the subjects reported a history of pelvic and genital infections, chronic diseases, and endocrine abnormalities, risk factors such as use of medication and smoking habits were not consistently recorded. Also, the effect of ejaculatory abstinence duration was not controlled.

Wider implications of the findings: Our study suggests a link between ROS-induced H19/Ig2 methylation and seminal plasma metabolites with semen quality. We speculate that deterioration of semen quality and sperm DNA integrity in specimens with moderate/high ROS levels is mediated by alterations in the methylation status of H19/Ig2 genes and SP metabolite concentrations.

Trial registration number: N.A.

O-025 Comparative proteomic analysis of seminal plasma proteins from fertile men with normal or high levels of reactive oxygen species

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Study question: Does seminal plasma proteome play a role in the preservation of sperm fertilizing ability in men with high levels of reactive oxygen species (ROS)?

Summary answer: Increased antioxidant activity and endopeptidase inhibitory mechanisms in seminal plasma may contribute to the preservation of the fertilizing potential of men with high ROS levels.

What is known already: During spermatogenesis, spermatozoa shed majority of their cytoplasm and thereby lose most of their antioxidant defenses. Therefore, they become dependent on antioxidant protection provided by seminal plasma which is largely made up of secretions from the male accessory glands. It is known that an imbalance between ROS production and antioxidant protection results in oxidative stress, which is linked with impaired sperm function and fertilization, ultimately contributing to male infertility. Seminal plasma also plays a major role in preventing premature capacitation. However, the molecular mechanisms behind the importance of seminal plasma in the preservation of male fertilizing ability are largely unknown.

Study design, size, duration: Semen analysis including liquefaction, viscosity, volume, and pH was performed in 17 semen samples. Samples were divided into two groups according to the ROS levels measured by chemiluminescence: Control group – ROS level <102RLU/s/million sperm ($n = 8$); ROS positive group – ROS level >102 RLU/s/million sperm ($n = 9$). Proteomic analysis of seminal plasma samples was performed to identify possible differential expressed proteins (DEPs) between the experimental groups, which were further selected for validation by Western Blot (WB).

Participants/materials, setting, methods: Semen samples were voluntarily provided by proven fertile men (men with at least one child). Seminal plasma was collected after centrifugation of liquefied semen at 4000Xg and protein concentration was estimated by the BCA assay. A pool of 5 samples from each experimental group was used for quantitative proteomic analysis (LC-MS/MS). The main core networks were identified by functional enrichment using STRING software and key DEPs were selected for validation by WB.

Main results and the role of chance: A total of 44 proteins were found to be differentially expressed between the experimental groups. Serine-type endopeptidase inhibitor activity (GO:0004867, false discovery rate $p = 0.00006$) and antioxidant activity (GO:0016209, false discovery rate $p = 0.00523$) were among the main molecular functions in which these DEPs were involved. In the ROS positive group, semenogelin-1 and semenogelin-2, which are proteins involved in seminal coagulum formation, were underexpressed ($p = 0.00074$, $p = 0.00023$, respectively). On the other hand, some proteins involved in antioxidant defense, haptoglobin (HP) and peroxiredoxin-4 (PRDX4) were overexpressed ($p = 0.00349$, $p = 0.00099$, respectively). Similarly, Serpin B6 (SERPINB6), a serine protease inhibitor, was also overexpressed in ROS positive group ($p = 0.00424$). The expression of these DEPs was identified by WB, for control ($n = 8$) and ROS positive ($n = 9$) groups, and fold variation to the control group was calculated by densitometry. MedCalc software was used for statistical data analysis. Firstly, the Tukey test was used for outlier detection, followed by a two-tailed Mann-Whitney test. The protein expression obtained by WB for the selected DEPs was not statistically significant between the experimental groups: SEMG1 ($p = 0.5286$) SEMG2 ($p = 0.2002$), HP ($p = 0.3865$), PRDX4 ($p = 0.6304$) and SERPINB6 ($p = 0.6304$).

Limitations, reasons for caution: The distinct results obtained between proteomics and WB could be explained by the differences in the sensitivity and reproducibility of these methods. Furthermore, only 5 of the 44 DEPs identified by proteomics were validated by WB.

Wider implications of the findings: Different expression pattern of seminal plasma proteins in fertile men with high levels of ROS may be responsible for the preservation of the spermatozoa fertilizing capacity. This may be attributed to the overexpression of proteins with antioxidant and endopeptidase inhibitor activity. Other DEPs must be investigated to test this hypothesis.

Trial registration number: not applicable.

O-026 Exosomal miRNAs in seminal plasma are markers of the origin of azoospermia and can predict the presence of sperm in testicular tissue

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Study question: Are exosomal microRNAs (miRNAs) in seminal plasma (SP) useful as markers of the origin of azoospermia and the presence of sperm in the testis?

Summary answer: The potential of SP miRNAs contained in exosomes as biomarkers for selecting azoospermic individuals with real chances of obtaining spermatozoa from the testis is shown.

What is known already: There are no precise non-invasive diagnostic methods for classifying the origin of the sperm defects and the spermatogenic reserve of the testis in azoospermic infertile men. The diagnosis of such individuals is often based on the practice of biopsies. In this context it is reasonable to study the presence of organ-specific markers in human semen that contains fluid from the testis and the male reproductive glands, which could help in the diagnosis and prognosis of azoospermia. Additionally, SP contains high concentrations of small extracellular vesicles (sEVs), particularly exosomes, which originate from multiple cellular sources in the male reproductive tract.

Study design, size, duration: Case and control prospective study. This study compares the miRNA content of exosomes in semen samples obtained from 9 normozoospermic fertile individuals (control group); 14 infertile men diagnosed with azoospermia due to spermatogenic failure and 13 individuals with obstructive azoospermia and conserved spermatogenesis. Additionally, three severe oligozoospermic individuals ($<5 \times 10^6$ sperm/ml) were included in the study.

Participants/materials, setting, methods: A differential high-throughput miRNA profiling analysis using miRNA qPCR panels (Exiqon) was performed in

SP exosomes from azoospermic and fertile individuals. Unpaired two-tailed t test was used to analyze the differences in expression of miRNAs. Validation of miRNA candidates was performed by RT-qPCR analysis in a larger cohort of individuals. Multivariate logistic regression and receiver operating characteristic (ROC) curve analysis of the expression values were used to predict/identify azoospermia origin, as well as residual spermatogenesis.

Main results and the role of chance: A total of 623 miRNA were included in the study and 397 miRNAs (63,7%) were consistently detected in samples from the groups of the study and statistically analysed, which revealed altered patterns of miRNA expression in infertile patients. We focused on the miRNAs that were differentially expressed between azoospermia as a result of an obstruction in the genital tract (having conserved spermatogenesis) and azoospermia due to spermatogenic failure, and described the expression values of one miRNA (miR-A*) in exosomes from semen as a predictive biomarker test for the origin of azoospermia with high sensitivity and specificity (>90%). Furthermore a model that included the miR-B* and the miR-C* expression values is also described as useful for predicting the presence of residual spermatogenesis in individuals with severe spermatogenic disorders with diagnostic accuracy.

*Note: The specific names of the miRNAs included in the model are not shown as a patent is being applied for their use as diagnostic markers.Please, consider all this information as confidential material.

Limitations, reasons for caution: Further studies which involve a larger cohort of individuals would be needed.

Wider implications of the findings: Our findings contribute to the search for the most valuable genetic markers which are potentially useful as tools for predicting the origin of azoospermia and the presence of residual spermatogenesis.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 06: NON-INVASIVE OOCYTE AND EMBRYO ASSESSMENT

Monday 2 July 2018 Room 117 10:00–11:30

O-027 Is follicular size at oocyte pick-up (OPU) a predictor of blastocyst development? A study evaluating morphokinetic development and cumulus cell (CC) gene expression

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Study question: Is follicular size at OPU a predictor of blastocyst development when evaluated by morphokinetic variables and CC gene expression?

Summary answer: Follicular size correlated significantly with blastocyst quality and viability as reflected in morphokinetic variables and is a more reliable indicator than CC gene expression.

What is known already: A correlation between follicular size and embryo development as reflected in morphokinetics was found in our previous study, (Kahraman et al, 2017). We conducted this further study of CCs obtained from follicles of different sizes, for the first time evaluating both morphokinetic development to the blastocyst stage and gene expression of CCs.

Study design, size, duration: This prospective cohort study was based on 2495 COCs belonging to 184 patients and was performed between July 2014 and September 2015. Patient inclusion criteria: age ≤ 39 years, BMI < 30 kg/m^2, ≥ 8 oocytes retrieved, < 2 previous treatment cycles, and exclusion criteria: recurrent pregnancy loss, severe endometriosis, PGD or PGS, oocytes > 24, embryo transfer (ET) on day 5, PCOS, uterine anomaly, severe male infertility.

Participants/materials, setting, methods: Morpokinetic variables for cleavage events (t2) up to the expanded blastocyst stage were annotated according to follicular size. Five genes (TNFAIP6, PTGS2, HAS2, PTX3 and GDF9) were evaluated. CC gene expressions were analysed according to patient specific variables: age, BMI, AMH and follicular size. Embryos reaching (eRB) or failing to reach blastocyst (eFRB), developing into bad (BQ), top (TQ) and good quality (GQ) blastocysts were analysed using generalized linear mixed models (GLMMs) with logit link.

Main results and the role of chance: When morphokinetics of embryos coming from large and small follicles were compared, those from small follicles were found to develop faster than embryos coming from large follicles for all cleavage timings. The highest rate of top and good quality blastocysts was achieved in embryos coming from large follicles, a higher rate of direct cleavage and developmental arrest were observed in embryos obtained from small follicles. There was also a correlation between blastocyst quality and CC gene expression of HAS2 and GDF9, but only when evaluated together with follicular size, age, BMI and AMH. Furthermore, no such correlation was found in the case of other gene expressions studied. Our study suggests that, for clinical purposes, follicular size together with morphokinetic variables are a more practical indicator than CC gene expression of the likelihood of oocytes developing into transferable blastocyst.

Limitations, reasons for caution: Results were obtained in a defined IVF setting and cannot necessarily be translated in another laboratory.

Wider implications of the findings: Our study suggests that, for clinical purposes, follicular size together with morphokinetic variables are a more practical indicator than CC gene expression of the likelihood of oocytes developing into transferable blastocyst.

Trial registration number: clinicaltrials.gov NCT02230449.

O-028 Acetyl-CoA enrichment from acetate is higher in cumulus cells associated with mature oocytes

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Study question: Is enrichment of acetyl-CoA from acetate in individual cumulus cell complexes (CCC) associated with oocyte maturity?

Summary answer: Enrichment of acetyl-CoA from acetate is higher in cumulus cells associated with mature metaphase II oocytes compared to immature oocytes.

What is known already: Cumulus cells have been reported by others to be highly glycolytic. However, in previous research we found that cumulus cells had low labeling of the acetyl-CoA pool derived from glucose. In contrast, labeling with acetate was remarkably high into acetyl-CoA, which is indicative of active metabolism. The objective of this study was to develop a method to study metabolism in individual CCC to determine if this enrichment of acetyl-CoA from acetate is associated with corresponding oocyte maturity.

Study design, size, duration: This prospective, controlled, and blinded study was conducted at a private fertility center and collaborating university biochemistry research laboratory. Patients were screened using strict inclusion/exclusion criteria, and all patients received the same controlled ovarian stimulation protocol. CCC were transported to the biochemistry research laboratory, where they were individually analyzed. Acetyl-CoA enrichment from acetate was measured in 490 individual sets of CCC from 31 patients pursuing in vitro fertilization (IVF) in 2017.

Participants/materials, setting, methods: CCC were removed from oocytes after oocyte retrieval procedure and placed individually into labeled microcentrifuge tubes for metabolic testing. CCC were incubated with stable isotope ^{13}C-labeled acetate substrate. The % relative enrichment of acetyl-CoA was determined using liquid chromatography-high resolution mass spectrometry (LC-HRMS), which maintains a quantitative substrate to product relationship. Therefore, the total number of cells in the CCCs, which varies between oocytes, did not factor into the metabolic analysis.

Main results and the role of chance: Statistical significance was set at $P<.05$, and data were analyzed with SAS/STAT software. The mean % enrichment of acetyl-CoA in CCC from mature metaphase II oocytes (45.9 ± 1.2) was significantly higher ($p < 0.05$) than in those CCC associated with immature prophase I and metaphase I oocytes (39.2 ± 1.4). In addition, the mean %

enrichment of acetyl-CoA in CCC collected from women less than 34 years of age (45 ± 1.45) was higher than ($p < 0.05$) in those CCC from woman greater than 34 years of age (40.8 ± 2.2).

Limitations, reasons for caution: Our ongoing clinical trial is being conducted to determine if % enrichment of acetyl-CoA in CCC can be considered to be predictive of oocyte maturity. In addition, biochemical analysis of CCC using LC-HRMS may not be economically feasible in most clinical IVF settings.

Wider implications of the findings: The % acetyl-CoA enrichment from acetate, which is indicative of active metabolism, was found to be higher in individual CCC associated with mature oocytes compared to immature oocytes. This stable isotope tracing methodology of individual CCC may be a promising new non-invasive approach to study metabolism in associated oocytes.

Trial registration number: clinicaltrials.gov/ct2/show/NCT02793752

O-029 Oocyte meiotic spindle morphology is a predictive marker of blastocyst ploidy. A prospective cohort study

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Study question: Is oocyte meiotic spindle (OMS) morphology at the time of intra-cytoplasmic sperm injection (ICSI) a predictive marker of blastocyst ploidy?

Summary answer: OMS morphology is a predictive marker of blastocyst ploidy. Oocytes with normal meiotic spindle morphology are associated with a significantly higher rate of blastocyst euploidy.

What is known already: Euploid embryos result in higher pregnancy rates, fewer miscarriages and the birth of healthy offspring. However, preimplantation genetic testing (PGT) is invasive and costly while morphologic or morphokinetic markers are unreliable. Oocyte aneuploidy is the main cause of embryonic aneuploidy and can result from OMS abnormalities leading to chromosome alignment and segregation errors. The oocyte meiotic spindle can be non-invasively visualised using polarised light microscopy (PLM). Morphologically normal meiotic spindles are positively associated with cleavage stage embryonic euploidy and pregnancy. The association of OMS morphology with blastocyst ploidy has not previously been investigated.

Study design, size, duration: This is a prospective analysis of patients (194 patients, 221 cycles) undergoing ICSI with the intention to biopsy blastocysts for PGT between 2014 and 2017. At the time of ICSI, OMS morphology was visualized by PLM and classified as: normal, dysmorphic, translucent, not visible or in telophase. Day 5/6 blastocyst biopsy was performed on suitably progressing embryos by embryologists blind to the OMS classification. Whole genome sequencing was used to analyse the biopsied trophectoderm cells.

Participants/materials, setting, methods: The association between spindle morphology and embryo PGT results was evaluated using generalized equation estimating regression methods while accounting for non-independence of data. The additional value of OMS morphology to morphology and female age in predicting euploidy was also examined. All analyses were performed both by a) 'intention-to-treat (ITT)" where oocytes not resulting in biopsied blastocysts were considered equivalent to an aneuploid blastocyst and b) 'as treated analysis (ATA)" where only biopsied blastocysts were analysed.

Main results and the role of chance: Mean female age was 37.8 years (95% CI: 37.2-38.4) and number of oocytes collected per cycle was 12.1 (95%CI: 11.2-13.1). Overall, OMS morphology was classified for 2056 oocytes at ICSI. Most oocytes had a normal spindle($n = 1080$), followed by dysmorphic($n = 463$), not visible($n = 268$), translucent($n = 157$) and in telophase($n = 88$). OMS morphology was strongly associated with the probability of the oocyte being fertilized ($p < 0.001$), producing a day-3 embryo ($p < 0.001$) and resulting in a biopsied blastocyst ($p < 0.001$). Oocytes with normal spindles showed the best potential followed by dysmorphic, translucent, not visible & telophase spindles. The presence of a normal OMS was positively associated with euploidy compared to oocytes with all other OMS types combined, either per ITT: odds ratio (OR): 2.16 [95%CI: 1.64-2.85] or ATA: OR: 1.57 (95%CI: 1.11-2.21). The type of OMS was associated with the probability of euploidy (ATA:$p = 0.043$ & ITT: $p < 0.001$). Blastocysts originating from oocytes with normal spindles had the highest probability of being euploid (44.7% of biopsied blastocysts) (dysmorphic:40.3%; translucent: 26.4%, not visible:21.9 and telophase: 0%). Even after controlling for female age, blastocyst quality (top vs. good) and developmental stage (blastocyst vs. expanding vs. hatching), the presence of a normal spindle was strongly associated ($p = 0.008$) with the probability of euploidy (normal:44.8% vs. other:33.8%).

Limitations, reasons for caution: This study was performed in patients with an indication for PGT and hence might not be representative of the general IVF population. The oocyte meiotic spindle reflects oocyte quality and only the maternal contribution to embryo ploidy. Its assessment requires specialized equipment and staff training.

Wider implications of the findings: Non-invasive visualisation of oocyte meiotic spindle morphology can be used as a marker of its genetic normality and reproductive potential. If validated in randomized controlled trials this selective tool could benefit both patients and fertility clinics by optimising embryo selection and cycle outcome.

Trial registration number: Not Applicable.

O-030 Non-invasive imaging of embryo metabolism in response to anoxia using fluorescence lifetime imaging microscopy (FLIM)

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Study question: How does embryo metabolic function depend on oxygen availability at different stages of development?

Summary answer: Depending on developmental stage, mouse embryos displayed distinct metabolic responses to anoxia. This yields new insight for the study of embryo metabolism and, therefore, viability.

What is known already: Fluorescence lifetime imaging microscopy (FLIM) can be used to non-invasively and quantitatively measure the metabolic state of embryos. FLIM measures the fluorescence of both NADH and FAD. These endogenous molecules are integral to cellular metabolism and are auto-fluorescent, thus avoiding the need to expose embryos to any potentially unsafe reagents or dye.

Study design, size, duration: We conducted a prospective observational study utilizing a mouse model. Using an engineered on-stage incubation system, embryos/oocytes were probed by dropping oxygen from 5% to 0% over 90 minutes, monitoring metabolic state throughout. Additionally, the use of an O2-sensitive fluorophore allowed us to plot metabolic state as a direct function of O2 concentration. Experiments were conducted over one year, in triplicate.

Participants/materials, setting, methods: Samples were equilibrated to 5% O2 for 30 minutes. 0% O2 gas was flushed into the culture system for 90 minutes, causing media O2 concentration to drop according to an exponential decay, after which 5% O2 gas was restored for 30 minutes. Metabolic measurements were obtained every 6 minutes to capture the dependence of metabolic state on O2 concentrations and mitochondrial function/recovery from anoxia. Embryos were subsequently cultured until the blastocyst stage in standard conditions.

Main results and the role of chance: Mouse embryos between 1-cell and 6-8 cell stages showed similar metabolic responses to transient anoxia, with an increase in NADH fluorescence intensity [1-cell: 36.6 ± 5.7%] (reflective of total concentration) and a decrease in FAD intensity [1-cell: -13.7 ± 1.4%]. These trends then reversed with restoration of oxygen. Morulae and blastocysts, however, showed less of a metabolic response to anoxia, with minimal change in NADH [Blastocyst: 8.3 ± 5.5%] and FAD [Blastocyst: -4.7 ± 3.6%] intensity.

This finding is in line with previous evidence of distinct metabolic shifts that occur as embryos develop into blastocysts. However, our observation that

oxygen dependence decreases at blastocyst stage was surprising, as oxygen consumption is known to increase at blastocyst. This suggests more subtle mechanisms for metabolic regulation, and provides a direct framework for further elucidating these metabolic shifts.

Of note, the decrease in oxygen dependence does reflect a likely adaptation to known physiologic changes in vivo. As embryos develop, they pass into the uterus, which is known to have a lower oxygen concentration (2%). Transient O2 deprivation proved not to be a significant long-term perturbation, as embryos exposed to anoxia subsequently developed to blastocysts at >80%.

Limitations, reasons for caution: Although these findings represent an innovative mechanism of embryo evaluation, a defined standard for interpreting metabolic FLIM parameters has not been fully developed. The hardiness of the mouse model may represent that caution is still needed when applying this technique to human embryos and gametes.

Wider implications of the findings: We have also shown that FLIM-based metabolic imaging provides a highly sensitive basis for detecting metabolic state, which is known to be essential for viability. This study thus provides further evidence that this technique could serve as a powerful tool for assessing embryo quality in the future.

Trial registration number: not applicable.

O-031 A pilot study to investigate mechanical characteristics of oocytes as a predictor of preimplantation embryo development

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Study question: To determine if mechanical properties of oocytes can be obtained without harming subsequent development, and be used to predict blastulation.

Summary answer: Obtaining mechanical measurements of oocytes does not affect subsequent fertilization or development, and for a subgroup of oocytes, mechanical properties may predict blastulation.

What is known already: In multiple tissues, cells' mechanical characteristics often predict their intrinsic health and function. Many characteristics of oocytes noted by embryologists, including the ease of piercing the oolemma during ICSI or blastocyst expansion, are direct manifestations of an oocyte or embryos' mechanical properties. Our device was designed to provide objective measurements of these properties using a pipette to aspirate the cell with a uniform step negative pressure. Using this device, the mechanical properties of zygotes has been observed to predict blastulation in humans, and in a mouse model, measurement of the oocyte predicted fertilization after conventional insemination and blastocyst development

Study design, size, duration: This was a prospective split cohort study of patients undergoing IVF-ICSI at two private fertility clinics collaborating with an academic embryology lab over 6 months. Nineteen patients and the corresponding 100 measured and 368 unmeasured control oocytes from clinic A (primarily blastocyst culture), and 20 patients and the corresponding 116 measured and 111 unmesaured control oocytes from clinic B (primarily Day 3 culture) were included in the analysis.

Participants/materials, setting, methods: Patients consenting to participation and planning to undergo IVF-ICSI with >10 oocytes retrieved were approached for consent to have at most, half of their oocytes, measured with the micropipette aspiration device. The aspiration depth over time was measured and applied to a modified Zener bulk mechanical model to obtain mechanical parameters: k0, k1, tau, eta0, eta1, and zona pellucida thickness. Oocyte fertilization, day 3 morphology, and blastocyst development were recorded and compared to mechanical properties.

Main results and the role of chance: There was no observed differences in fertilization rates (74% vs. 78%, p = NS), good day 3 development per oocyte (44% vs.37%, p = NS), and a trend towards higher blastocyst development after measurement, (55% vs. 45%, p = 0.09) when comparing the measured and unmeasured oocytes. There was no correlation between maternal age or zona pellucida thickness and any of the five mechanical parameters, and the parameters do not seem to predict normal fertilization of the oocyte; however, among fertilized oocytes, 94% of oocytes with eta0 in the lowest quartile, 90% of oocytes with k1 in the top quartile, 88% of oocytes with tau in the lowest quartile, and 88% of oocytes with eta1 in the lowest quartile made blastocyst, all being significantly higher blastulation rates than oocytes in other quartiles ($p < 0.05$). No trend was observed to be predictive among other parameters.

Limitations, reasons for caution: The higher than anticipated blastocyst development rate (>70%/2PN) and good prognosis patients included in the study may limit the application of these preliminary findings to a more generalized patient population.

Wider implications of the findings: In this pilot, mechanical parameters of oocytes are highly specific but not sensitive for blastulation, and have potential for a non-invasive test of oocyte competence. Measurement is safe and does not affect fertilization or embryo development. Additional investigation with a more diverse patient population is needed to confirm these findings.

Trial registration number: NCT02530892

O-032 Comprehensive assessment of human embryo development and estimation of implantation

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Study question: Can we improve our ability to assess embryo developmental and implantation potential by investigating the possible relationship between EEVA scores and embryo-derived microRNAs or sHLA-G?

Summary answer: Our preliminary results are consistent with the study hypothesis, suggesting a correlation between EEVA scores and expression profiles of both sHLA-G and microRNAs (miRNAs)

What is known already: Early prediction of embryo viability and implantation potential remains challenging although several non-invasive strategies have been developed. Among the latter, the EEVA (Early Embryo Viability Assay) system represents a time-lapse approach that applies a patented algorithm to automatically classify Day 3 embryos in five categories, according to their highest (SCORE-1, S1) or lowest (SCORE-5, S5) probability of reaching blastocyst stage. Furthermore, a central role of sHLA-G for successful implantation and pregnancy maintenance by cytokine secretion modulation inducing immunotolerance is well established. Moreover, recent studies have suggested that miRNAs are involved in molecular signaling during maternal-conceptus dialogue aiming at implantation success.

Study design, size, duration: This is a prospective randomized pilot study. Human embryos obtained during routine IVF/ICSI cycles were continuously monitored with the EEVA system just after fertilization check and EEVA scores were generated on day 3. After day 3, embryos were cultured up to blastocyst stage. Immediately before vitrification or transfer, 25 µl of spent blastocyst media (SBM) of 90 samples were individually processed for miR-20a-5p and miR-30c-5p analysis. Furthermore, 10 µl of SBM were assayed for sHLA-G protein quantification.

Participants/materials, setting, methods: MicroRNAs isolation was conducted with miRCURY RNA Isolation Kit for Biofluids (EXIQON) and miRNAs profiles evaluated by miRCURY LNA™ /Universal RT-SYBR® Green PCR assay (EXIQON). UniSp6 and Unisp2 were used as reference genes. sHLA-G levels were assessed by Bio-Plex Multiplex immunoassays (Bio-Rad). Data distribution was analyzed by Kolmogorov-Smirnov and Shapiro-Wilk tests. Correlation was investigated using Spearman's rho and Wilcoxon test. MiRNAs and sHLA-G expression levels among EEVA categories were compared using Kruskall-Wallis and Mann-Whitney post-hoc tests.

Main results and the role of chance: Out of 90 samples, 24 were S1, 16 S2, 16 S3, 16 S4 and 18 S5. MiR-20a-5p, miR-30c-5p and sHLA-G expression levels followed a non-normal distribution. sHLA-G concentrations increased with decreasing EEVA scores (negative correlation: r=-0,476, p = 0,000) and differed significantly among EEVA categories (Kruskall Wallis, p = 0,001). Pairwise comparisons as a post-hoc analysis revealed significantly increased sHLA-G levels in EEVA score 1 (ES1) group compared to EEVA score 5 (ES5) group (p = 0,000, ES1 mean rank = 54,16 vs ES5 mean rank = 22,31). MiR-20a-5p expression profiles were also negative correlated with EEVA scores normalizing miR-20a-5p CT values both respect to UniSp2 plus UniSp6 (r=-0,277, p = 0,01) or respect to UniSp2 (r=-0,283, p = 0,03) and UniSp6 (r=-0,285, p = 0,01) only. Nevertheless miR-20a-5p was not differentially expressed across EEVA categories (Kruskall-Wallis, p = 0,165) consequently multiple comparisons were not carried out. Unexpectedly, we found a positive relationship between EEVA scores and miR-30c-5p normalizing mir-30c-5p CT values both relatively to UniSp2 plus UniSp6 (r = 0,357, p = 0,001) than for UniSp2 (r = 0,294, p = 0,008) and UniSp6 (r = 0,354, p = 0,001) only. Statistical analysis comparing miR-30c-5p profiles between EEVA categories highlighted differential expression levels (Krusall-Wallis, P = 0,039) with statistically significant increased concentrations in SBM from ES5 compared to ES1 (p = 0,04, ES5 mean rank = 51,22 vs ES1 mean rank = 29,7).

Limitations, reasons for caution: Current findings are preliminary, therefore further evaluations are needed.

Wider implications of the findings: MiRNAs and sHLA-G characterization is a good non-invasive approach for embryo evaluation and provides helpful information regarding his implantation potential and his physiological state. Therefore, profiling miRNAs and sHLA-G from SBM might be integrated with EEVA scores predictivity to enhance embryo comprehensive assessment and selection for transfer.

Trial registration number: None.

SELECTED ORAL COMMUNICATIONS

SESSION 07: BEFORE ART: FERTILITY AWARENESS, ASSESSMENT AND PRESERVATION

Monday 2 July 2018 Room 116 10:00–11:30

O-033 A systematic review of fertility-related psychological distress and reproductive concerns in cancer patients of reproductive age: informing on improved oncofertility psychological care

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Study question: What is the level of psychological distress and reproductive concerns of cancer patients of reproductive age (<45 years) throughout cancer treatment and survivorship.

Summary answer: Significant anxiety, depression and trauma related stress persist into survivorship, with female cancer patients particularly at risk of long term fertility-related psychological distress.

What is known already: Actual or perceived infertility brought about by cancer disease and treatment leads to significant psychological distress and impairments to later quality of life for cancer survivors. However, research has yet to investigate the degree of psychological distress and reproductive concerns at different oncology treatment time points; diagnosis, treatment, survivorship. One way to diminish the impact of long term distress is via the provision of appropriate fertility-related psychological care throughout the cancer journey. A systematic review of the oncofertility literature would inform on an improved model of fertility-related psychological care.

Study design, size, duration: A systematic review of the literature conducted in January 2018 utilised electronic databases Medline, EMBASE, PSYCH Info, Web of Science and SCOPUS. Search terms reflected subheadings of cancer, psychological distress and fertility. Inclusion criteria included peer reviewed articles published in English, that reported on fertility-related psychological distress or reproductive concerns of cancer patients, diagnosed at reproductive age (<45 years). No limitations were placed on patient gender, cancer type or time since diagnosis.

Participants/materials, setting, methods: An initial search identified 701 potentially relevant studies, with full text of 174 studies further screened for eligibility. A second reviewer rated a subsection of the studies at full text to ensure inter-rater reliability. Any discrepancies on inclusion or exclusion were further discussed. Quality of all included studies was assessed via the Mixed Method Appraisal Tool, with all studies of reasonable quality. A total of 40 studies were included within the review.

Main results and the role of chance: Fertility-related psychological distress experienced by patients throughout the cancer journey were reported in 34 papers. Clinical levels of anxiety and depression were reported in one third of newly diagnosed patients, with anxiety and worry persisting through to survivorship. Trauma related stress were reported in survivorship, with stress greatest in female patients. Devastation, loss of control and loneliness were negative emotional responses experienced throughout the cancer journey. Psychological distress was related to perceived quality of oncofertility care.

Reproductive concerns were reported in 34 papers. Reproductive concerns were higher in younger and infertile patients, those whom life's narrative had been disrupted. Unfulfilled desire for a child in female patients was associated with greater trauma symptoms, higher depression and poorer mental health. Prevalence of pregnancy-related concerns were high, linked with depression or trauma in some samples. Menopause concerns were linked to greater emotional distress and higher symptoms of depression. Other common reproductive concerns linked to negative emotional expression included concerns about future children and current or future relationships.

Limitations, reasons for caution: Included studies do not focus exclusively on fertility-related psychological distress. As such, there is the risk that vital information has not been captured by current literature. The limited number of interventions also hinder the ability to draw definitive conclusions about how services may be improved to better incorporate psychological care.

Wider implications of the findings: Cancer patients and survivors would greatly benefit from fertility-related psychological support implemented into standard practice. The provision of this care may serve to mediate some of the later life impacts to psychological health and reproductive quality of life in cancer survivors of reproductive age.

Trial registration number: not applicable.

O-034 Ten pathways to elective egg freezing: A binational qualitative study of what leads healthy women to fertility preservation

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Study question: Why are healthy women freezing their eggs?

Summary answer: We identified 10 different pathways leading to EEF, mostly revolving around women's lack of stable partnerships with men committed to marriage and parenting.

What is known already: The literature on oocyte cryopreservation suggests that EEF is being used primarily to defer or delay childbearing among women pursuing education and careers. However, emerging empirical data from both anonymous surveys and interview-based studies suggest that the lack of a stable partner might be the primary motivation for EEF.

Study design, size, duration: From June 2014 to August 2016, 150 women (114 in the United States, 36 in Israel) who had completed at least one cycle of EEF were interviewed by two senior medical anthropologists, one in each country.

Participants/materials, setting, methods: Study participants were recruited through 4 American IVF clinics (2 academic, 2 private) and 3 in Israel (1 academic, 2 private). In-depth, semi-structured, but open-ended interviews were audio-recorded, transcribed, and entered into a qualitative data analysis program (Dedoose) for thematic analysis, along with detailed interview summaries.

Main results and the role of chance: The majority (85%) of women in the study were without partners, reflecting 6 different life circumstances that led them to EEF (i.e., being single, divorced or divorcing, broken up, deployed overseas, single mother by choice or circumstance, career planner). EEF undertaken for career planning was the least common of these pathways. Those with partners (15%) faced 4 different life circumstances leading to EEF (i.e., not ready to have children, relationship too new or uncertain, partner refuses to have children, partner has multiple partners). As seen in these pathways, most women had already pursued and completed their educational and career goals, but had been unable to establish lasting reproductive partnerships. With only one exception (i.e., overseas deployment), these pathways varied relatively little among American and Israeli women in the study.

Limitations, reasons for caution: As a binational study, women were recruited somewhat differently between the two countries, and interviewed by two anthropologists in two languages. The number of participants was unequal, reflecting national differences in population size. These were the main limitations of this interview-based study, which is the largest to date.

Wider implications of the findings: Pathways to EEF may or may not be similar for women in other national settings. Further in-depth, interview-based research is essential. Furthermore, clinicians must be aware of the role that partnership 'troubles" play in the lives of EEF patients and make patient-centered care for single women a high priority.

Trial registration number:

O-035 Two-year follow-up of a randomised controlled trial: knowledge and reproductive outcome after online fertility education

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Study question: What are the long-term effects of fertility education on knowledge and reproductive outcome?

Summary answer: Participants in the intervention group retained some knowledge after two years and the partnered women had another child more quickly than those in control group1.

What is known already: Fertility education improves knowledge at least in the short term. Attitudes toward childbearing and its timing can change after exposure to educational materials.

Study design, size, duration: In the original randomised controlled trial (RCT), knowledge of reproductive-aged participants was assessed before (T1) and immediately after (T2) receiving one of three information brochures: fertility (intervention group), intake of folic acid during pregnancy (control group 1), or family policies in Japan (control group 2). This follow-up study was conducted two years later in January 2017 (T3).

Participants/materials, setting, methods: Of the T1 participants (n = 1,455), 383 men and 360 women (51%) responded to the T3 survey. Fertility knowledge measured with the Japanese version of the Cardiff Fertility Knowledge Scale (CFKS-J) and fertility status (e.g., new births, new medical consultations, and the timing of new birth) were assessed.

Main results and the role of chance: Baseline (T1) characteristics of the T3 participants were well-balanced between groups, whereas T3 participants were older, married, and more educated compared to those lost to follow-up. Group comparisons of the T3 scores on the CFKS-J showed no differences. Within-group comparison showed that, in the intervention group, the T3 score was significantly higher than the T1 score (4.5 and 3.6 percent higher among men and women, respectively) and significantly lower than the T2 score (6.0 and 11.5 percent lower among men and women, respectively). There were no differences between groups in the proportions of those who had a new birth or those who had a new medical consultation by T3. Among those who had a partner at T1, the proportion of those who had a new birth in the first year was higher in the intervention group than in control group 1 (folic acid): 8.8% versus 1.4% (odds ratio [OR] = 6.8, 95% confidence interval [CI]: 0.72 to 327.3) among men and 10.6% versus 2.3% (OR = 5.1, 95% CI: 1.00 to 49.5) among women.

Limitations, reasons for caution: High attrition rate may limit the generalizability of these findings.

Wider implications of the findings: Effects of one-time education were limited but retained. Follow-up education might help people retain knowledge and facilitate reproductive decisions. In view of the high attrition rate, especially among young populations, educational strategies should be explored.

Trial registration number: UMIN Clinical Trials Registry number 000016168

O-036 Randomized controlled trial on the effect of an online decision aid for young female cancer patients considering fertility preservation

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Study question: Does the use of an online decision aid about fertility preservation (FP) in addition to FP counseling, reduce decisional conflict, compared to FP counseling alone?

Summary answer: The additional use of the online decision aid (DA) significantly reduced decisional conflict (DC) in female cancer patients compared to FP counseling alone.

What is known already: Female cancer patients may be confronted with impaired fertility due to cancer treatment. Nowadays, several options to preserve fertility are available, however having to decide whether or not to opt for FP, within the short time frame after cancer diagnosis and before treatment start is challenging. Various studies have already shown that there is a high DC amongst these women and support is highly in demand. According to previous research, DA's may be a helpful support in decision-making. To our knowledge, there is no evaluated DA about FP for German speaking women that, furthermore covers several cancer types.

Study design, size, duration: The study is a randomized-controlled trial including 51 women, who were referred to one of the participating fertility centers after having been diagnosed with cancer. Participants were either assigned to the control group (counseling only) or to the intervention group (counseling and additional use of the online DA immediately after counseling). Recruitment was ongoing from July 2016 to December 2017 at a total of 8 fertility centers in Switzerland and Germany.

Participants/materials, setting, methods: The online DA was developed by an interdisciplinary team of reproductive specialists, gynecologists and psychologists. Participants were asked to complete an online questionnaire at three time points: after counselling (control group) or after counseling and the use of the DA (intervention group) (T1) as well as one month (T2) and 12 months later (T3). The survey comprised questions about fertility-related knowledge, decisional conflict, decisional regret, attitude towards FP, willingness to undergo FP and socio-demographic data.

Main results and the role of chance: First analyses of the Decisional Conflict Scale show a statistically significant difference between the intervention and control group regarding decisional conflict at T1 ($p = 0.008$). Women, who used the DA in addition to counseling, showed a significantly lower total score on the Decision Conflict Scale ($M = 23.95$, $SD = 13.48$), compared to those in the control group ($M = 36.11$, $SD = 17.26$). The mean score of three out of the four subscales of the Decisional Conflict Scale were also significantly lower for the intervention group compared with the control group. First analyses show, that there seems to be an immediate positive effect of the DA by reducing DC about FP in female cancer patients. It is expected that the final analyses of the study will provide further insight in aspects such as patients' fertility-related knowledge and decisional regret.

Limitations, reasons for caution: Education was high in two thirds of the participants. It is difficult to say, whether the DA might be equally effective in women with a lower educational background. Further analyses of the final data must be conducted in order to complete the evidence about the effectiveness of the DA.

Wider implications of the findings: This study contributes to extending the range of nowadays available DA's about FP, as it is to our knowledge the first randomized controlled trial evaluating a DA covering several cancer types. The DA seems to serve as a helpful complement of the decision-making process for patients and professionals alike.

Trial registration number: NCT02404883 (clinicaltrials.gov)

O-037 Impact of fertility assessment and counselling on life changing decisions in women of reproductive age – status one year after the initial consultation

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Study question: What was the impact of attending fertility assessment and counselling on fertile women´s decisions and subsequent choices regarding their family formation, childbearing or relationship?

Summary answer: The fertility and assessment clinic provided an individualised approach that was frequently a catalyst for change concerning childbearing decisions and behaviour.

What is known already: Over the past 30 years women and men have postponed family formation in high income societies. Fertility assessment and counselling has been suggested as a method to reduce the negative consequences of delayed childbearing. There is a need to investigate the impact of this type of intervention to see if counselling influences choices on the critical issues of delayed childbearing and gaps in fertility awareness and knowledge.

Study design, size, duration: Follow up data from a longitudinal semi-structured qualitative interview study including 20 women aged 35-40 years seeking individual fertility counselling. The interviews were conducted one year after their consultation at the clinic.

Participants/materials, setting, methods: Study participants were five single and 15 cohabiting women, residents in the Capital Region of Copenhagen, Denmark and who had sought individual fertility assessment and counselling. Since their consultation at the counselling clinic, five women had gone through fertility treatment, three had delivered and seven had attempted to become pregnant. The interviews took place in their own homes or at the clinic. Data were analysed by qualitative content analysis.

Main results and the role of chance: Twenty different women, twenty different stories. The overall theme was: 'Knowledge increased'. The women increased their knowledge after they had attended the counselling. 'Catalyst for change', 'Staying in limbo' and 'Peace of mind' were subthemes. Some of the women saw the counselling as a catalyst for change – they changed behaviour and relationship status. Five started fertility treatment (with their partner or as a future solo mother), two left their partner and seven attempted to become pregnant. The women stopped thinking about the pros and cons of childbearing and acted instead. The counselling initiated conversations with their partner about childbearing. Some of the women felt that they were still in limbo as they were still in doubt concerning childbearing. The consultation had not given them an answer with a clear deadline, and this frustrated them. But for the others, the consultation gave them peace of mind because they realised that they had time to figure out their future plans concerning childbearing.

Limitations, reasons for caution: The study participants had all chosen to seek individual fertility counselling and all women were of advanced reproductive age over 35 years. Hence, the results may not be directly transferred to the general population in regards to attitudes towards family formation and concerns of reproductive lifespan.

Wider implications of the findings: Our study highlights the impact of fertility assessment and counselling intervention which included an increase in knowledge. What the women did with it depended on their current life circumstances, fertility status and readiness. The clinic allows for an individualised approach which is necessary given the unique nature of childbearing decisions.

Trial registration number: Not applicable.

O-038 'Doing it in the right order' – Childless young men's intentions regarding family formation

G.M. Malling[1], T. Pitsillos[2], T. Tydén[2], S. Ziebe[3], B. Friberg[4], K. Hammarberg[5], L. Schmidt[1]

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Study question: Which factors influence young men's decision-making regarding timing of family formation?

Summary answer: Social and societal expectations, following the right chronology, being ready to give up individuality, and ensuring security influence young men's preferred timing of family formation.

What is known already: Postponing family formation increases risk of involuntary childlessness or having fewer children than desired. Despite Denmark and Sweden offering good opportunities to combine work life with having children, the age of childbearing has increased significantly in the last 40 years. Most previous studies regarding family formation and fertility awareness have focused on university-educated women. The few existing studies of men's attitudes to family formation indicate that prerequisites for men are to be in a stable relationship, having completed education, ensured a stable job, and having sufficient personal maturity.

Study design, size, duration: This qualitative study assessed fatherhood intentions among childless men in Denmark and Sweden in their last year of university education or vocational training. In total, 17 Danish and 12 Swedish male students were interviewed. The interviews were conducted between February-September 2017 and ranged between 30 and 90 minutes.

Participants/materials, setting, methods: The participants were recruited face-to-face or via postings at their educational institutions, and by snowball methods. Inclusion criteria were: being male, childless, aged 20-30 years and in the last year of education. The interviews were conducted wherever the participants preferred, either at their educational institution, their home, or in an office. The 29 interviews were transcribed and the content analysed through a thematic content analysis.

Main results and the role of chance: Study participants were on average 25 years old (range 20-30). Through the analysis five subcategories emerged: 1) Expectations from the society; 2) Influence from within the social circle; 3) Enablers for earlier family formation; 4) Giving up freedom and individuality; 5) Ensuring security and stability.

Almost all participants wanted children in the future. The young men wanted to find their path in life before having children. In relation to life events, they felt that they were required to follow the right chronology – first education, accommodation and career, then children. Further, they expressed the need to explore some of the many other life experiences available to young people in contemporary society, including travel, to figure out what they wanted in life. Moreover, being ready to have children was associated with being ready to give up individuality and freedom. While many stated that they felt mature enough, this was not equated with feeling ready to have children. Other factors influencing participants' preferred timing of parenthood were being in a stable relationship, having appropriate housing, ensuring financial security, and having fulfilled career aspirations. Further, both Danish and Swedish men expressed reflecting themselves in their parents in relation to age at first child.

Limitations, reasons for caution: Despite following the same interview guide, the interview technique between countries may have differed. This may have influenced the dialogue. Men who want children may have been more likely to agree to participate than men who do not want children.

Wider implications of the findings: In order to promote earlier family formation and prevent infertility this study highlights the need to educate young men about the limitations of fertility. Exploring the many opportunities of today's society and chasing a career is not limited by age, however fertility is.

Trial registration number: Not applicable

INVITED SESSION

SESSION 08: LABORATORY MODELLING TO UNDERSTAND ENDOMETRIAL FUNCTION

Monday 2 July 2018 Room 211 + 212 11:45–12:45

O-039 Endometriosis and associated pain

E. Greaves[1]

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Endometriosis is a complex chronic inflammatory condition associated with debilitating pain and infertility that affects 176 million women worldwide. Current research aims to understand the aetiology and pathophysiology of the disorder and develop novel effective non-surgical therapies that lack the unwanted side effects of current medical management. Tools for endometriosis research fall into two broad categories: patient-derived tissues and fluids (and cells isolated from these sources) and laboratory models based on the use of cells or animals. There are many different models (in vitro and in vivo) reported in the literature, all have their advantages and drawbacks and choice of model depends largely upon the research question. Rodent models of endometriosis in particular exist in many different variations and have different utilities depending whether the study is designed to explore pain or infertility. The rat has been used most consistently for studying mechanisms of endometriosis-associated pain with a number of different behaviour read-outs including those used to measure mechanical hyperalgesia, allodynia and anxiety. Many different versions of mouse models of endometriosis exist (with refinements and standardisation being a current focus in the field) and their utility for the assessment of pain in the condition is becoming a priority. In summary, hypothesis-driven endometriosis research can only be facilitated with careful experimental design and selection of the most appropriate human tissue from patients with and without endometriosis and combinations of physiologically relevant in-vitro and in-vivo laboratory models.

O-040 Implantation and early pregnancy, the maternal contribution

C. Simon Valles[1]

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Abstract text

Human reproduction is a very inefficient process with a probability of conception during a given menstrual cycle around 30%. In the last 40 years, reproductive medicine has emerged as one of the most rapidly developing areas of medical science propelled by clinical and basic research advances in ovarian physiology, induction of ovulation, and embryology. Despite all technological advances in the understanding of the "seed", the maternal endometrium has been the forgotten organ. Nowadays, when a morphologically normal blastocyst is transferred into a seemingly normal uterus, cycle reproductive success is still limited, and sadly, has not significantly improved since the 1990´s. This is the main reason the clinical community turn its attention to embryonic implantation as the end-point not only for the reproductive success but also for the obstetric outcome and the conditioning of the origin of some adult diseases.

The human endometrium is a hormonally regulated organ that is the anatomic pre-requisite to initiate and control human pregnancy while is a sentinel for protecting the upper female reproductive from infections by a variety of microbes. The transition from anatomical to molecular medicine has led to the discovery of the transcriptomic signature of endometrial receptivity and its clinical application in patients with implantation failure through personalized embryo transfer. The endometrial cavity has been classically considered to be a sterile organ, but reports challenging this dogma support the existence of an endometrial microbiota composed of different microorganisms (specifically *Lactobacillus* spp., *Mycoplasma hominis*, *Gardnerella vaginalis,* and *Enterobacter* spp.) that might affect the reproductive outcome. The maternal decidua controls conception and the course of pregnancy in humans. Also, failed decidualization is associated with a spectrum of reproductive defects including failed implantation and clinical miscarriage but also late gestational complications such as preeclampsia. New emerging data reinforce the concept that suboptimal uterine conditions lies upstream of not only inefficient conception, but also failed trophoblast differentiation and invasion driving placentation, paving the way to better diagnosis, prevention and treatment of late gestational diseases.

In this presentation, I will revisit the modern knowledge of the "soil" concept and its basic and clinical contribution to the embryo implantation process.

INVITED SESSION

SESSION 09: DATA REPORTING SESSION

Monday 2 July 2018 Room 111 + 112 11:45–12:45

O-041 Data from the ESHRE PGD Consortium

O-042 How do ESHRE members use and evaluate the ESHRE guidelines?

S. Gameiro[1], N. Vermeulen[2], M. Sousa Leite[3]

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Introduction: From 2013 ESHRE has been publishing evidence-based guidelines to support care providers and patients making every-day decisions about appropriate and effective health care {Vermeulen, 2017 #508}. So far ESHRE has mainly focused on the development and the dissemination of the guidelines and less on ensuring that they are being appropriately implemented across Europe. The current study reports on dissemination, implementation and impact of the first four published ESHRE guidelines: Management of Endometriosis (ENDO; Dunselman et al., 2014), Routine Psychosocial Care (RPC; Gameiro, et al., 2015), Premature Ovarian Insufficiency (POI; European Society for Human Reproduction and Embryology Guideline Group on POI, 2016) and Recurrent Pregnancy Loss (RPL; European Society for Human Reproduction and Embryology Guideline Group on RPL, 2018).

Methods: The online survey assessed dissemination (knowledge that the guidelines were published [Y/N] and download [Y/N]), implementation (using guidelines in daily practice [Y/N] and made changes to practice according to recommendations [Y/N]) and impact (perceived patient benefit [Y/N] and referral of patients to the guidelines [Y/N]). It further investigated perceived quality of the guidelines (1 'very poor' to 5 'excellent'), and barriers [open question] and beneficial support (list of available support options) for their implementation. The survey was online from February 20th to April 3rd 2018. It was advertised via the ESHRE website, social media and mailings to the ESHRE membership. Participants were offered the opportunity to win a free registration for the ESHRE Annual Meeting in Barcelona or an ESHRE Campus Course.

Results: A total of 723 ESHRE members accessed the survey.

540 (82.1%) participants know ESHRE published the ENDO guideline. From these, 356 (65.9%) downloaded it, 225 (41.7%) use it in their daily practice, 166 (30.7%) are changing their practice, 105 (19.4%) think that patients benefited from the guideline, and 48 (8.9%) referred them to it.

227 (54.6%) participants know ESHRE published the RPC guideline. From these 119 (52.4%) downloaded it, 67 (29.5%) use it in their daily practice, 43 (18.9%) are changing their practice, 37 (16.3%) think that patients benefited from the guideline, and 29 (12.8%) referred them to it.

205 (56.6%) participants know ESHRE published the POI guideline. From these 111 (54.1%) downloaded it, 69 (33.7%) use it in their daily practice, 44 are changing their practice, 33 (16.1%) think that patients benefited from the guideline and referred them to it.

199 (59.5%) participants know ESHRE published the RPL guideline. From these, 113 (56.8% downloaded it and 63 (31.7%) intend to change their practice.

The overall perceived quality of the guidelines was: ENDO 4.18 (0.71), RPC 4.11 (0.62), POI 4.26 (0.62), and RPL 4.26 (0.68).

Data on perceived barriers to implementation will be subjected to content analysis. Regarding perceived beneficial support, 180 (68.4%) participants would do an e-learning course on the guidelines, 164 (64.3%) would use an app, 166 (63.8%) would use a step-by-step manual, 153 (59.8) would use a printed pocket guideline, and 128 (50.0%) would attend a campus course.

Discussion: Considering the multi-faced dissemination strategies used by ESHRE, one would expect higher knowledge and access to the guidelines. Although ESHRE members perceive the quality of the guidelines to be very good and show interest in learning about how to implement them, only around one-third actually use them in their clinical practice. It is therefore not a surprise that perceived patient benefit is low. Results suggest that the current ESHRE guidelines strategy is insufficient to ensure their implementation. Knowledge about perceived barriers to implementation will inform about additional support that ESHRE can provide to its community. On-line media seems to be the members' preferred channel to receive such support.

INVITED SESSION
SESSION 10: FERTILITY SOCIETY OF AUSTRALIA EXCHANGE LECTURE

Monday 2 July 2018 Room 113 + 114 + 115 11:45–12:15

O-043 Rapid, non-invasive measurements of metabolic balance can stratify embryos based on ploidy

B. Arman[1], L. Sandeman[1], C. Carbone[1], F. Bell[1,2], M. Lane[1,2]

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[2]*Monash IVF, Monash IVF, Melbourne, Australia*

Aim: To determine whether measurements of glucose uptake and hydrogen peroxide production in human embryo secretome can be used to predict aneuploidy and subsequent viability.

Method: Samples of spent culture media (n = 123) from patients (mean maternal age 36.6 ± 0.6, mean maternal BMI 26.3 ± 0.8) undergoing IVF or ICSI with pre-implantation genetic screening (PGS) were collected following incubation of individual embryos in G2 Plus (Vitrolife) from day 3 to day 5. Samples were frozen and blinded before analyses of glucose (ultra-microfluorometric analysis) and hydrogen peroxide (Amplex UltraRed) concentrations. These concentrations were correlated to embryo ploidy status, morphology, and pregnancy outcomes. Statistical analysis was conducted using a Student's t-test.

Results: Metabolic profile was not correlated with either blastocyst expansion or inner cell mass or trophectoderm quality. When combining the rate of hydrogen peroxide production and glucose update to approximate mitochondrial function as a proportion of substrate uptake, then euploid embryos had a significantly higher metabolic ratio than aneuploid embryos (+2.9 fold, $p < 0.05$). Within euploid embryos, pregnancy rates were associated with an increase in glucose uptake (+3.0 fold, $p < 0.05$).

Conclusion: Metabolic balance between substrate uptake and pathway utilisation improves the ability to stratify embryos into viable and non-viable cohorts. This study also confirms that morphology is a poor predictor of viability and metabolic output. These results suggest that metabolic balance of embryos could be used as a rapid and inexpensive non-invasive screening tool for ranking embryo suitability for transfer. Further studies are required to determine the metabolic consequences of individual aneuploidies.

INVITED SESSION
SESSION 11: WHAT ABOUT THE CHILDREN?

Monday 2 July 2018 Room 117 11:45–12:45

O-044 Health of ICSI children

U.B. Wennerholm

University of Göteborg, Department of OB/GYN\rInstitute for Women's & Children's Health, Göteborg, Sweden

It is over 25 years since the first child was born after ICSI treatment. Since then there has been an ongoing debate on the use and safety of the technique. Initially, ICSI was used to treat severe forms of male factor infertility but today it is also used to treat mild male factor infertility, mixed male/female infertility, unexplained infertility and fertilization failures. Both the latest European ESHRE report and the global ICMART report reveal an increasing global use of ICSI, with one-third of fresh assisted reproductive technology (ART) cycles using conventional IVF and two-thirds using ICSI. Due to the invasiveness of the ICSI procedure as well as the arbitrary selection of the spermatozoon and genetic

and epigenetic parental factors, concerns have been expressed about the health of ICSI children.

Adverse perinatal outcomes in ART, which includes ICSI and conventional IVF techniques, are associated mainly with ART's higher rates of multiple pregnancies. In ART singletons, the rate of very preterm birth and very low birth weight is about two to three times higher than in the general population. When comparing ICSI and conventional IVF, most large studies have found similar or lower risks of very preterm and preterm birth, very low birth weight, low birth weight and peri/neonatal mortality.

Many large national registry studies show a slight increase in birth defects in ART children compared to the general population. Evidence regarding birth defects in ICSI versus IVF is less clear but most large cohort studies show no difference in the incidence of birth defects between ICSI and IVF newborns, and the different sperm sources used in ICSI do not seem to influence the rate of birth defects negatively. However, the incidence of de novo and inherited sex chromosomal abnormalities in ICSI offspring is slightly higher, which probably relates to the genetics of the infertile couples. Some studies, mainly case-control studies have reported associations between ART (but not to a specific reproductive technique) and imprinting disorders such as Beckwith-Wiedemann syndrome, Angelman syndrome and Prader Willi syndrome. These disorders may be related to the underlying infertility problem. All are rare disorders and the absolute risk of an imprinting disorder after ICSI/IVF is extremely small.

Studies of growth and physical health are few and limited to childhood. Looking at general physical health including such factors as hospitalizations, childhood illnesses, surgical interventions and medical therapies, there are similar results for ICSI and IVF-conceived children. Results are also similar for age and gender-matched controls in the general population. Most large cohort studies do not show any increase in childhood cancer in general in ART children and provide no evidence of increased numbers of adverse outcomes in the ICSI group.

Most studies comparing children up to eight years of age born after ICSI, IVF and spontaneous conception, suggest their neurocognitive development is comparable. Sperm source or individual semen parameters do not affect neurodevelopment. Two large recent studies reporting associations between ICSI and autism and autistic disorders should be interpreted with caution, since the absolute risks of autism and autistic disorders are small.

A few longitudinal reports on reproductive health in ICSI adolescents and young adults have also been published recently. Onset of puberty and pubertal development is similar for ICSI and spontaneously conceived boys and girls, but ICSI-conceived men seem to have lower sperm concentrations and total sperm counts than age-matched, spontaneously conceived controls.

Further monitoring and research is necessary before firm conclusions about the safety of ICSI, as opposed to IVF, can be drawn. Large well-designed cohort studies are still needed, taking into account parental factors and technical procedures as well as selection and surveillance biases.

O-045 Psychological and medical follow-up of children born after Pre-implantation Genetic Testing (PGT)

J. Nekkebroeck[1], B. Maryse[2]

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Abstract text

Psychological and medical follow-up of children born after **Pre-implantation Genetic Testing** (PGT)

Follow-up studies on the health of PGT (and PGT-Aneuploidy) children are essential in order to provide reassurance about the safety of the technique (ESHRE 2007; ESHRE PGD Consortium 2012).

Psychological health is translated in the cognitive and motor development of children. Pioneering studies did not differentiate between children born after PGT or PGT-A (Banerjee, 2008; Nekkebroeck, 2008) but found reassuring results for cognitive development at age 2 (Nekkebroeck, 2008). In the study of Banerjee et al. (2008) the scores for locomotor capacities of the PGT(-A) children were significantly lower than those of the ICSI group. In the study of Nekkebroeck et al. (2008) PGT(-A) children presented normal motor development compared to ICSI and naturally conceived (NC) counterparts.

Only a few studies focused solely on PGT children. Thomaidis et al. (2012) examined 31 PGT children of different ages, 22.6% showed mild motor retardation quotients but there was a lack of matched controls. The same was the case in the study of Sacks et al. (2016) where 4-to-5 year old PGT children showed normal neuropsychological development. Winter et al. (2014) examined 5-to-6 year old PGT singletons for cognitive and motor development and found comparable scores to their matched ICSI and NC controls.

Psychological health is also defined in terms of psycho-social development and family functioning. In Nekkebroeck et al. (2008) the socio-emotional development of PGT(-A) children aged 2 did not differ from the control groups (ICSI and NC) neither did parental stress and mental health status. For PGT children aged 5-6 similar results were found for psychosocial development (Winter et al., 2015). In terms of family functioning the NC mothers, experienced more parental stress while PGT/ICSI mothers had higher feelings of competence and were more goal directed.

The neonatal and medical outcomes of PGT/PGT-A children compared to ICSI and sometimes NC are also described and are mainly reassuring.

Two months after birth PGT/PGT-A children were examined and compared with ICSI new-borns in research of Liebaers et al. (2010). More perinatal death was observed in PGT/PGT-A multiples than in ICSI multiples. Gestation, birthweight and major malformation rates were similar. In another neonatal follow-up study of 995 children (singletons and multiples) born after PGT no differences regarding: mean term, prematurity, birth weight, very low birth weight, perinatal death, major malformations and neonatal hospitalizations with ICSI singletons and multiples. However, fewer multiples born after PGT presented a very low birth weight in comparison to ICSI multiples (Desmyttere et al. 2012).

At age 2 no differences between PGT(-A) singletons regarding weight, height and head circumference were found when compared to ICSI singletons. Compared to NC children the birth weight tended to be slightly lower for PGT (-A) children. The latter were more frequently born after Caesarean section than ICSI children (and NC) but there were no more congenital malformations, hospital admissions and surgical interventions compared with ICSI/ NC children (Desmyttere et al., 2009). When comparing 2-year-old twins and singletons again no differences were observed compared to ICSI counterparts. In a Dutch cohort of PGT-A children there were no differences in blood pressure and anthropometrics at age 4 and 9 compared to IVF/ICSI (Seggers et al., 2013; Kuiper et al., 2018).

Conclusion: Methodological weaknesses are encountered in many follow-up studies on PGT(-A) children (e.g. no distinctions between PGT/PGT-A children, including singletons as well as multiples, including children of different ages, no matched controls, small samples sizes). Luckily, most studies with or without these flaws indicate that young PGT(-A) children develop very similar to ICSI and NC children. Continuous follow-up remains necessary to ensure this positive outcome at older ages.

INVITED SESSION

SESSION 12: DEBATE: IMMUNOSUPPRESSION FOR IMPLANTATION FAILURE - IS IT WORTH IT?

Monday 2 July 2018 Room 211 + 212 14:00–15:00

O-046 Pro

S. Quenby[1]

University Hospital Coventry Warwick Medical School, Division of Reproductive Health, Warwick- Coventry, United Kingdom

Implantation failure is an increasing problem. This is a result of advances in ART so that success rates following embryo transfer are now significantly better than intercourse. Hence today a lack of positive pregnancy test or a transiently positive pregnancy test after embryo transfer is now a source frustration from the

patient and clinician's perspective. Implantation and embryo-maternal interactions are complex and still the subject of intensive research. However, some features of this interaction are known. The embryo needs to be recognised by the endometrium as being of high quality, a function that is achieved by the process of decidualisation. The decidualised endometrium then needs to respond to this embryo in a controlled fashion with an initial inflammatory response that then needs to be modulated to allow trophoblast invasion. There are many lines of evidence that indicate that failure to respond to an embryo, excessive inflammation, lack of local glucocorticoid steroid synthesis and failure to down regulate inflammation are all associated with implantation failure and early pregnancy loss. This means that there are many plausible biological mechanisms by which immunosuppression could improve implantation. Randomised controlled clinical trials in this area have all been disappointing however, they have lacked a personalised approach. The clinicals trials have demonstrated safety of immunosuppression in implantation failure. It is possible that immunosuppression given to the right patient at the right time will improve pregnancy outcome.

O-047 Con

INVITED SESSION
SESSION 13: MEIOSIS AND GENOME STABILITY
Monday 2 July 2018 Room 111 + 112 14:00–15:00

O-048 Dissecting gene function in oocyte meiosis

M. Schuh[1]

Max Planck Institute for Biophysical Chemistry, Department of Meiosis, Göttingen, Germany

The Department of Meiosis at the Max Planck Institute for Biophysical Chemistry investigates mechanisms that are involved in chromosome segregation during meiosis in mammalian oocytes, the progenitor cells of eggs. This topic is of great interest for fundamental research because chromosome segregation during meiosis is still much more poorly understood than in mitosis, especially in mammals. It is also of direct medical relevance because aneuploidy in eggs is a leading cause of pregnancy loss and several congenital disorders such as Down's syndrome. Our main aim is to understand how defects at the interface between chromosomes and cytoskeletal structures lead to aneuploid eggs and pregnancy loss in mammals. To this end, we study how the meiotic spindle is organized, how it segregates the chromosomes and how the spindle interacts with actin to drive the meiotic divisions. We are also interested in the mechanisms that lead to an increase in aneuploidy in eggs with advanced maternal age.

We are also very active in developing new tools to study meiosis in mammalian oocytes. For instance, we have recently developed a method, called Trim-Away, that can be used to acutely remove endogenous proteins from oocytes and embryos. We have also established methods that can be used to study the causes of chromosome segregation errors directly in live human oocytes. In this presentation, I will talk about our latest research on the causes of the maternal age effect, which describes the decline in female fertility with advanced maternal age.

O-049 DNA damage and genome stability in the preimplantation embryo

INVITED SESSION
SESSION 14: FEMALE FERTILITY PRESERVATION
Monday 2 July 2018 Room 113 + 114 + 115 14:00–15:00

O-050 Fertility preservation - ovarian stimulation, when, how and in whom?

A. Germeyer[1]

Universitäts-Frauenklinik, Gyn. Endocrinology and Reproductive Medicine, Heidelberg, Germany

Fertility preservation is getting more and more important for young women with gonadotoxic therapies, as their chance for survival is highly improved due to better therapies. Furthermore as childbearing is postponed due to social factors, such as female carriers, women are at increased cancer risk before their family planning is complete.

Therefore cancer survivors nowadays have an increased desire for parenting and the wish to conceive their genetic offsprings.

Currently available techniques for fertility preservation include ovarian suppression with GnRHa, ovarian stimulation and egg collection, ovarian cryopreservation and ovarian transposition in cases of radiation of the lower abdomen.

Ovarian stimulation for collection of mature oocytes is a, well established, option for fertility preservation in women at risk. The chance for later conception with these cryopreserved oocytes depends on the age at retrieval and the number of oocytes available.

An ovarian stimulation is feasible, when 2 weeks are available prior to the start of gonadotoxic therapies.

In contrast to conventional ovarian stimulation for in vitro fertilization, ovarian stimulation can be started in any cycle phase (so called random start) with similar outcome. In order to reduce the risk for ovarian hyperstimulation syndrome, despite a high oocyte yield, a dose intensified antagonist protocol followed by ovulation induction with GnRHagonist is highly recommended. Gonadotropin doses used are typically 50IE higher than in conventional ovarian stimulation, to optimize the number of oocytes retrieved. In women with estrogen dependent tumors, such as hormone receptor positive breast cancer patients, an aromatase inhibitor (Letrozol® 5 mg/d) is given during ovarian stimulation to minimize the estrogen production. When time is limited until gonadotoxic therapies, in vitro maturation can be an option in women with a high antral follicle count, as seen in PCOS. IVM is performed without prior ovarian stimulation however chances of conception with the recovered cells are lower than after conventional ovarian stimulation. On the other hand, if more than 4 weeks are available until gonadotoxic therapies are started, a double stimulation can be performed with a first stimulation at random start and a second stimulation started 2 days after ovum pickup, in order to increase the number of oocytes available.

Recovered mature oocytes can then either be frozen without or after fertilisation (preferably with intracytoplasmic sperm injection in order to reduce the risk of fertilization failure).

The only premise for the use of ovarian stimulation as fertility preservation technique is a postpubertal stage with remaining ovarian reserve at the time of consultation prior to the gonadotoxic therapy.

O-051 Use and efficiency of cryopreserved material for women with serious disease

SELECTED ORAL COMMUNICATIONS
SESSION 15: REPRODUCTIVE SURGERY
Monday 2 July 2018 Room 117 14:00–15:00

O-052 Validation of the Endometriosis Fertility Index (EFI) to predict pregnancy in an infertile argentine population

L. Solari[1], **G. Botti**[2], **G. Percivalle**[1], **M. Gutierrez**[1], **S. Marin**[1], **M.E. Mackey**[3]

[1]*Sanatorio Centro, Centro de Cirugía Ginecológica Mini Invasiva, Rosario, Argentina*
[2]*Sanatorio Centro, Centro de Cirugía Ginecològica Mini Invasiva, Rosario, Argentina*

[3]PROAR, Bioestadìstica, Rosario, Argentina

Study question: To evaluate if Endometriosis Fertility Index (EFI) is useful to estimate the reproductive outcome in infertile argentine patients with diagnosis and surgical treatment of endometriosis.

Summary answer: The EFI was validated as a useful tool to estimate the reproductive prognosis of infertile argentine patients diagnosed with endometriosis.

What is known already: Adamson y Pasta developed a scoring system, being the only classification of endometriosis for infertile patients that predicts the spontaneous pregnancy rate after laparoscopy and allows to determine what type of fertility treatment is appropriate for each patient. After being validated in the North American population, retrospective studies in other countries such as France, Italy, Belgium and China have also evaluated their usefulness, although it had not yet been studied in our country.

Study design, size, duration: Retrospective cohort study. We analyze medical records of 90 infertile patients (April 2011 to January 2015) with endometriosis diagnosed by laparoscopy. Data from surgical factors for categorization according to EFI was obtained from videos of laparoscopies and historical factors were collected from medical records. The pregnancy rate was evaluated after 12 months follow-up period. Patients were divided according to EFI in group 1 (0-4), 2(5-7), 3 (8-10), cumulative pregnancy rates among these groups were compared.

Participants/materials, setting, methods: We included infertile patients diagnosed with endometriosis by laparoscopy performed by the same surgical team. Patients with a uterine factor, an abnormal hysterosalpingogram, or whose partner had a severe male factor were excluded. The treatment performed was fulguration of peritoneal endometriosis lesions, adhesiolysis and endometriomas cystectomy. A double-blind retrospective review of these videos was performed to obtain the data of the surgical factors to categorize patients.

Main results and the role of chance: The probability of spontaneous pregnancy or pregnacy with ovulation induction or intrauterine insemination (IUI) within 12 months after laparoscopy was significantly higher in those with the greater EFI category compared with those with lower EFI category. The total pregnancy rate was 48.8%, 18.8% in group 1, 37.8% in group 2 and 66.66% in group 3. These rates presented a statistically significant linear trend ($p = 0.003$). When comparing the groups it is observed that the group 3 pregnancy rate was higher with respect to group 2 (RR: 1.76, 95% CI 1.10–2.81), and group 1 (RR: 3.67, 95% CI 1.03–13.08), both differences statistically significant. The pregnancy rate of the second group was double in relation to the group with lower EFI (RR: 2.09, 95% CI 0.56–7.79), however this difference was not statistically significant ($p = 0.22$). The 65.9 % of pregnancies achieved within the first 6 months after surgery.

Limitations, reasons for caution: A disadvantage of this system is the subjectivity when performing the Least Function Score because is possible to find differences between the surgeons. Also the calculation of EFI can be time consuming for the surgeon.

Wider implications of the findings: In patients with higher EFI category, the probability of spontaneous pregnancy or pregnancy with ovulation induction or IUI was increased within 12 months after laparoscopy. This allows us to validate the EFI as a useful tool to estimate the reproductive prognosis of infertile argentine patients diagnosed with endometriosis.

Trial registration number: Not applicable.

O-053 Whether the surgical indications for septate uterus are reasonable in infertile patients before in vitro fertilization?

X. Li, Y. Ouyang, Y. Wen

Reproductive & Genetic Hospital CITIC-Xiangya, Imaging Department, Changsha, China

Study question: Whether the surgical indications are reasonable for infertile patients with septate uteri before in vitro fertilization-embryo transfer (IVF-ET)?

Summary answer: The surgical indications for septate uteri proposed in this study are reasonable in infertile patients before IVF-ET.

What is known already: Up to date, there is no uniform surgical indications for septate uteri. This anomaly has been commonly considered to be associated with poor reproductive outcomes. However, the optimal surgical timing for septate uteri is still controversial. Should preventive treatments be given to reduce complications?

The incidence of uterine malformations in infertile patients is much higher than that in the general population. Normal uterine morphology is a prerequisite for successful embryo implantation, therefore, proper treatments are necessary for septate uteri before IVF-ET. Then, what kind of surgical indications are reasonable?

Study design, size, duration: A retrospective study was conducted of 937 infertile patients with septate uteri and 1578 patients with normal uteri who obtained singleton pregnancies via IVF-ET from January 2014 to December 2015.

Hysteroscopic metroplasty was performed before IVF-ET if patients meet at least one indication: (1) the septum depth ≥10 mm; (2) the septum depth between 5-10 mm accompanied by unexplained recurrent miscarriage or infertility history; (3) recurrent failures of IVF-ET. Conversely, patients would be managed expectantly.

Participants/materials, setting, methods: This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). The patients with septate uteri were divided into non-surgical ($n = 518$) and surgical ($n = 419$) groups and the uterine cavities were normal after surgery. The septate uteri were diagnosed according to the ESHRE-ESGE system. 1578 infertile patients with normal uteri were selected as the control group. The pregnancy and obstetric outcomes were compared among non-surgical, surgical and normal groups.

Main results and the role of chance: Multivariate regression analysis showed a significantly higher early pregnancy loss (EPL, OR 1.49 (1.07-2.07), $p = 0.018$) risk and much lower rates of term delivery (OR 0.72 (0.54-0.95), $p = 0.023$) and live birth (OR 0.67 (0.50-0.91), $p = 0.010$) in the surgical group than those in the non-surgical group; compared with the normal group, the surgical group showed significantly higher risks of EPL (OR 1.23 (1.08-1.40), $p = 0.002$), small gestational age at delivery (OR 1.26 (1.02-1.57), $p = 0.041$), low birth weight (OR 1.32 (1.03-1.70), $p = 0.019$), and the rates of term delivery (OR 0.79 (0.70-0.88), $p \leq 0.001$) and live birth (OR 0.79 (0.70-0.88), $p \leq 0.001$) were much lower; all pregnancy and perinatal outcomes were statistically similar between the non-surgical and normal groups ($P > 0.05$).

In the surgical group, the rates of miscarriage (OR 3.10 (2.08-4.61), $p \leq 0.001$) and ectopic pregnancy (OR 13.25 (6.46-27.17), $p \leq 0.001$) were significantly lower and the live birth rate (OR 0.05 (0.03-0.09), $p \leq 0.001$) was much higher than before surgery. According to the ROC curves, the cut-off values for surgery were septum length = 6.85 mm and the rate of internal indentation/uterine wall thickness = 0.895.

Limitations, reasons for caution: Firstly, all pregnancy outcomes were obtained via telephone calls or faxes, thus, some details were not studied. Secondly, the control group was not screened randomly, which may cause selection and confounding biases. Thirdly, not all patients received surgeries in our hospital, the inconsistency of surgical effects was inevitable.

Wider implications of the findings: The surgical indications proposed in this study can be used to guide proper treatments for infertile patients with septate uteri before IVF-ET.

Trial registration number: None.

O-054 Fertility after salpingotomy versus salpingectomy in women with tubal pregnancy; an individual patient data meta-analysis

N. Netteb[1], P. Capmas[2], H. Fernandez[2], P. Hajenius[1], B.W. Mol[3], M. Van Wely[1], F. Mol[1], O.N. Behalf of the DEMETER study group[2], O.N. Behalf of the ESEP study group[1]

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Study question: Before surgery for ectopic pregnancy: can we distinguish women who would benefit from salpingotomy in terms of a subsequent ongoing pregnancy, based on patient characteristics?

Summary answer: Salpingotomy did not improve fertility rates, although a small benefit could not be excluded. No subgroup was detected that would benefit more from salpingotomy.

What is known already: Tubal ectopic pregnancy can be surgically treated by salpingectomy in which the affected tube is removed, or by salpingotomy in which the ectopic pregnancy is removed and the tube is preserved. Salpingotomy bears the increased risk of persistent trophoblast, requiring additional treatment. Our conventional meta-analysis including data of two RCTs (DEMETER, ESEP) did not show a large benefit from salpingotomy, but a small benefit could not be excluded.

Study design, size, duration: We performed a meta-analysis with individual patient data (IPD) of RCTs based on a search of international scientific databases in collaboration with the Cochrane gynaecology and fertility group. We selected trials comparing salpingotomy and salpingectomy for tubal ectopic pregnancy and collected data on fertility prospects after surgery. The primary outcome was an ongoing pregnancy by natural conception. We aimed to determine the pregnancy chance over time, which will be analysed by survival methods.

Participants/materials, setting, methods: Follow up times were calculated from randomisation until last menstrual period of the ongoing pregnancy. All analyses were performed on an intention-to-treat basis. The estimated log hazard ratios were combined across studies using standard fixed-effect and random-effects meta-analysis methods. Subgroup effects were estimated by treatment-by-covariate interaction terms within studies and combining these across studies similarly. We explored the treatment modification for maternal age, serum hCG pre-operatively and size of tubal ectopic mass on ultrasound.

Main results and the role of chance: We identified two relevant trials, containing data of 653 women randomised to salpingotomy or salpingectomy. The cumulative ongoing pregnancy rate by natural conception was 58.3% after salpingotomy and 55.0% after salpingectomy (log rank p = 0.537). The mean time to an ongoing pregnancy was 21.9 months (95% CI 19.1 – 24.7) after salpingotomy and 23.8 (95% CI 21.1–26.5) after salpingectomy, resulting in a hazard ratio of 1.07 (95% CI 0.86–1.35). There was no interaction between maternal age, serum hCG level, size of tubal ectopic mass and treatment on ongoing pregnancy chance. We found no associations with maternal age or size of tubal ectopic mass. Serum hCG < 2,335 IU/l was associated with a higher ongoing pregnancy chance with a cumulative ongoing pregnancy rate of 63.8% after salpingotomy and 60.5% after salpingectomy (fecundity rate ratio 1.18; 95% CI 0.87–1.61; log rank p = 0.278).

Limitations, reasons for caution: In the ESEP study women with contralateral tubal disease were excluded whereas such women were not excluded in the DEMETER study. In the DEMETER study all women treated by salpingotomy also received a single shot methotrexate postoperatively. Analyses of other pre-specified treatment modifications are ongoing.

Wider implications of the findings: This IPD meta-analysis facilitates shared-decision- making. Salpingectomy might be the preferred treatment in view of safety of the intervention. Women with a preference for maximizing their pregnancy prospects might still opt for salpingotomy. Future studies need to take costs into account.

Trial registration number: DEMETER NCT00137982, ESEP ISRCTN37002267

O-055 Ovarian Reserve after uterine artery embolization in women with morbidly adherent placenta

A. Mohr Sasson[1], M. Spira[1], R. Rahav[1], D. Manela[1], E. Schiff[1,2], S. Mazaki-Tovi[1,2], R. Orvieto[1,2], E. Sivan[1,2]

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[2]*Tel Aviv University, Sackler School of Medicine, Tel Aviv, Israel*

Study question: The aim of this study is to assess ovarian reserve in women after preservative cesarean section using uterine artery embolization (UAE) due to morbidly adherent placenta.

Summary answer: Women post preservative cesarean section using UAE due to placenta accrete have lower ovarian reserve compare to controls matched by age.

What is known already: Conservative management of morbidly adherent placenta can be considered when fertility preservation is desired, however, concern has been raised regarding the effect of embolization on ovarian function. One possible potential risk to fertility, is the exposure of the ovaries to ionizing radiation, being a procedure that is carried out under fluoroscopic control using contrast media for X-ray angiograms. Other mechanism, is the potentially unintentional migration of embolization material through anastomoses between the uterine and ovarian vessels. To the best of our knowledge, this is the first study evaluating hormonal status after UAE in patients with morbidly adherent placenta.

Study design, size, duration: A prospective historical study including all pregnant women admitted to a single tertiary care center, since November 2011 until July 2016 with a diagnosis of morbidly adherent placenta that had a successful preservative cesarean section delivery with bilateral uterine arteries embolization.

Participants/materials, setting, methods: Inclusion criteria included gestational age >24 weeks, singleton pregnancy and placenta increta / percreta. Exclusion criteria included maternal age >43 years at the time of recruitment and women after cesarean hysterectomy. Control group included women attending the infertility clinic due to male factor, or single women conceiving via sperm donation, matched by age. Blood samples were collected from all patients on day 2-5 of menstruations for hormonal profile and anti mullarian hormone levels.

Main results and the role of chance: 59 women underwent preservative cesarean section and uterine artery embolization due to morbidly adherent placenta during the study period. 21 women met inclusion criteria (33.9%) and were matched controls (n = 40). Median time for post operation blood sampling was 55 month (IQR 22.5-90.5). One woman was breastfeeding, 2 had intra uterine devices, and all other women reported they were not using hormonal treatment for contraception at the time of blood sampling. The study group had higher gravidity and higher rates of cesarean section as expected (2 IQR:2-4.75 vs 1 IQR:0-1, p = 0.001), (1IQR:1-2 VS 0, p = 0.001), respectively. Circulating levels of E2 and FSH did not differ significantly between the two groups (p = 0.665, p = 0.396, respectively). Anti mullarian hormone (AMH) was lower in the study group (0.8 IQR 0.44-1.80) compared to the controls (2.08 IQR 1.68-3.71),(p = 0.001). This finding was consistent in linear multivariate regression analysis where the group of cesarean section using bilateral artery embolization due to placenta accrete was significantly predictive for the levels of AMH (B=-1.308, p = 0.012).

Limitations, reasons for caution: Limitations of the study is the relatively small study group, though rarity of the condition, and the lack of information regarding baseline AMH levels before the procedure.

Wider implications of the findings: UAE in morbidly adherent placenta might be associated with reduced ovarian reserve, nevertheless, the alternative of hysterectomy is definite and do not offer solution to women desiring future fertility. Our findings support the use of UAE, while giving the proper consultancy on the possible effect on ovarian reserve.

Trial registration number: The study protocol was approved by the Institutional Review Board (ID 3178-16-SMC) and was supported by the National Institutes of Health (NCT02821702).

SELECTED ORAL COMMUNICATIONS

SESSION 16: BASIC RESEARCH IN GAMETE AND EMBRYO BIOLOGY

Monday 2 July 2018 Forum (Auditorium) 15:15–16:30

O-056 Maternal STAT3 regulates oocyte maturation and development of early embryos through autophagy

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Study question: What is the role of maternal STAT3 signaling in preimplantation embryonic development?

Summary answer: Maternal STAT3 signaling is important for optimal oocyte maturation and cleavage-stage embryo development via autophagy activation.

What is known already: JAK2/STAT3 signaling is known as an intracellular signaling pathway. JAK2 is the member of Janus protein tyrosine kinase family and recruits and phosphorylates STAT3. Phosphorylated STAT3 is dimerized and translocated into the nucleus to regulate transcriptional activity. In addition, cytoplasmic STAT3 regulates the autophagy machinery in cells. In preimplantation embryos, STAT3 is constitutively active during fetal stage and shown to be important for embryonic development as demonstrated by embryonic lethality in STAT3 knockout mice. However, its role during early cleavage stage embryos is unknown due to the presence of maternal STAT3 in early embryos before zygote gene activation.

Study design, size, duration: GV-stage oocytes and preimplantation embryos were obtained from ICR mice (3-4 weeks of age) for quantification of STAT3 expression. In addition, these oocytes and embryos were used to examine the role of maternal STAT3 in oocyte maturation and embryonic development by inhibiting phosphorylation of JAK2 and STAT3 using STAT3 selective inhibitors, JAK2 selective inhibitors and JAK2/STAT3 signaling inhibitors.

Participants/materials, setting, methods: The expression levels of STAT3 in oocyte and embryos at different stages were measured using real-time RT-qPCR. GV-stage oocytes, zygote and 8 cell-stage embryos were cultured with different inhibitors to compare the proportions of oocytes underwent maturation and embryonic development. The spindle structure in cultured oocytes was examined using tubulin staining. To assess the autophagy activity, expression of microtubule-associated-protein-light-chain 3 (LC3) at mRNA and protein levels were measured by real-time RT-qPCR and immunofluorescence staining, respectively.

Main results and the role of chance: The expression levels of STAT3 peaked in oocytes and gradually decreased toward 4 cell-stage embryos. It was not detected after 8 cell-stage embryos, suggesting its maternal origin. In GV-stage oocytes, nuclear maturation rate was significantly decreased when both JAK2 and STAT3 signaling were inhibited (Control: 73.8 ± 5.4%, treat: 44.1 ± 4.9%, $P < 0.05$). When embryos were cultured with the STAT3 inhibitor S3I-201, embryo development was significantly suppressed at 4 cell-stage (Control: 94.5 ± 5.0%, STAT3 selective inhibitor: 9.7 ± 3.8%, $P < 0.01$), whereas JAK2 inhibitor Febratinib showed a weaker suppressive effects (Control: 94.5 ± 5.0%, JAK2 selective inhibitor: 57.9 ± 15.7%, $P < 0.01$). If both JAK2 and STAT3 inhibitors were used, they showed synergistic effect on suppression of embryonic development from 2 cell-stage embryos onwards (2 cell-stage; Control: 96.4 ± 2.1%, JAK2/STAT3 inhibitor: 31.3 ± 4.8%, $P < 0.01$, 4 cell-stage; Control: 94.5 ± 5.0%, JAK2/STAT3 inhibitor: 28.1 ± 4.4%, $P < 0.01$). Of note, embryo development was not affected when phosphorylation of JAK2 and/or STAT3 was inhibited after 8 cell-stage embryos. Furthermore, LC3 expression was significantly decreased in these embryos, indicating decline in autophagy activity.

Limitations, reasons for caution: Our study was performed using mouse oocytes and preimplantation embryos. Therefore, this result could not be directly translated to human cases.

Wider implications of the findings: Although similar studies using human oocytes are required to confirm the importance of STAT3 activity, our findings may provide a new strategy to help the patients with impaired oocyte maturation by culturing their GV-stage oocytes with STAT3 activators to induce oocyte maturation.

Trial registration number: Not applicable.

O-057 The human sperm basal body contributes a complex matrix of proteins important for proper preimplantation development of the oocyte upon fertilization

F. Amargant[1], A. Pujol[2], A. Ferrer-Vaquer[1], M. Durban[1], M. Martínez[1], M. Barragan[1], I. Vernos[3], R. Vassena[1]

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Study question: What does the sperm basal body contribute to the human zygote to ensure embryo early development?

Summary answer: The spermatozoon provides 288 centrosomal proteins to the zygote to form a functional centrosome, which is required in order to achieve optimal preimplantation developmental rates.

What is known already: During fertilization in the human species, the sperm provides a centrosome, the main microtubule organizing center (MTOC) essential for the development of a new organism, to the zygote. In somatic cells, the centrosome is comprised by 2 centrioles surrounded with pericentriolar material (PCM) composed of several essential proteins for its activity; the current view is that the human sperm basal body (BB) does not possess PCM, and PCM proteins are recruited from the oocyte cytoplasm after fertilization. Although alterations in the sperm centrosome function have been linked to infertility, its role in human preimplantational development has not been tested directly.

Study design, size, duration: To identify the sperm BB components, proteomic analysis was carried out on 4 experimental samples prepared from mixtures of 4 normozoospermic ejaculates. To study the BB function, headless sperm tails containing the BB, were generated by microsurgery, and injected into MII oocytes, which were further activated parthenogenetically ($n = 15$ sham and $n = 15$ tail injections) by sequential passages of ionomycin 10 µM. Morphokinetic recording of developing parthenotes was carried out every 5 minutes during 5 days.

Participants/materials, setting, methods: For proteomics studies, sperm were sonicated and the tail fraction, including the BB, was spun through a 30% sucrose cushion. Tails were solubilized with Laemmli Buffer and analyzed by mass spectrometry. In the functional study, sham- and tail-injected oocytes were cultured in SAGE and their development recorded. Times analyzed included second polar body extrusion, cell divisions and blastocyst expansion. Parthenotes were fixed at different times through D5 and tubulin, MTOCs, and DNA analyzed by immunofluorescence.

Main results and the role of chance: We identified 2,312 proteins as components of the sperm tail including the BB (validated by immunofluorescence with centrin). Gene ontology analysis identified 18% of the proteins as cytoskeletal, including 288 proteins from centrioles and PCM. As expected, most parthenotes (80%) arrested their development before reaching blastocyst stage, while morphokinetic developmental timings in both groups did not differ in any of the annotated times. However, while tail-injected parthenotes arrested throughout *in vitro* culture and irrespectively of developmental stage, 75% of the arrested sham-injected ones (lacking the sperm derived centrosome) could not proceed through compaction, and arrested around the time of embryonic genome activation. None of the sham- injected parthenotes that arrested before compaction, had detectable MTOCs in immunofluorescence, while MTOCs were visible in all those that developed past compaction. Altogether, our data suggest that the sperm BB is more complex than previously described; the high number of centriolar and PCM components it contains may provide an efficient conversion to a functional centrosome in the fertilized oocyte. The presence of MTOC-like structures in parthenotes suggests a developmental control of PCM protein expression during the early phases of development and a correlation between the presence of MTOCs and successful compaction.

Limitations, reasons for caution: The main limitations of these studies concern the sample size of the injection experiments and the fact that up to one third of the tails injected may not contain a BB, hence diluting the tail- injected parthenotes phenotype.

Wider implications of the findings: Our proteomic and functional results do not support the current view of "naked" centrioles in the human sperm BB; rather, they indicate that the centrioles are transferred to the oocyte at fertilization together with PCM proteins that may participate in the efficient assembly of the first centrosome of the zygote.

Trial registration number: Not applicable.

O-058 Human fertilization is completely blocked by egg Juno protein inhibition

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Study question: Is Juno protein present at the membrane surface of human oocyte and involved in fertilization process?

Summary answer: Juno Protein is expressed on the plasma membrane of human oocyte and its inhibition by a monoclonal antibody completely blocks the gamete fusion.

What is known already: Gamete membrane fusion is the culminating event of the fertilization process, driving by poorly understood molecular mechanisms. Until now, three molecules have been proven to be essential: The egg's CD9 tetraspanin, the Izumo1 sperm protein and Juno, its receptor on egg. Gene invalidation of these three proteins causes a drastic decrease of mice fertility by the loss of gamete fusion ability. CD9 and Izumo have been described in human and the Izumo1-Juno interaction is conserved within several mammalian species, including human. The presence of Juno on human oocyte was not yet reported and its role in fertilization is not known.

Study design, size, duration: We investigate, in human, the presence of Juno on egg membrane surface and study its potential involvement in membrane gamete interaction during fertilization.

Participants/materials, setting, methods: Monoclonal antibodies against human Juno (anti-hJuno mAb) were produced by immunization of mice with HEK cells transfected with the putative human Juno sequence (HEK-hJuno). These antibodies were used for immunostaining experiments and *in vitro* fertilization assays on human gametes (GERMETHEQUE Biobank).

Main results and the role of chance: Three hybridoma's supernatants, tested by immunostaining, specifically revealed HEK-hJuno cells. The 3 purified monoclonal antibodies FJ2E4 (IgG1), FJ8E8 (IgG1) andFJ4F5 (IgG2a) recognized in western blot of HEK-hJuno extracts a protein with an expected MW of 25 kDa. Using these 3 anti-hJuno mAb for immunostaining, we identified the presence of Juno protein at the plasma membrane of human eggs. The distribution of the fluorescent signal appeared heterogeneous on fresh oocytes whereas it was homogeneous and finely punctuated when the eggs were fixed before staining; suggesting a relocation of the protein induced by the binding of the specific antibody. Furthermore, we highlighted a progressive expression of Juno depending on oocyte maturity. Finally, we showed that human oocyte inseminated in presence of anti-hJuno mAb wouldn't be fertilized by human sperm. These results suggest that anti-hJuno mAb block the interaction site of Juno protein with Izumo1 receptor, and, as in mouse model, that Juno is involved in membrane gamete interaction during human fertilization.

Limitations, reasons for caution: In order to respect French bioethics laws, functional tests have been performed using zona-free eggs, which is different from physiologic conditions.

Wider implications of the findings: This is the first report of the presence of Juno on human oocyte and of its involvement in human fertilization. It allows going further in the understanding of molecular mechanisms that drive gamete fusion; a crucial challenge in occidental and developing countries where infertility affects 16% of genitally active couples.

Trial registration number: not applicable.

O-059 PLCz knock-out sperm reveals the sole effect of altering calcium signals during assisted oocyte activation on later mouse embryogenesis

M. Ferrer Buitrago[1], L. Tilleman[2], V. Thys[1], A. Hachem[3], F. Van Nieuwerburgh[2], L. Leybaert[4], P. De Sutter[1], D. Deforce[2], J. Parrington[3], B. Heindryckx[1]

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Study question: Does a differing Ca^{2+} signature during assisted oocyte activation (AOA) have a later impact on pre-implantation mouse blastocysts?

Summary answer: Variations of Ca^{2+} at fertilization during ICSI-AOA do not prove detrimental for development of the blastocyst and its associated transcriptional status in mouse.

What is known already: Mammalian fertilization occurs in combination with a series of cytoplasmic Ca^{2+}oscillations initiated by the sperm-borne phospholipase C zeta (PLCz). Oocyte activation is achieved in response to the sum of individual Ca^{2+} spikes, irrespective of the Ca^{2+} oscillatory pattern. Hence, several pharmacological agents can induce oocyte activation by stimuli that diverge profoundly from the physiological Ca^{2+} signature. Although still controversial, some studies showed that altering the Ca^{2+} oscillatory regime at fertilization affects blastocyst establishment and post-implantation development. Novel genome-edited PLCz-null mouse sperm provide a neat way for evaluating the sole effect of different Ca^{2+} signals during ICSI-AOA on later embryogenesis.

Study design, size, duration: Assisted oocyte activation (AOA) was performed after ICSI of PLCz-null sperm into mouse oocytes. Four AOA strategies were applied: AOA1: human recombinant PLCz (Ca^{2+} oscillations), AOA2: strontium (Ca^{2+} oscillations), AOA3: ionomycin (single Ca^{2+} transient) and AOA4: TPEN (zinc chelator; absence of an initial Ca^{2+} trigger). Embryonic development and blastocyst transcriptome analysis were compared to control conditions: C1 (*in vivo* fertilization), C2 (ICSI using normal activating sperm) and C3 (parthenogenesis; induced by strontium and cytochalasin-D).

Participants/materials, setting, methods: MII oocytes were collected from B6D2F1 females after ovarian hyperstimulation. Frozen sperm samples were thawed before ICSI, according to standard procedures. Embryos were cultured for 5 days in sequential media. RNA isolation was carried out in individual blastocysts following manufacturer instructions (PicoPure-RNAi kit. Arcturus). RNA-seq was carried out on the NextSEq500. Data analysis was conducted in R in 9380 genes with a cpm >5 in at least 4 samples per group.

Main results and the role of chance: PLCz-null sperm were mostly not able to activate mouse oocytes (activation rate 9%). Failed fertilization was rescued in response to AOA, with all AOA strategies showing similar efficiencies in oocyte activation (91 ± 9%) compared to controls (87 ± 11%). The lack of PLCz did not compromise pre-implantation embryonic development, and blastocyst stage was achieved in all groups. Blastocyst formation rate (bfr) was significantly decreased in C2 (52%) in comparison to C1 (98%) and C3 (80%), reflecting the deleterious effect of ICSI in mouse embryonic development. We further evaluated the efficiency of the distinct AOA methods to support embryonic development compared to C2. The use of strontium (AOA2) yielded the highest bfr (75%, $p < 0.05$) within the AOA groups. Ionomycin (AOA3) resulted in similar output compared to C2 (49% vs 52%), but significantly reduced compared to C1 and C3. Despite the decreased bfr observed after using human recombinant-PLCz (AOA1; 29%) or TPEN (AOA4; 30%), differences were not statistically significant compared to C2. Both, AOA1 and AOA4 strategies showed significantly lower bfr compared to C1, C3 and AOA2. The transcriptional profile of individual blastocysts was further analyzed by RNA-seq, which did not identify significant differences in global gene expression within and between the groups.

Limitations, reasons for caution: RNA-seq analysis included only coding regions of well-annotated genes. This analysis involves only pre-implantation events.

Wider implications of the findings: Our findings provide a novel insight in understanding the role of Ca^{2+}oscillations beyond meiotic resumption in mammals. The use of PLCz-null sperm represents a potent translational model to explore the impact of AOA throughout early and late embryogenesis.

Trial registration number: (Non applicable).

O-060 Early steps of human embryo implantation explored in a 2D in vitro model

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Study question: Can we use a 2D *in vitro* adhesion model to investigate molecular pathways involved in the early steps of human embryo implantation?

Summary answer: Human embryo implantation is initiated by apposition and adhesion and these steps are associated with different expression profiles of adhesion and extracellular matrix (ECM) transcripts.

What is known already: The few adhesion and ECM proteins analysed for human embryo implantation are derived from similarities found in the mouse model and leukocyte trans-endothelial migration. Both human and murine embryos have previously been co-cultured with Ishikawa cells in a 2D *in vitro* adhesion model. Only few data is reported on gene expression in human preimplantation blastocysts, however these studies do not provide large scale molecular knowledge to understand fundamental pathways involved during the implantation process.

Study design, size, duration: Vitrified human blastocysts were warmed and co-cultured in a 2D *in vitro* adhesion model, in which Ishikawa cells are used to represent the human endometrial epithelium. Attachment of the embryos to the Ishikawa cells was assessed in this study. Loosely (apposition) and firmly (adhesion) attached embryos were compared with unattached embryos. The early steps of implantation were investigated both on the observational (microscopy) and the molecular level (quantitative PCR (qPCR) and immunofluorescence (IF)).

Participants/materials, setting, methods: Six days post fertilisation (dpf) human embryos were co-cultured on the Ishikawa cells for 12 h, 24 h (7dpf) or 48 h (8dpf); and attachment rate and morphological development were investigated. Gene expression of 84 adhesion and ECM genes was analysed by qPCR. Proteins were localised by IF staining. Neutralizing antibody was used for functional blocking experiments. Data is reported on 109 human embryos. Mann-Whitney *U* and two-way ANOVA tests were used for statistical analysis ($P < 0.05$).

Main results and the role of chance: According to our *in vitro* attachment data, the majority of the embryos attach on the polar trophectoderm. We are also the first to show that human embryo implantation is initiated by apposition and followed by adhesion; the majority of the 6dpf embryos co-cultured for 12 h were loosely attached and this progressed to a firm attachment in 85.7% of the 7dpf embryos. HCG positive embryonic outgrowth formation at 8dpf indicates differentiation of the trophectoderm to the invasive syncytiotrophoblast.

Gene expression analysis was performed on loosely attached and unattached embryos co-cultured for 12 h with the Ishikawa cells. Loosely attached embryos were distinguished from the unattached embryos by the additional expression of *THBS1*, *TNC, COL12A1, CTNND2, ITGA3, ITGAV,* and *LAMA3*. Loose embryo attachment was also associated with the significant upregulation of *CD44* ($P = 0.014$). *TNC* and *THB1* were validated on protein level in 7dpf firmly attached embryos. THBS1 showed cytoplasmic localisation in the embryonic cells and TNC was present on the membrane of trophectoderm cells. Functional blocking of only TNC protein with a neutralizing antibody in 48 h co-cultured embryos did not affect the attachment rate nor hCG secretion.

Limitations, reasons for caution: The study was performed *in vitro* and made use of a carcinoma cell line. Also the sample size of the study was limited. Contamination of the analysed embryos by Ishikawa cells can be excluded due to the different gene expression profiles.

Wider implications of the findings: Taken our data together we have provided a "proof of concept" for fundamental understanding of the early steps of human embryo implantation. Confirmation of the data on a larger sample size and larger gene expression analysis will provide insights of defective molecular pathways involved in implantation failure.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 17: TRIALS AND REVIEWS IN CLINICAL ANDROLOGY

Monday 2 July 2018 Room 211 + 212 15:15–16:30

O-061 Effect of annexin-V positive sperm-cells removal with magnetic activated cell sorting (MACS) for ICSI procedures in couples with male infertility (teratozoospermia); A randomized controlled trial

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NASCERE, Reproductive Health Division, Mexico City, Mexico

Study question: Does the use of MACS and incubation with annexin-V conjugated microbeads (ANMB) before ICSI in couples with male infertility (teratozoospermia) improve the live-birth delivery rate?

Summary answer: The use of MACS-ANMB in men with teratozoospermia improves the development and quality of oocytes/embryos, consequently improving fertilization(FR), implantation(IR), pregnancy(PR), and live-birth-delivery rates.

What is known already: It is proved that human spermatozoa play an extensive role in the reproductive physiology, beginning with the fertilization process, continuing with early and late stages of embryo development, that ultimately impact on implantation and pregnancy rates. The expression of early apoptotic markers as plasma membrane translocation of phosphatidylserine (PS) is associated with abnormal embryo development and suboptimal results of FR, IR and PR. Annexin-V magnetic cell separation of non-apoptotic spermatozoa is simple, fast, inexpensive and highly specific, however, its role in late stages of embryo development and live-birth rate is unclear.

Study design, size, duration: A prospective, randomized, triple-blinded, and controlled trial was conducted using a parallel two-arm study. We included a total of 200 couples with teratozoospermia undergoing ICSI assigned in a 1:1 proportion either to the experimental or the control group. Samples from the study group were prepared by swim-up followed by MACS-ANMB to remove Annexin-V positive sperm cells. The study was conducted from June-2012 to March-2017 in a single reproductive center in Mexico City.

Participants/materials, setting, methods: Only those samples exhibiting teratozoospermia (morphology <4% according to Kruger's strict criteria) were included in the study. In addition, patients who presented with poor ovarian response ("Bologna" criteria) were eliminated. Samples were randomized in two groups: i)swim-up followed by MACS-ANMB, and ii)swim-up. All embryos were analyzed until day 5-6 (blastocyst stage) and all transfers were done on the same embryo stage. Statics: Mann-Whitney-test, Fisher-exact-test, and Person's-correlation were done to analyze the results ($p < 0.05$, significant).

Main results and the role of chance: Different results were obtained between groups, however, patients demographics were similar. The Annexin-V positive fraction was negatively correlated with sperm concentration ($r = -0.5435$ $p = <0.0001$), progressive motility ($r=-0.2975$ $p = 0.02$), and total motility ($r = -0.2975$ $p = 0.02$); and was positively correlated with type D morphology ($r = 0.4397$ $p = 0.0006$). Regarding embryo development and reproductive results, comparison between MACS-ANMB ($n = 100$), and control group ($n = 100$) showed the following results: i) FR of 40.8% (95% IC, 27.4-65.0) versus 45.2% (95% IC, 14.7-69.0) $p = 0.04$, ii) embryo survival on day-3 of 97.3% (95% IC, 89.7-100) versus 98.9% (95% IC, 96.8-100) Non Significantly (NS), iii) mean quality of embryos on day-3 of 2.9 (95% IC, 2.8-3.0) versus 2.9 (95% IC, 2.9-3.0) NS, iv) blastocyst development on day-5 of 61.2% (95% IC, 51.7-70.6) versus 48.8% (95% IC, 30.2-66.6) $p = 0.02$, v) quality of embryos on day-5 of 2.8 (95% IC, 2.6-3.1) versus 3.4 (95% IC, 3.3-3.6) $p = 0.001$, vi) percentage of arrested embryos of 32.9% (95% IC, 23.5-42.3) versus 49.9% (95% IC, 36.8-63.0) $p = 0.04$, vii) IR of 25.5% (95% IC, 12.7-67.1) versus

12.5% (95% IC, 6.2-32.8) p = 0.001, viii) PR of 51.1% (95% IC, 25.5-65.7) versus 26.1% (95% IC, 13.3-34.2) p = 0.001, and ix) live-birth delivery rate 24.5% (95% IC, 12.2-72.0) versus 9.5% (95% IC, 4.7-27.9) p=<0.0001, respectively.

Limitations, reasons for caution: In this study, we only included men with teratozoospermia, not evaluating other men with different types of male infertility. Despite the fact that we selected sperm-cells without indicators of apoptosis and measure its impact on live-birth delivery rate, we didn't evaluate its effect on chromosomal abnormalities.

Wider implications of the findings: Through ICSI there is the possibility of injecting a spermatozoon with an apoptotic process that by natural selection would be condemned to die generating suboptimal results in ART; with the help of MACS-ANMB we improve live-birth delivery rate in men with teratozoospermia and type-D motility.

Trial registration number: The main intervention of the study involved the handling of the sperm samples rather that human beings, clinical trial registration was not required.

O-062 Sperm Selection by either PICSI or MACS in cases with abnormal Sperm DNA fragmentation index for ICSI, a randomized control trial

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Study question: which sperm selection technique PICSI or MACS is better for dealing with abnormal sperm DNA fragmentation patients undergoing ICSI.

Summary answer: there is no significant difference in cleavage, blastulation, implantation, pregnancy or ongoing pregnancy rates between both techniques.

What is known already: Sperm DNA fragmentation (SDF) has shown a negative correlation with fertilization rate, embryo quality, and ongoing pregnancy rate, and a positive correlation with miscarriage rate.

PICSI and MACS have been developed for selecting a healthy mature non apoptotic sperm for ICSI to obtain the best embryo quality and achieve higher ongoing pregnancy rates.

Sperm membrane binding to hyaluronic acid on a PICSI Dish can improve the likelihood of obtaining a sperm with lower SDF. MACS depends on the binding of protein Annexin V to phosphatidylserine which is a marker for apoptosis, giving an elution of sperm with lower SDF.

Study design, size, duration: Prospective randomized controlled trial enrolled 286 patients undergoing ICSI in Ganin Fertility Center, from November 2016 and still recruiting. On the day of ICSI patients with abnormal DNA fragmentation index were randomized into 2 arms; 1st arm is PICSI and it contains 145 Patients, the 2nd arm is MACS and it contains 141 Patients. Patients embryological parameters are recorded then pregnancy rate, implantation rate and ongoing pregnancy rate is followed up.

Participants/materials, setting, methods: Couples undergoing ICSI were included if the female age is below 40 years and had at last 5 mature oocytes, male must have abnormal DFI (> 19%) using TUNEL assay. Cases were randomized to the 2 groups, semen processing is done by double layer density gradient method then the resulted pellet is either placed on hyalornan dot of PICSI dish or labeled by Anexin V microbeads for further separation on the MACS column.

Main results and the role of chance:

Point of comparison	PICSI	MACS	P value	Significance
Total no. of cases	145	141	/	/
Average no. of MII oocytes	14.8	14.4	/	/
Primary outcome:				
Ongoing pregnancy rate (%)	56.3 %	50.8%	P = 0.3456	NS
Secondary outcome:				
Cleavage rate (%)	76.20%	75.56%	P = 0.5977	NS
Blasulation rate (%)	63.60%	60.61%	P = 0.0555	NS
Blastocyst quality rate (%)	62.70%	60.70%	P = 0.3172	NS
Pregnancy rate (%)	63.8%	59.10%	P = 0.4081	NS
Implantation rate (%)	38.35%	35.40%	P = 0.4214	NS

Limitations, reasons for caution: the number of enrolled patients is not enough to be 80% statistically powered so patient recruitment is open to complete 400 patients. The results could be more confident if it was applicable to use both of the techniques for injecting sibling oocytes (case controlled study).

Wider implications of the findings: Most of the published research agree with the benefit of using a sperm selection technique whether it's PICSI or MACS. This comparison will tell us novel data about ongoing pregnancy rate but more studies are needed to evaluate the live birth rate.

Trial registration number: Clinical trial registration number: NCT03398317

O-063 Antisperm-antibodies prevalence and relationship with seminal parameters and with post-coital test outcome. A retrospective analysis on over 10.000 men

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Study question: To determine antisperm-antibodies (ASA) prevalence and the relationship of auto-immunization degree with seminal parameters and with post-coital test (PCT) outcome.

Summary answer: Only a 100% positivity of IgG-MAR test, detected in 2% of the study population, was significantly associated with lower sperm quality and poor PCT outcome.

What is known already: Although the IgG-MAR test has been recommended by WHO as an integral part of semen analysis for the screening of antisperm-antibodies (ASA), the prevalence of positive results and their relationship with seminal parameters as well as the cut-off values of clinical relevance are controversial.

Study design, size, duration: Retrospective analysis on 12.296 consecutive men attending the Andrologic Unit of the University/Hospital of L'Aquila for the evaluation of fertility potential.

Participants/materials, setting, methods: Immunological screening with IgG-MAR test was performed to all ejaculates as integral part of semen analysis. Positive samples (≥10%) were tested also for IgA-ASA. The prevalence of positive IgG-MAR tests and the relationship of the degree of the sperm-autoimmunization with seminal parameters were analyzed. The outcome of PCT performed in couples where the male partner exhibited a positive MAR test was also analyzed.

Main results and the role of chance: Excluding from the analysis semen samples with not-executable MAR-test due to azoospermia or severe oligo-asthenozoospermia, the prevalence of positive IgG-MAR test in the remaining 10.025 men was 4% (398), 3.4% (342) and 2% (200) using a cut-off of 10%, 50% and 100% positive MAR-test. Only 100% positive MAR-tests were significant associated with a higher prevalence of the mixed pattern of positivity, as well as with concomitant occurrence of IgA-ASA. Only 100% positive MAR-tests were significantly associated with a lower median value of total number of

spermatozoa: 69×10^6 vs 132×10^6 (p = 0,0003) in 50%-99% positive MAR test and vs 120×10^6 (p < 0,0001) in negative samples. Furthermore, Only 100% positive MAR-tests were significantly associated with a lower median value of progressive motility: 40% vs 53% (p < 0,0001) in 50%-99% positive MAR test and vs 54% (p < 0,0001) in negative samples. In 120 PCT performed in couples where the male partner exhibited a positive MAR, only 100% positive MAR-tests were significantly associated with a higher percentage of poor PCT outcome: 78% vs 30% (p < 0.0001) in 50%-99% positive MAR test and 17% (p < 0.0001) in >50% positive MAR-test. This association was independent from cervical mucus score and total progressive motile sperm in the ejaculate.

Limitations, reasons for caution: Only surrogate infertility-related endpoints have been analysed in this study. However, as the impairment of sperm penetration through the cervical mucus represents the primary mechanism of ASA interference with fertility, the PCT outcome may represent a suitable endpoint.

Wider implications of the findings: In the context of the controversial clinical relevance of ASA, this study, the largest reported, provides a more reliable estimate of their prevalence. Although 50%- positive MAR-test has been indicated by WHO as clinically-relevant cut-off, only 100%- positive MAR-tests were significantly associated with lower sperm quality and poor PCT outcome.

Trial registration number: not applicable.

O-064 Antioxidants in the treatment of male factor infertility: Results from the double blind, multi-center, randomized controlled Males, Antioxidants, and Infertility (MOXI) trial

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Study question: Among couples with male factor infertility, do antioxidants improve male fertility, as measured by semen parameters and sperm DNA integrity at 3 months, and pregnancy by 6 months of treatment?

Summary answer: Antioxidant treatment of the male partner does not improve semen parameters, sperm DNA integrity, or in vivo pregnancy rates in couples with male factor infertility.

What is known already: Clinical trials suggest that antioxidants have a positive effect on sperm motility, DNA integrity, and pregnancy rates in couples undergoing assisted reproductive technologies; however, there are significant gaps in our knowledge of their impact on male fertility. To date research studies have used: 1) small sample sizes, usually less than 50 subjects, 2) heterogeneous populations and a variety of single antioxidants, 3) changes in semen parameters or DNA integrity as the endpoint, rather than clinical outcomes, and 4) antioxidants in conjunction with in vitro fertilization with intracytoplasmic sperm injection (ICSI) when assessing effectiveness.

Study design, size, duration: 174 couples were enrolled in a multi-center, double blind, randomized, placebo-controlled trial of a daily antioxidant formulation, containing 500 mg vitamin C, 2000IU vitamin D3, 400IU vitamin E, 1 mg folic acid, 20 mg zinc, 200 mcg selenium, and 1000mg L-carnitine. Males were treated for a minimum of 3 months and a maximum of 6 months. Couples attempted to conceive naturally during the first 3 months and with clomiphene citrate with intrauterine insemination in months 4 through 6.

Participants/materials, setting, methods: Males with sperm concentration ≤ 15 M/ml, motility ≤ 40%, normal morphology ≤ 4%, or DNA fragmentation > 25% were eligible. Female partners were ≤ 40 years old, with documented tubal patency and ovulation. Semen parameters and DNA fragmentation were assessed at randomization and following 3 months of treatment. Data are presented as median (interquartile range) or mean ± standard deviation.

Main results and the role of chance: After 3 months of treatment, change in sperm concentration differed slightly between the antioxidant [-4.0 (-12.0, 6.0) M/ml] and placebo groups [+3.2 (-9.0, 15.5) M/ml] (p = 0.03). However, there were no significant differences between the two groups in the change in morphology, motility, or DNA fragmentation. Among the 66 oligospermic men at randomization, concentration did not differ at 3 months [8.5 (4.8,15.0) M/ml versus 15.0 (6.0,24.0) M/ml; p = 0.30] between antioxidant and placebo groups. Among the 76 asthenospermic men, motility did not differ at 3 months (34 ± 16.3% versus 36.3 ± 15.6%; p = 0.93). Among the 40 men with teratospermia, normal morphology did not differ at 3 months [2.0 (0.6,4.5)% versus 3.0 (2.0,4.0)%; p = 0.28]. Among the 34 men with high DNA fragmentation, DNA fragmentation did not differ at 3 months [28.9 (21.6,36.5)% versus 28.8 (21.6,37.4)%; p = 0.68]. In the entire cohort, cumulative pregnancy rates did not differ at 3 months (10.5% versus 9.1%; p = 0.76) or at 6 months (22.1% versus 29.6%; p = 0.26) between the antioxidant and placebo groups, respectively.

Limitations, reasons for caution: While the trial was adequately powered to examine changes in semen parameters, the trial is underpowered to assess differences in pregnancy rates between antioxidants and controls. However, the DSMB recommended not proceeding with the larger trial, given the results of the internal pilot.

Wider implications of the findings: Antioxidants do not appear to improve semen parameters or DNA fragmentation among men with male factor infertility. While previous data suggest that antioxidants improve pregnancy rates in in vitro fertilization, these data suggest they do not improve in vivo conception.

Trial registration number: NCT02421887

O-065 Features of metabolic disorder in late adolescence are negatively associated with testicular function aT 20 years of age; evidence from a birth cohort,

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Study question: In a population of men within a birth cohort, are features of an adverse cardiometabolic profile in late adolecence associated with impaired testicular function at 20 years of age?

Summary answer: Despite the majority of men being only 20 years old, with a normal BMI, features of cardiometabolic disorder were associated with signs of testicular impairment.

What is known already: An adverse cardiometabolic profile in adulthood has been reported to be associated with impaired testicular function; however no data exists from younger men. Hence it is unclear whether the metabolic disorder predates the adverse testicular function, or whether it is the lower serum testosterone concentration that predisposes the man to metabolic disorder in later life.

Study design, size, duration: The Raine Study recruited 2900 women in pregnancy and retained the 2868 children born to form the Raine Study cohort. At 17 years of age males underwent a liver ultrasound for fatty liver (n = 490), and serum cytokines were measured (n = 520). At 20 years, measures included blood biochemistry (n = 618), a DEXA scan (n = 634), and a testicular assessment performed; semen sample (n = 365), testicular ultrasound (n = 404) and serum testosterone, LH, FSH and inhibin B concentrations were assayed (n = 620).

Participants/materials, setting, methods: Fasting bloods were analysed for serum IL-18 by ELISA, tumor necrosis factor receptors (TNFR1 & 2) by cytometric bead array, and liver enzymes, insulin, glucose, lipids, high sensitivity CRP (hsCRP) and uric acid. Homeostasis model assessment (HOMA) was calculated and insulin resistance (IR) was defined by a HOMA>4. DEXA measurement was performed for lean and total fat mass.

A cluster analysis was used to derive high-risk groups with features consistent with the metabolic syndrome.

Main results and the role of chance: Participants that had testicular assessment were similar clinically to those that declined participation. The prevalence of metabolic syndrome was 4.1% (NCEP-ATPIII definition) (40) and 5.4% (IDF definition).

After adjustment for age, BMI, abstinence, a history of cryptorchidism and presence of a varicocele; total sperm output was reduced in men with a higher IL-18, and serum TNFR1, and sperm concentration was negatively associated with IL-18 (all $p < 0.05$). After adjustment, seminal volume was negatively associated with serum hsCRP, and serum testosterone was positively associated with serum HDL-cholesterol and lean mass, and negatively with serum insulin (all $p < 0.05$).

In an unadjusted dichotomized analysis of men within the high risk metabolic cluster compared to those low risk, their testosterone (3.6 ng/ml *vs* 4.8 ng/ml) and inhibin B (167.9 pg/ml *vs* 223.7 pg/ml) concentrations were lower ($p < 0.001$ for both). Participants with IR had a smaller testicular volume (12.8mls *vs* 15.2mls, $p = 0.010$), lower testosterone (3.2 ng/ml *vs* 4.6 ng/ml, $p < 0.001$) and inhibin B (172.4 pg/ml *vs* 217.8 pg/ml, $p = 0.001$), and higher serum FSH concentrations (6.1iu/l *vs* 4.3iu/l, $p = 0.046$).

Ultrasound evidence of fatty liver was associated with a reduction in; total sperm output (68.0×10^6 *vs* 126.0×10^6, $p = 0.044$), testosterone (4.0 ng/ml *vs* 4.7 ng/ml, $p = 0.005$) and inhibin B (209.1 pg/ml *vs* 218.4 pg/ml, $p = 0.032$) concentrations.

Limitations, reasons for caution: Although this study had a very good rate of recruitment there is potential for recruitment bias, and the possibility of confounding. Univariate associations were substantiated in multivariable analyses that controlled for confounding for age and BMI at 20 years, cryptorchidism and presence of a varicocele.

Wider implications of the findings: This study demonstrated a potential negative association of adverse cardiometabolic features in adolescence with testicular function by 20 years of age. Despite the majority of men having a normal BMI, and being relatively young, a significant minority were already showing some features of the metabolic syndrome and impaired testicular function.

Trial registration number: NHMRC project grants (nos 634557, 35351417, 403981)

SELECTED ORAL COMMUNICATIONS

SESSION 18: HEALTH RISKS IN CHILDREN BORN AFTER ART

Monday 2 July 2018 Room 111 + 112 15:15–16:30

O-066 Body composition and blood pressure of 6-year-old singletons born after Pre-implantation Genetic Testing (PGT): a matched cohort study

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Study question: Do children conceived by PGT have an altered body composition or higher blood pressure?

Summary answer: Blood pressure and anthropometric measurements including body mass index (BMI) and indices of peripheral and central adiposity were comparable between PGT and ICSI children.

What is known already: Although neonatal outcomes after PGT conception have been found comparable to those after ICSI, few studies have reported on the medical outcome at older ages. We previously found that at age 2, mean body mass index tended to be lower in PGT children while the prevalence of congenital anomalies was similar. Others reported that blood pressure and anthropometrics did not differ in 4- and 9-year old children conceived after IVF/ICSI with or without aneuploidy testing (PGT-A). However, outcomes in PGT-A children cannot be extrapolated to PGT children given the dissimilar indications and parental reproductive background.

Study design, size, duration: In this single-center study, singletons conceived by PGT reaching between 5 and 6 years during the period May 2011 and June 2017 were matched as closely as possible for gender, age, maternal educational level and birth order to peers born after ICSI. Biopsy of cleavage-stage embryos was performed solely for the purpose of PGT (PGT-A cycles were excluded) and all fresh embryo transfers in PGT and ICSI cycles were carried out at day 5.

Participants/materials, setting, methods: Anthropometrics (weight, height, BMI, skinfold thickness, waist and mid-upper arm circumference) and blood pressure readings of 87 singletons born after PGT, including PGT for mongenic defects (PGT-M) and chromosomal structural rearrangements (PGT-SR), but not for aneuploidies (PGT-A), were compared with results of 87 peers born after ICSI.

Main results and the role of chance: From the 124 eligible PGD families, 110 could be contacted, 23 declined and 87 (70.2%) children participated of whom 39 (45%) were males. The reasons for declining were 'no time' and 'living too far from the hospital'. Neonatal characteristics, including birth weight Standard Deviation Score (SDS) and gestational age and parental characteristics, including maternal weight gain, smoking and alcohol consumption during pregnancy, maternal age, current maternal and paternal BMI were comparable between the groups (all $p > 0.05$). Mean age was 5.5 years in the PGT group and 5.6 years in the ICSI group ($P = 0.2$). All anthropometric measurements, including BMI SDS, waist and mid-upper arm circumference SDS were comparable between the PGT (-0.23; 0.27; 0.17 respectively) and ICSI (-0.29; 0.10; 0.11) group (all $P > 0.05$). Furthermore, skin thickness derived indices of peripheral and central adiposity were comparable (PGT: 14.7 mm; 11.6 mm and ICSI: 15.5 mm; 11.5 mm) as well as the calculated total body fat mass (PGT: 13.7% and ICSI: 13.9%) (all $P > 0.05$). Finally, also blood pressure SDS were comparable between the two groups (all $P > 0.05$). Results did not change taking neonatal (birth weight SDS, gestational age, birth order) and parental (pre-pregnancy maternal BMI, maternal educational level) characteristics into account.

Limitations, reasons for caution: The non-participating PGT children did not differ from the participating group in terms of gender, birth order, gestational age, birth weight or maternal educational level, which makes participation bias less likely.

Wider implications of the findings: Our results point to no adverse effect of embryo biopsy at cleavage-stage and fresh embryo transfer at day 5. However, more and long-term follow-up studies are indicated as there are currently no data available for PGT children born after trophectoderm biopsy and/or transfer of warmed embryos after vitrification.

Trial registration number: not applicable.

O-067 Birthweight and gestational age following blastocyst transfer compared to cleavage stage embryo transfer: an analysis of 68,042 singleton births

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Study question: Are there any differences in birthweight and gestational age in singleton births conceived after blastocyst transfer as compared to those following cleavage stage embryo transfer?

Summary answer: Birthweight and gestational age outcomes of singleton pregnancies following fresh blastocyst transfer are similar to those subsequent to cleavage-stage embryo-transfer.

What is known already: Previous observational studies and meta-analysis suggest that risks of preterm birth (PTB) and high birthweight babies (HBW) in singleton pregnancies are higher following blastocyst transfer than after embryo transfer at cleavage stage. The published studies have limitations including small sample sizes and inability to adjust for a number of potential confounders. Hence there is a need for definitive results based on analysis of a single large national dataset.

Study design, size, duration: This is a cohort study using anonymized Human Fertilisation Embryology Authority (HFEA) data from the United Kingdom (UK). This study included all fresh in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) cycles between the years 1999 and 2012 that resulted in a singleton birth after the transfer of two different types of embryo: cleavage-stage (2-3 days of culture) and blastocyst-stage embryo (5-6 days).

Participants/materials, setting, methods: IVF or ICSI cycles with singleton births following the fresh transfer of cleavage stage or blastocyst stage embryo(s) were included in the study. Logistic regression was used to assess the association between the stage of embryo transferred (cleavage stage or blastocyst) and birthweight and gestational outcomes adjusting for potential confounders. Risk ratios (RR) with 99.5% confidence intervals (CI) were used as a measure of strength of associations.

Main results and the role of chance: Out of 68,042 singleton pregnancies, 11,243 occurred after blastocyst-stage embryo transfer while 56,799 resulted from cleavage-stage embryo-transfer. The proportion of low birth weight babies in the two groups were 8.9% and 9.3% respectively ($p = 0.12$). The risk of having a low birthweight baby (aRR 0.93, 99.5% CI 0.83-1.05) and that of having a high birthweight baby (aRR 0.93, 99.5% CI 0.83-1.05) were comparable after adjusting for maternal age, IVF/ICSI, duration of infertility, primary cause, number of eggs, and previous pregnancy. The proportion of babies born preterm was 1.9% in the blastocyst-stage group and 1.8% in the cleavage stage group ($p = 0.68$). The risks of preterm birth (aRR 1.03, 99.5% CI 0.91-1.18) and very preterm birth (aRR 1.00, 99.5% CI 0.78-1.28) were similar between the two types of embryo transfer strategies. The odds of congenital anomalies was similar between the groups (aOR 0.89, 99.5% CI 0.69-1.16).

Limitations, reasons for caution: As the anonymized HFEA data presents gestational age and weight in bands rather than actual values, we were unable to adjust birth weight according to gestational age and calculate parameters such as Z scores. Additionally, one woman could have been represented more than once in this cycle based analysis.

Wider implications of the findings: Our findings provide reassurance to patients undergoing blastocyst-stage embryo-transfer, in contrast to other studies that have reported increased risk of high birthweight babies and preterm delivery. Follow up studies from randomised trials or individual patient data meta-analysis of registry based data will be needed to answer the question definitely.

Trial registration number: not applicable.

O-068 Diabetes risk among children born after assisted reproductive technology: An early population-based examination

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Study question: What is the risk of developing diabetes among children born after Assisted Reproductive Technology (ART)?

Summary answer: ART children have a low absolute risk of developing diabetes by age 19, but the relative risk compared with non-Art children approaches statistical significance.

What is known already: Data from animal studies suggest a potential risk for diabetes among children born after ART, which may be mediated by epigenetic changes. In humans, however, the majority of diabetes cases develop later in life, most of which represent type-2 diabetes. In contrast, type-1 diabetes is less common overall, but more prevalent among children. One recent study found increases in both types among all children ≤19 years. The existing literature has not had sufficient follow-up time to evaluate diabetes development after ART. Early detection however, could have tremendous value, as many diabetic complications can be prevented or mitigated with current therapy.

Study design, size, duration: We examine the association between ART use and diabetes risk through 2011 within a historical population cohort consisting of all residents of Denmark. We examine the time to development of diabetes using population-based clinical registries for diabetes, and other national datasets. We focus on children born during the first six years of the Danish ART registry, i.e., born between 1994 and 1999. We use insulin status as a crude initial proxy for diabetes type.

Participants/materials, setting, methods: All children born in Denmark between 1994 and1999 are captured in the birth registry and eligible for the study. We examine the numbers of children who develop diabetes, as captured by the diabetes disease registry. We then compare the risk of developing diabetes among children born after ART treatment (using the ART registry) with that of children born without treatment (i.e., spontaneous births). We use proportional hazard models to assess the time to disease onset.

Main results and the role of chance: The maximum follow-up time was 19 years, i.e., for children born in 1994. Between 1994 and 1999, there were 5,912 live births after ART treatment and 400,131 spontaneous births. Among these, a total of 1,986 children developed diabetes by 2012 as captured by the national disease registry, or 4.9 per 1,000 spontaneous birth children and 5.9 per 1,000 children born after ART. The mean age at diabetes detection was 10.2 and 8.7 years, respectively; 82.0% and 82.9% were receiving insulin among spontaneous birth and ART children. After multivariate adjustment, children born after ART trended towards being more likely to develop diabetes (hazard ratio = 1.4; p-value = 0.08), but this association was not statistically significant at conventional thresholds (i.e., p-value = 0.05).

Limitations, reasons for caution: This study has limited follow-up time and numbers of total children in each group, decreasing both the power to detect events during childhood and adolescence, and the ability to detect disease later in life. The comparisons also reflect associations with diabetes and do not imply causality.

Wider implications of the findings: There is a potential increased risk and earlier onset of diabetes associated with ART treatment, compared with spontaneous births, though these preliminary analyses have limited statistical power. Ongoing work will increase the amount of follow-up time, thus improving both power and the ability to examine risks during early adulthood.

Trial registration number: Not applicable.

O-069 The risk of urogenital anomalies in children conceived with ICSI using fresh or frozen embryo cycles

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Study question: Does the risk of urogenital anomalies in children conceived with intracytoplasmic sperm injection (ICSI) vary by use of fresh or frozen embryo cycles?

Summary answer: Relative to natural conceptions, urogenital anomalies were increased in children conceived with ICSI using fresh cycles but not frozen cycles, accounting for infertility aetiology.

What is known already: Urogenital anomalies are among the most commonly diagnosed congenital anomalies, affecting up to 16 per 1000 births per year. Higher rates of urogenital anomalies are also observed among births conceived using assisted reproductive technologies (ART), particularly ICSI. Male factor infertility, the most common indication for ICSI, is also a risk factor for some urogenital anomalies. There is evidence linking embryo freezing to improved perinatal outcomes. Previous studies of urogenital anomalies in ART populations have not investigated whether embryo freezing modifies the risk of anomalies, nor accounted for the possible role of male factor infertility.

Study design, size, duration: This study involved a whole of population data-linkage cohort for the state of South Australia, comprising 308,933 births and terminations of greater than 20 weeks gestation, from January 1986 to December 2002. Congenital anomalies are notified until children reached age 5 years.

Participants/materials, setting, methods: The South Australian Birth Cohort links data from infertility clinics with government perinatal and birth defects registries. Urogenital anomalies are coded to the British Paediatric Association modification of ICD-9 codes. Using children conceived naturally as the reference, we examined the risk of urogenital anomalies among children conceived with ICSI overall, and separately, ICSI with frozen or fresh cycles, using logistic regression with adjustment for potential confounders. The role of male factor infertility was also considered.

Main results and the role of chance: There were 308,933 births and terminations, including 1410 resulting from ICSI (79% using fresh cycles, 21% using frozen cycles). Male factor infertility only was the most common reason for treatment in the fresh and the frozen ICSI cycles (75% vs 73%, respectively). Overall, the risk of urogenital anomalies was increased in children conceived with ICSI compared with natural conceptions (adjusted OR = 1.89, 95% CI: 1.39-2.58). The increased risk was observed in ICSI with fresh cycles (n = 1115, adjusted OR = 2.22, 95% CI: 1.62-3.04) and remained when patients with male factor infertility were excluded from this group (adjusted OR = 2.08, 95% CI: 1.10-3.93). There was no increased risk of urogenital anomalies in children conceived with ICSI using frozen cycles (n = 291, adjusted OR = 1.04, 95% CI: 0.43-2.48).

Limitations, reasons for caution: There was not sufficient statistical power to investigate specific subtypes of urogenital anomalies. Residual confounding may be present, as not all patient- and treatment-related risk factors for urogenital anomalies, for example severity of male infertility, number of embryos and embryo quality, were fully captured in available data.

Wider implications of the findings: The risk of urogenital anomalies associated with ICSI may be reduced through the use of frozen embryo transfer cycles. These findings contribute robust evidence to enhance understanding of the relative contribution of patient and treatment factors to the increased risk of adverse perinatal outcomes among children conceived using ART.

Trial registration number: not applicable

O-070 Does transfer of multiple embryos affect perinatal outcomes of the resulting singleton live births? Analysis of 113 784 singleton live births following ART

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Study question: Does transfer of two or more embryos affect perinatal outcomes of resulting singleton live births following assisted reproductive technology (ART)?

Summary answer: The risk of adverse perinatal outcomes is higher in singleton births following transfer of two or more embryos mainly associated with plurality at conception.

What is known already: Singleton pregnancies following ART are at a higher risk of adverse perinatal outcomes compared to spontaneous conceptions. Underlying infertility, altered endocrine milieu following ovarian stimulation, *in vitro* gamete manipulation and embryo culture are some of the suggested reasons for adverse perinatal outcomes. Earlier studies have found an increased risk of preterm birth (PTB) and low birth weight (LBW) in singletons following transfer of multiple embryos versus single embryo transfer (SET). However, these studies did not address the contributory role of plurality at conception, the initial presence of more than one gestational sac.

Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), which collects the data prospectively on all ART cycles performed in the UK. Data from 1991 to 2011 involving 508 410 fresh and 131 334 frozen autologous ART cycles resulting in 95 779 and 18 005 singleton live births respectively were analysed for perinatal outcomes based on the number of embryos transferred.

Participants/materials, setting, methods: Fresh or frozen ART cycles were analysed separately to compare perinatal outcomes of PTB and LBW in singleton live births resulting from transfer of two or more embryos versus SET. Logistic regression was performed adjusting for female age, infertility causes, previous live birth and embryo stage. Subgroup analyses was planned within ART cycles for perinatal outcomes of singleton live births following either initial single or initial multiple gestational sacs versus singleton live birth following SET.

Main results and the role of chance: There was a significantly higher risk of PTB (aOR 1.14, 95% CI 1.06–1.23) and LBW (aOR 1.14, 95% CI 1.06–1.23) in singleton births following transfer of two or more embryos compared to SET in fresh cycles. There was a significantly higher risk of PTB (aOR 2.70, 95% CI 2.37-3.05) and LBW (aOR 2.76, 95% CI 2.44–3.13) in singleton births with initial multiple gestational sacs compared to those following SET with fresh transfers. There was no significant difference in the risk of PTB (aOR1.08, 95% CI 1.00–1.16) and LBW (aOR 1.08, 95%CI 1.00-1.16) singleton live births when there was an initial single gestational sac after fresh transfer of two or more embryos compared to SET. There was no significant difference in the risk of PTB (aOR 1.05, 95% CI 0.91–1.21) and LBW (aOR 0.95, 95% CI 0.81-1.12) in singleton births after transfer of two or more embryos compared to SET in frozen cycles. There was a significantly higher risk of PTB (aOR 2.13, 95% CI 1.55–2.93) and LBW (aOR 2.61, 95% CI 1.87-3.64) in singleton live births with initial multiple gestational sacs compared to those following SET in frozen cycles.

Limitations, reasons for caution: While the analysis was adjusted for a number of known confounders, the dataset had no information on other confounders like smoking, body mass index, previous obstetric history or medical history of women during pregnancy to allow for adjustment.

Wider implications of the findings: Current study results suggests that there is an increased risk of adverse perinatal outcomes following transfer of more than one embryo in singleton live births following ART and plurality at conception or the initial multiple gestation reduced spontaneously to singleton gestation plays the major contributory role.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 19: THE LINK BETWEEN REPRODUCTIVE ENDOCRINOLOGY AND METABOLISM: NEWS FROM THE LAB

Monday 2 July 2018 Room 113 + 114 + 115 15:15–16:30

O-071 Genetic Deconstruction of Hypothalamic Neural Circuits for Obesity-induced Infertility

P. Wang[1], J. Xu[2], C. Bartolome[2], Y. Xinchi[2], C. Low[2], D. Kong[2]

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Study question: To investigate how the brain, especially the hypothalamus, adjusts reproductive performance according to metabolic cues and contributes to the pathophysiological process of obesity-induced infertility.

Summary answer: In obesity, hyperactive agouti-related protein (AgRP) neurons shut down reproductive performance by innervating preoptic area (POA) of the hypothalamus, where critical reproduction-regulating neurons reside.

What is known already: Epidemiological evidence suggests that obesity is tightly related to infertility, and given the role of the hypothalamus in coordinating both metabolism and reproduction, such obesity-induced infertility is likely mediated by the hypothalamus. We propose that the AgRP neurons in arcuate nucleus of the hypothalamus to be the key neurons in this pathophysiological process, given that AgRP neurons are, a) known as a major regulator of body weight and energy balance; b) heavily project to the POA of the hypothalamus, a key reproduction-regulating region; c) dysfunctions of AgRP neurons can result in severe obesity, which can cause infertility.

Study design, size, duration: To test the sufficiency and necessity of AgRP neurons in obesity-induced infertility, genetic engineered mice were randomized into control group and treatment group. Typically, each group includes 8-10 mice and 3 weeks were waited to allow sufficient expression of the viral vectors, which are used to manipulate AgRP neurons.

Participants/materials, setting, methods: AgRP-IRES-Cre mice of 6-8 weeks age were used in the current study to achieve cell-type-specific neuronal manipulation. Taking advantage of recently developed Cre-enable viral tools, in vivo inhibition and activation of AgRP neurons was carried out using DREADDs (designer receptors exclusively activated by designer drugs) and optogenetic methods. Subsequenlty, routine evaluation of reproductive performance was followed. CRISPR/Cas9 technique was also used to knock out leptin receptor in AgRP neurons.

Main results and the role of chance: To test the necessary role of AgRP neurons in mediating obesity-induced infertility, hyperphysiological neuronal activity of AgRP neurons in high-fat-diet-induced obese mice was suppressed using inhibitory hm4Di/DREADDs methods. In these obese mice, meliorated reproductive performance, as indicated by recovered estrous cycle and improved pregnancy rate (33.3% Vs. 100%, $P < 0.05$), was observed following chronic AgRP neuronal inhibition. On the other hand, to identify the sufficient role of AgRP neurons, we activated AgRP neurons in mice of normal body weight using excitatory hm3Dq/DREADDs methods. After chronic AgRP neuronal activation, impaired reproductive performance was observed, as manifested by lower luteinizing hormone (LH) levels (0.75 ± 0.12 Vs. 0.31 ± 0.03, $P < 0.05$), reduced GnRH transcription and disrupted estrous cycles. Next, we aimed to clarify the downstream neural network of AgRP neurons. To selectively manipulate AgRP afferents to the POA without affecting other AgRP-neuron projection sites, optogenetic terminal stimulation was used. After 30-minutes stimulation, significantly lowered LH levels (0.81 ± 0.20 Vs. 0.25 ± 0.04, $P < 0.001$) and GnRH transcription was observed. Moreover, to identify the molecular mechanism underlying AgPR neurons hyperactivity in obesity and the subsequent infertile phenotype, we selectively knockout leptin receptor in AgRP neurons in adult female mice and found disrupted estrous cycles and impaired pregnancy rate (100% Vs. 20%, $P < 0.05$).

Limitations, reasons for caution: The neural mechanisms discovered in this study has only been tested in mice. In the future, we are planning to extend this results to patients and identify is this neural circuit could be used as a therapeutic target for obesity-induced infertility.

Wider implications of the findings: This study is likely to provide a better understanding of the neural basis of obesity-induced infertility, which will likely accelerate the development of novel therapies of infertility. Moreover, utilizing newly developed genetic approaches with neuron-specific and real-time characteristics will open a new vista for studying central reproductive modulation.

Trial registration number: B2014-94 (approved by institutional animal care and use committee of Tufts University)

O-072 DNA methylation modifications potentially participate in the intergenerational epigenetic inheritance of prenatal androgenized mice

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Study question: Were there any epigenetic marks underpin the potential intergenerational epigenetic inheritance of prenatal androgenized (PNA) mice?

Summary answer: Prenatal androgenized could induce metabolic abnormalities in F2 offspring through paternal germline, when DNA methylation modifications were the potential epigenetic marks passing the phenotype.

What is known already: Polycystic ovarian syndrome (PCOS) could be recapitulated by prenatal androgenization across species, suggesting a developmental etiology for this disorder, when there are increasing evidences that certain acquired traits could be transmitted from one generation to the next. It arouses our interests that whether prenatal androgenization induced PCOS traits could be transmitted to the next generation and whether there exist epigenetic information carrier passing the phenotype.

Study design, size, duration: Twenty-four pregnant mice (F0) were injected with 200 ug/day dihydrotestosterone (PNA group) or sesame oil (Control group) at embryonic day 16-18. The offspring mice were studied as F1 when maternal F2 offspring and paternal F2 offspring were generated after adult female F1 and male F1 mating with normal individuals, respectively. The phenotypes of offspring mice were separately determined at 3 month's old and 8 month's old.

Participants/materials, setting, methods: Male and female C57BL/6J mice (F0) were paired and female F1 were studied with PCOS diagnostic and metabolic traits while male F1 were investigated with metabolic phenotypes. The PCOS diagnostic and metabolic traits of maternal and paternal F2 female individuals were determined to evaluate the intergenerational epigenetic effect. Single cell whole genome bisulfite sequencing (scWGBS) on F1 oocytes and WGBS on F1 sperms were performed to analyze their DNA methylation changes.

Main results and the role of chance: PNA female F1 mice had disrupted estrous cycles, elevated serum testosterone, increased preantral ovarian follicles, impaired glucose tolerance and unaffected insulin sensitivities as previously reported. We did not find any PCOS diagnostic traits or metabolic abnormalities in PNA maternal female F2 offspring as long as 8 month's old. Surprisingly, we found PNA male F1 mice represented significantly impaired glucose tolerance ($P < 0.01$) and insulin sensitivities ($P < 0.05$). More importantly, although PNA paternal female F2 offspring did not manifested PCOS diagnostic traits, they showed markedly reduced body weights ($P < 0.01$) and a clear tendency of glucose tolerance impairment ($P = 0.06$) at 3 month's old. And their glucose tolerance were even deteriorated ($P < 0.01$) and insulin sensitivities were also decreased ($P < 0.01$) at 8 month's old. In searching for epigenetic information carriers, we performed single cell whole genome bisulfite sequencing (scWGBS) on oocytes of female F1 mice and WGBS on sperms of male F1 mice. Bioinformatics analysis indicated the DNA methylation patterns of female F1 oocytes in PNA and control group did not clustered while PNA F1 sperms clearly clustered from control. We also identified 217 differentially methylated regions (DMRs) in PNA F1 sperm epigenome which could potentially be epigenetic information carriers pass the phenotype.

Limitations, reasons for caution: The exact role of DNA methylation alterations in prenatal androgenization induced epigenetic inheritance were not fully validated, and these observations were shown only in mouse species.

Wider implications of the findings: This study provide the first evidence for prenatal androgenization inducing intergenerational epigenetic inheritance potentially through paternal germline by DNA methylation modifications, shading light on explanations of PCOS prevalence and inheritance.

Trial registration number: not applicable.

O-073 Exploration of chemerin system in human granulosa cells: a new insight for polycystic ovarian syndrome

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Study question: Characterization of chemerin system in human granulosa cells from PCOS patients and cell line and modulation of its signaling activities using a CMKLR1 nanobody (C4910).

Summary answer: The chemerin system is differentially expressed and active in human granulosa cells and could have a role on PCOS syndrome.

What is known already: Studies on fertility disorders related to metabolic abnormalities have highlighted the importance of the role of hormones

produced by adipose tissue (adipokines) on the appearance of pathologies such as polycystic ovary syndrome (PCOS). Chemerin is a new adipokine able to bind three G protein-coupled receptors: CMKLR1 (chemokine-like receptor 1), GPR1 (G protein-coupled receptor 1), and CCRL2 (Chemokine (CC motif) receptor like 2). Recently, a role for the chemerin/CMKLR1 pathway in ovarian follicle function and steroidogenesis has been reported in mammals. In human granulosa (hCGs) and in the human ovarian granulosa-like tumour cell line (KGN), chemerin inhibited IGF-1-induced steroids production.

Study design, size, duration: Six groups of 10 patients aged 22 to 41 years old: control (undergoing in vitro fertilization for men infertility), obese (BMI > 30), PCOS (presenting two of the three Rotterdam criteria), ECHO (presenting only enlarged polycystic ovaries by ultrasound), PCOS-O (PCOS and obese) and ECHO-O (ECHO and obese). Cultured KGN cells were stimulated (5, 10, 30 and 60 minutes) or not with human recombinant chemerin (200 ng/ml, n = 3) and/or with C4910 (10^{-8} and 10^{-9} M, n = 1).

Participants/materials, setting, methods: Patient's clinical and biological parameters as well as granulosa cells were obtained in collaboration with the CHRU Bretonneau (Tours, France). The mRNA expression of chemerin and its receptors in granulosa cells was made by RT-qPCR.

After stimulation, proteins from KGN cells were extracted and co-immunoprecipitation experiments (homodimerization / heterodimerization of the CMKLR1) and western blot (signaling pathway) were carried out.

Main results and the role of chance: Chemerin mRNA was highly expressed in granulosa cells of obese (3.56 ± 0.51), PCOS-O (5.14 ± 1.02) and ECHO-O (2.47 ± 0.29) patients as compared to control (0.16 ± 0.02), PCOS (0.18 ± 0.02) and ECHO (0.28 ± 0.05) patients and we observed opposite profile for CMKLR1. The mRNA expression of CCRL2 was lower in PCOS (0.013 ± 0.001), obese (0.011 ± 0.001) and ECHO-O (0.013 ± 0.002) patients as compared to control (0.017 ± 0.002), ECHO (0.014 ± 0.002) and PCOS-O (0.014 ± 0.001). In parallel, we found that in KGN cells CMKLR1 and CCRL2 receptors formed a heterodimer after 5 minutes of stimulation with chemerin and gradually increased until 60 minutes. We also showed that chemerin rapidly activates (5 minutes) MAPK ERK 1/2, P38 and Akt phosphorylation and more slowly (30 minutes) AMPK and β-arrestin phosphorylation in KGN cells. Preliminary in vitro experiments of KGN cell co-incubated with chemerin and the CMKLR1 nanobody (C4910) showed a lower phosphorylation of P38 after 5 minutes than in cells only incubated with chemerin.

Limitations, reasons for caution: The characterization of the chemerin system in granulosa cells was carried out in the KGN cell line, we will confirm our results in primary granulosa cells from control women and patients.

Wider implications of the findings: According to the literature, chemerin system was expressed in granulosa cells and a deficit of CMKLR1 seems protect against follicular development disruptions in PCOS patients. We also described the chemerin signaling involved in inflammation and steroidogenesis that was partially blocked by a potential therapeutic nanobody targeting CMKLR1.

Trial registration number: not applicable.

O-074 Anovulation, insulin resistance and Akt/mTOR signaling pathway activated in LepRb-mutated mice

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Study question: What is the reproductive alteration of female mice with mutation in leptin receptor(LepR)? And What is the mechanism?

Summary answer: The mutated female mice exhibited anovulation, follicle loss, obesity and insulin resistance, with a decreased phosphorylation of IRS-1/2 and an increased activation of Akt/mTOR pathway.

What is known already: Leptin and LepR play an important role in female reproduction. In the downstream pathways of LepR, protein kinase B (Akt)/mammalian target of rapamycin (mTOR) took part in primordial follicle activation. The phosphatase and tensin homolog deleted on chromosome 10 in humans (PTEN) is a main negative regulator of PI3K pathway. Intracellular insulin signal transduction was found to have interaction with LepRb signaling pathway via insulin receptor substrate (IRS). IRS was in the upstream pathway of Akt/mTOR in both LepR and insulin signal pathway, including IRS-1 and IRS-2, both involved in regulating blood sugar.

Study design, size, duration: From September 2013 to December 2015, adult heterozygous male and female LepR-mutated mice were raised in an animal care facility of Obstetrics and Gynecology Hospital, Fudan University; consequently, fetus mice were delivered, the female ones of which were genotyped by PCR analysis of toe/tail-tip DNA at age 4 weeks, before grouped into homozygous (HOM) LepR-mutated mice and Wild Type littermates, each group being composed of 10.

Participants/materials, setting, methods: At age of 10 weeks, a glucose tolerance test was performed after a 6-h (8:30-14:30) fast. Glucose concentrations were measured in blood collected by venous bleeding from tail vein, before and 30, 60, and 120 min after a bolus i.p. injection of glucose at 0.75 g/kg. Fasting insulin and anti-mullerian hormone (AMH) was detected. We compared the reproduction and metabolic characteristics in the mice, analyzing the expression of Akt/mTOR, PTEN and IRS in the ovaries.

Main results and the role of chance: The results showed that 10-week old female mutated HOM exhibited no estrus periods, infertility, obesity, insulin resistance, declined AMH levels in the serum and ovaries, reduced primordial follicles, primary follicles, secondary follicles, antral follicles and hardly no corpus lutea (all $P < 0.05$). The phosphorylation of downsream Akt, mTOR, p70 ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein-1 (4E-BP1) of LepR were all elevated in the ovaries of the mutated female mice. They also presented a decreased phosphorylation of IRS-1, IRS-2, and PTEN, and a strengthened phosphorylation of Forkhead box O-3a (FOXO-3A) in the ovaries, when compared with WT mice.

Limitations, reasons for caution: We have not examined follicles and detected the LepR mutation, Akt/mTOR and IRS pathway in clinical obese women.

Wider implications of the findings: Clinical trials enrolled obese women will be designed and programed in the Ob and Gyn Hospital. Ovarian function and LepR mutation will be detected.

Trial registration number: The current basic study has no trial registration number.

O-075 GDNF-induced down-regulation of miR-145-5p enhances human oocyte maturation

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Study question: To examine the effects of glial cell line-derived neurotrophic factor (GDNF) on human *in vitro* oocyte maturation and to investigate the involvement of microRNAs (miRNAs) in GDNF-induced oocyte maturation.

Summary answer: Down-regulation of miR-145-5p may contribute to GDNF-induced enhancement of oocyte maturation and of cell viability against cell apoptosis.

What is known already: The maturation rate and the developmental potential of embryos derived from in vitro maturation (IVM) oocytes are significantly lower than those of embryos derived from oocytes matured in vivo. Though GDNF and miRNAs have been shown to regulate oocyte maturation, little is known about the underlying molecular mechanisms.

Study design, size, duration: A total of 200 human immature oocytes were used to evaluate the effects of GDNF on oocyte maturation. The involvement of miRNAs in GDNF-induced oocyte maturation were identified by comparing the miRNA expression profiles of cumulus cells either with or without GDNF stimulation. Human immature cumulus-oocyte complexes (COCs) were collected from infertile patients who underwent IVF treatment between January 2015 and November 2015.

Participants/materials, setting, methods: The experiments were performed in an IVF center. Immature oocytes were cultured with/without GDNF stimulation. Human miRNA arrays were used to examine the miRNA expression patterns of cumulus cells (CCs) either with/without GDNF stimulation. Luciferase reporter assays were performed to verify the targets of miRNA-145-5p. MiR-145-5p inhibitor and mimic transfections were performed to study downstream gene expression (GDNF, GDNF family receptor-alpha1(GFRA1), ret proto-oncogene (RET), and epidermal growth factor receptor (EGFR)) in human CCs.

Main results and the role of chance: During the IVM process, treatment with GDNF significantly increased the percentage of MII-stage oocytes and down-regulated the expression of miR-145-5p in cultured human CCs. Expression of MiR-145-5p in CCs is negatively correlated with oocyte maturation. Transfection with miR-145-5p mimic significantly decreased the mRNA and protein levels of GFRA1, RET, and EGFR in human CCs, whereas transfection with an miR-145-5p inhibitor reversed these effects. Treatment with GDNF inhibited cell apoptosis in cultured CCs, and this suppressive effect was reversed by transfection withthe miR-145-5p mimic.

Limitations, reasons for caution: The role of miR-145-5p was demonstrated by the fact that GDNF treatment down-regulated the expression of miR-145-5p and miR-145-5p mimic transfection decreased the expression of GFRA1, RET, and EGFR in human CCs. But we haven't directly shown that miR-145-5p mediate the effects of GDNF.

Wider implications of the findings: Our findings provide insight into the roles of GDNF and miR-145-5p in the regulation of oocyte maturation and may be clinically applied to improve the developmental potential of immature oocytes from infertile couples undergoing IVM.

Trial registration number: no.

SELECTED ORAL COMMUNICATIONS

SESSION 20: GENETIC DETERMINANTS OF HUMAN REPRODUCTION

Monday 2 July 2018 Room 117 15:15–16:30

O-076 Sperm imprinting integrity in seminoma patients?

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Study question: Does the sperm of patients with Testicular Germ Cell Tumor, in particular seminoma, present imprinted defects?

Summary answer: Seminoma patients with oligozoospermia presented sperm DNA methylation alterations on imprinted genes like those observed in oligozoospermic patients.

What is known already: Testicular Germ Cell Tumor such as seminoma is strongly associated with male reproductive problems commonly associated with the alteration of sperm parameters as described in testicular dysgenesis syndrome. Interestingly, numerous studies have reported that the precursor of germ cell cancer: Germ Cell Neoplasia in situ (GCNIS), present similarities to fetal gonocytes, specifically characterized by global DNA hypomethylation particularly on imprinting sequences. These disorders may have a common origin derived from perturbations of embryonal programming during fetal development. Presently, there is no available information concerning the sperm DNA methylation patterns of testicular cancer patients.

Study design, size, duration: A total of 92 cryopreserved sperm samples from men who gave their consent for research were included, 31 before seminoma treatment (S) and 61 in the context of ART procedures who served as controls: 31 normozoospermic (N) and 30 oligozoospermic (O). Among seminoma patients, 23 (74%) of them were normozoospermic (SN) and 8 (26%) were oligozoospermic (SO).

Participants/materials, setting, methods: DNA methylation levels of seven differentially methylated regions (DMRs) of imprinted genes [*H19/IGF2*: IG-DMR: CTCF3 and CTCF6 of *H19* gene; *IGF2*-DMRs (DMR0 & DMR2); *MEG3/DLK1*:IG-DMR; *SNURF*:TSS-DMR; *KCNQ1OT1*:TSS-DMR] were assessed by pyrosequencing. All analyses were performed after adjusting for age.

Main results and the role of chance: Comparisons of sperm DNA methylation levels between seminoma (S) and normozoospermic (N) samples showed a significant difference for the *SNURF* sequence ($p = 0.017$), but after taking into account the sperm parameters no difference was observed for both SN vs N and SO vs O. However, we confirmed a significant association between oligozoospermia (O) and imprinting defects for *H19*-CTCF6 ($p = 0.001$), *MEG3/DLK1* ($p = 0.017$), *IGF2*-DMR2 ($p = 0.022$) and *SNURF* ($p = 0.032$) in comparison with control group (N). Moreover, significant imprinting defects were detected in oligozoospermic seminoma patients (SO) compared to normozoospermic controls (N) for *H19*-CTCF6 ($p = 0.013$), *IGF2*-DMR2 ($p = 0.019$) and *SNURF* ($p = 0.001$).

Limitations, reasons for caution: Despite careful microscopic control, cellular contamination cannot be fully excluded. The pyrosequencing method limits investigations on DNA methylation to few CpGs, but yields better resolution in imprinted sequences than for larger scale analyses.

Wider implications of the findings: This study highlights the high risk of sperm imprinting defects in oligozoospermia cases, and shows that seminoma patients with normal spermatogenesis presented sperm imprinting integrity. These data disprove the idea of a common fetal imprinting defect in germ cells leading to both CGNIS and subfertility.

Trial registration number: not applicable.

O-077 Identification of X-linked CNVs in women with extremely skewed X chromosome inactivation and miscarriage

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Study question: Do changes in copy number of X-linked regions in the maternal genome may be cause of skewed X-chromosome inactivation (sXCI) in her lymphocytes and miscarriage.

Summary answer: Copy number variations (CNVs) affected X-linked genes are apparently associated with the formation of sXCI in mother and linked to the death of her embryo.

What is known already: Recent data provide evidence, that sXCI is associated with X-linked disease manifestation, cancer or adverse reproductive outcomes. Extremely sXCI (95% or above) is associated with an advanced incidence (>4-times) of idiopathic recurrent pregnancy loss based on meta-analysis of 12 datasets (Su et al., 2015). Skewed XCI may have a protective effect for woman health by suppressing X-linked lethal gene mutations or CNVs. Therefore, one can suggest, that inheritance of these mutations or CNVs by embryo may be incompatible with its normal development. If this true, the male embryos or female embryos with normal X-inactivation are in the elevated risk group.

Study design, size, duration: We investigated the status of X chromosome inactivation in 111 women with a history of spontaneous abortions with normal karyotype in anamnesis. Eight samples of DNA of the woman with extremely sXCI were analyzed using aCGH. The presence and inheritance of probably pathogenic CNVs were analyzed by quantitative Real Time PCR both in mother and miscarriage.

Participants/materials, setting, methods: X-chromosome inactivation was assessed by classical methylation-specific assay at the AR locus. DNA samples from 8 woman with extremely sXCI were searched for CNVs using SurePrint G3 Human CGH + SNP 4×180 K Microarray Kit (Agilent Technologies, USA). Array CGH was performed according to the manufactures recommendations. Confirmation studies were performed on AriaMX Real Time PCR System (Agilent Technologies, USA) with DNA samples from mother's lymphocytes and extraembryonic tissues of spontaneous abortions.

Main results and the role of chance: X-inactivation status was found to be skewed in 8 of 111 (7%) women. Their karyotype was analyzed by aCGH. In one case woman with sXCI had delXq24, 239 kb in size, affected 8 genes (*SLC25A43, SLC25A5-AS1, SLC25A5, CXorf56, UBE2A, NKRF, SEPT6, MIR766*). This deletion was confirmed by Real-Time PCR both in mother and spontaneous abortion with 46,XY karyotype. The mother, 29 years old, had a history of 5 miscarriages, 1 induced abortion and 1 healthy female child. DelXq24 encompassing UBE2A gene was revealed previously in two unrelated boys with mental deficiency, heart defects, dysmorphic features (Thunstromet al., 2014) and related to X-linked syndromic mental retardation, Nascimento type (MIM 300860). The X-inactivation study in both patients' mothers showed skewed X-inactivation (99%). Xq24 deletions were also among the most common deletions in HER2-positive breast cancer and affected genes *SLC25A43, SLC25A5-AS1, SLC25A5* (Gabrielson et al., 2016). Products of affected genes are involved in cell proliferation, differentiation and post-transcriptional regulation of gene expression. Knockdown of *SLC25A43* in non-tumor cells significantly inhibited cell cycle progression during G1-S transition and reduce proliferation rate. Probably a lack of these genes expression leads to the shifting to sXCI in deletion carriers with subsequent risk for adverse pregnancy outcomes.

Limitations, reasons for caution: The possible variation in the X-chromosome inactivation level in different tissues as well as tissue specific expression profiles of genes escaping X-inactivation should be considered.

Wider implications of the findings: Our data are applicable to understanding of mechanisms responsible for coordinated control of the expression level of X-linked loci responsible for early embryo development by epigenetic and cytogenetic processes. They will provide new strategies for PGD in women with recurrent pregnancy loss. This study is supported by RFBR grant 18-015-00437_a.

Trial registration number: Not applicable.

O-078 Does fertility status in varicoceles depends on DNA acetylation and methylation processes; cross-link with Hsp70-2 in an experimental trial using Wistar rats

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Study question: Does DNA acetylation, methylation and heat shock protein (Hsp70-2) correlate with varicocele-induced DNA damage? What is the cross-link between epigenetic factors, sperm DNA integrity and blastocyst rate?

Summary answer: Impaired Hsp70/DNA methyltransferase-1 (DNMT1)/ histone acetyltransferase (HAT)/ histone deacetylases-1 (HDA1) network adversely affects the epigenetic pathways during spermatogenesis, reduces sperm fertilization potential and down-regulates blastocyst rate.

What is known already: Misregulation of histone modification and replacement during spermiogenesis has been linked with varicocele-induced oxidative DNA damage. Indeed, the epigenetic mechanisms are core regulators of DNA relaxation, replication, repairmen and histone replacement/modification. The DNMT1 is a key maintenance methyltransferase during early stages of developing germ cells, which allows transcription to occur at later stages. On the other hand, the HAT and HDA1-induced acetylation and deacetylation are involved in chromatin-protamine remodeling and DNA repairmen. Moreover, the Hsp70-2 protects DNA strands against oxidative stress. Thus, the correlation between altered acetylation, methylation, histone-protamine replacement, Hsp70-2 expression, and the pre-implantation embryo development is still uncovered.

Study design, size, duration: Thirty mature Wistar rats were divided into control, control-sham and varicocele-induced groups (NO = 10 rats). The experimental left hand side varicocele was induced by semi-ligation of left renal vein adjacent to caudal Vena cava. The animals in the control-sham group were undergone a simple laparotomy without varicocele induction. The control group did not undergo surgical application. Following 60 days, the left testicles were dissected out and different molecular and biochemical analyses were conducted.

Participants/materials, setting, methods: The DNMT1, HAT, HDA1 expressions were analyzed using qRT-PCR. The Hsp70-2 expression was assessed by using quantitative RT-PCR, western blot and immunohistochemistry. To show DNA methylation, the immunoflourescent staining of 5 methylcytidine was conducted. Testicular total antioxidant capacity (TAC), gluthatione peroxidase (GSH-px), superoxide dismutase (SOD), catalase, malondialdehyde (MDA) levels and sperm DNA integrity were evaluated. Following in vitro fertilization, correlation between blastocyst rate and each assessed epigenetic factor was analyzed.

Main results and the role of chance: The varicocele group exhibited decreased mRNA of DNMT1, HAT and HDA1 versus control groups ($P = 0.001$). The mRNA and protein levels of Hsp70-2 and the Hsp70-2^+ cells distribution *per* mm^2 of tissue were decreased in varicvocele group versus control animals ($P = 0.01$). The varicocele group exhibited down-regulation of 5 methylcytidine, representing suppressed DNA methylation. The testicular TAC, GSH-px, SOD and catalase levels were down-regulated and the MDA content was up-regulated in varicocele group. The percentages of sperms with DNA fragmentation were increased in varicocele animals. Analyses of HAT and HDAT1 mRNA levels revealed an inverse correlation between HAT and HDAT1 and sperm DNA damage ($p = 0.02$, $r^2 = -0.912$) as well as the blastocyst rate ($P = 0.01$, $r^2 = -0.812$). No correlation was found between DNMT1 mRNA and sperm DNA damage. Meanwhile, the sperm DNA damage was negatively correlated with decreased levels of TAC ($P = 0.01$, $r^2 = -0.812$), GSH-px ($p = 0.02$, r^2: -0.721), SOD ($p = 0.001$, r^2=-0.816) and catalase ($P = 0.01$, $r^2 = -0.830$). An inverse correlation was found between decreased expression of Hsp70-2 and sperm DNA damage ($P = 0.02$, r^2: -0.761). No correlation was found between mRNA and protein levels of Hsp70-2 and blastocyst rate. A negative correlation was found between sperm DNA damage and the blastocyst rate ($P = 0.02$, r^2: -0.932).

Limitations, reasons for caution: The results of the current study may differ in different laboratory settings. Moreover, the species differences can affect the spermatogenesis-related epigenetic pathways, and some differences between rat and human spermatozoa can partially change the results belonging to fertilization outcomes. Furthermore, additional studies are needed to represent the birth rates.

Wider implications of the findings: Present study increases our understanding that, (i) varicocele down-regulates the intra-testicular acetylation and methylation ratio, (ii) down-regulates the Hsp70-2 expression and antioxidant status and (iii) Impaired acetylation in association with suppressed Hsp70-2 and diminished antioxidant status amplifies sperm DNA damage and lowers the blastocyst rate.

Trial registration number: N/A

O-079 Distribution of MTHFR isoforms carriers and level of homocysteine in an infertile population with assisted reproductive technologies (ART) cycle failures

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Study question: What is the impact of MTHFR isoforms in fertility? Can it be related to an increase of circulating homocysteine known to affect the gametes?

Summary answer: The proportion of MTHFR mutations carriers is significantly higher in the infertile population. The average level of homocysteine is significantly higher in the mutations carriers.

What is known already: MTHFR isoforms induce defective folic metabolism and affect the recycling of homocysteine to methionine. The increased levels of homocysteine due to MTHFR polymorphisms is responsible of oxidative stress (Guo, 2016). Oxidative stress is a common cause of infertility and ART failures.

MTHFR isoforms are responsible of spermatozoa anomalies, especially via a defect in nucleus compaction (Cornet, 2017). Furthermore, the IVF embryos originating from MTHFR isoforms carriers have high chromosomal problems for the heterozygotes and higher in the homozygotes patients (Enciso, 2017).

Study design, size, duration: From January 2016 to january 2018, we followed 198 women patients suffering from infertility and having had at least 2 ART (Assisted Reproductive Technologies) cycle failure. All those patients

were tested for MTHFR isoforms and we also measured their level of circulating homocysteine. We compared those results among our infertile population and with the general population.

Participants/materials, setting, methods: The presence of MTHFR isoforms was determined from a venous blood sample, using real time PCR with the RealFast™ assay (ViennaLab Diagnostic GMBH, Vienna, Austria).

The levels of homocysteine are measured with a competitive immunoassay using direct, chemiluminescent technology.

Main results and the role of chance: Among the infertile population of our study, 63.6% of the patients are carrying the MTHFR mutation (43.9% in a heterozygous and 19.7% in a homozygous state). This proportion is significantly more important ($p < 0.05$) than the proportion of patients carrying MTHFR mutations in the general population: 50.5% (Zappacosta, 2009).

The average level of homocysteine among homozygotes and heterozygotes patients is significantly higher than among the wild type patients (respectively 10.7 and 8.8 vs 7.9, $p = 0.01$ and $p = 0.02$). In total, the average level of homocysteine among MTHFR carriers (homozygotes+heterozygotes) is significantly higher than among non carriers (wild-type) patients (9.4 vs 7.9, $p = 0.02$).

Limitations, reasons for caution: The genetic analysis of the MTHFR gene needs to be enlarged to other mutations in order to screen all the patients in which fertility could be affected by oxidative stress. The proportion of MTHFR mutation carriers is probably more important and treatments could benefit to a larger number of patients.

Wider implications of the findings: Infertile patients with ART cycle failures should be tested for MTHFR mutations and their homocysteine levels should be measured. Indeed, the affected patients could benefit from a treatment available allowing to by-pass the problems linked to MTHFR impaired activity and thus decreasing oxidative stress, and improving their ART outcomes.

Trial registration number: None.

O-080 Novel loci for polycystic ovary syndrome (PCOS) identified in the largest genome-wide association study (GWAS) conducted to date

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Study question: We aimed to explore the genetic components of PCOS by conducting a GWAS in >285,000 women, including 20,410 PCOS cases.

Summary answer: Our GWAS identified 5 novel loci that were associated with PCOS at genome-wide significance ($p < 5 \times 10^{-8}$).

What is known already: Although the pathophysiology of PCOS is known to involve multiple, heterogeneous mechanisms, the molecular components of these mechanisms are still being uncovered. GWAS conducted in Han Chinese and European populations have identified 17 loci, overlapping with genes involved in the hormonal, metabolic, and cellular aspects of the disorder. However, since the estimated heritability of PCOS may be as high as 70%, it is likely that only a portion of its underlying genetic landscape has been uncovered. A more complete understanding is necessary for the development of novel molecular strategies to diagnose PCOS and target its various clinical features.

Study design, size, duration: In collaboration with the personal genetics company 23andMe, Inc., we conducted a GWAS in women of European ancestry who had consented to participate in research. Among these women were 20,410 PCOS cases (4 times larger than the largest published GWAS to date) and 265,397 controls. Inclusion or exclusion of subjects was determined by responses to online surveys.

Participants/materials, setting, methods: Case subjects self-reported being diagnosed and/or treated for PCOS. Control subjects self-reported not having been diagnosed or treated for PCOS. Participant samples were genotyped on 1 of 4 custom genome-wide genotyping arrays targeting 556-955k SNPs. Participant genotypes were imputed against the 1000 Genomes Project Phase 1 reference haplotypes. We performed a logistic regression under an additive model with age, top 5 principal components, and genotyping array version as covariates.

Main results and the role of chance: Of the 17 loci previously identified in PCOS GWASs, 8 reached genome-wide significance ($p < 5 \times 10^{-8}$) in our analysis, and a further 7 reached nominal significance ($p < 0.05$), all with a consistent direction of effect. Further, we report 5 novel loci achieving genome-wide significance for the first time in the context of PCOS: *ZBTB16* (rs1784692, $p = 2.1 \times 10^{-15}$, OR[95% CI] = 1.12[1.09-1.15]), *FTO* (rs8047587, $p = 4.3 \times 10^{-9}$, OR[95% CI] = 1.07[1.04-1.09]), *ZKSCAN5-FAM200A* (rs10278040, $p = 5.6 \times 10^{-9}$, OR[95% CI] = 1.16[1.10-1.22]), and *ZEB2* (rs13028626, $p = 3.3 \times 10^{-8}$, OR[95% CI] = 1.06[1.04-1.09]). In addition, we identified a potential association in *MYO10* locus (rs9312937, $p = 4.0 \times 10^{-8}$, OR[95% CI] = 1.06[1.04-1.08]). The *FTO* and *MYO10* loci have been linked to BMI-related traits in previous GWASs but never linked to PCOS at genome-wide significance. However, variants in *FTO* have been associated with PCOS in several candidate genetic studies. Interestingly, *MYO10* has been identified as a meiotic regulator in animal studies. The *ZBTB16* locus was previously identified in a PCOS GWAS but did not reach genome-wide significance. *ZBTB16* is involved in brown adipocyte bioenergetics and the development of metabolic syndrome, as well as androgen receptor activity. Similarly, the *ZKSCAN5-FAM200A* locus overlaps with a locus previously associated with levels of steroid hormones, such as progesterone and dehydroepiandrosterone sulfate.

Limitations, reasons for caution: The novel associations in *FTO*, *ZKSCAN5-FAM200A*, *ZEB2*, and *MYO10* loci need to be replicated in an independent population. Further, additional characterization is warranted to identify putative causal variants and genes that may underlie the observed novel associations. Lastly, this study used self-reported phenotypic data, which could impact the case-control stratification.

Wider implications of the findings: In the largest PCOS GWAS to date, we provide evidence of novel associations with 5 genetic loci. This work extends the understanding of the genetic underpinnings of PCOS and emphasizes androgenic and metabolic mechanisms in the pathophysiology of the disease.

Trial registration number: N/A.

SELECTED ORAL COMMUNICATIONS

SESSION 21: FEMALE FERTILITY PRESERVATION: CLINICAL AND LABORATORY

Monday 2 July 2018 Room 116 15:15–16:30

O-081 Potential leukaemic contamination in cryopreserved ovarian tissue

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Study question: Can xenografting of cryopreserved ovarian tissue from patients with leukaemia detect the presence of contaminating leukaemic cells within the tissue?

Summary answer: Leukaemic cells are present in ovarian tissue from the majority of patients' tissue examined and these cells multiply with time in an immunodeficient mouse.

What is known already: Two previous studies have examined the potential of ovarian tissue from leukaemia patients to harbour leukaemic cells. Although one study detected the presence of leukaemia in 5 out of 18 patients' tissue following xenografting, the other did not detect any contamination even in tissue found to be PCR positive for leukaemic cells. Similarly, in the former study, not all the tissue positive for leukaemic cells by PCR manifested in disease in the xenografted mice.

Study design, size, duration: To assess whether ovarian tissue from patients with leukaemia harboured leukaemic cells and whether these cells were capable of proliferation over time, cryopreserved ovarian tissue was transplanted into immunodeficient mice (3 mice per patient) and left for 24 weeks. At the completion of the study, the ovarian tissue and organs were removed, fixed and examined for evidence of leukaemic cells.

Participants/materials, setting, methods: Stored ovarian tissue from 18 patients with leukaemia (5 ALL, 9 AML, 4 CML) were donated to the study. Two slices of ovarian tissue were attached to the abdominal wall using non dissolvable sutures and 1 small piece placed under the kidney capsule in each NOD SCID mouse. At 24 weeks mice were examined for tumour development, blood taken, and spleen weight measured. One slice of ovarian tissue was prepared for PCR analysis.

Main results and the role of chance: Tumour development was observed in tissue from 13 out of the 18 patients examined (3/5 ALL, 6/9 AML, 4/4 CML) following xenografting. Generally 1 mouse out of the 3 xenografted with same patient tissue developed a tumour. Tumour was only associated with the grafted tissue in 5 mice, the remainder of tumours were detected in other organs or in nodes. Sectioning of tumours/nodes and staining with H&E showed infiltration with monomorphous leukaemic cells. In some mice other organs were also infiltrated with leukaemic cells and the spleens were enlarged to 2 to 6 times the normal size.

Tissue from 6 patients which had been cryopreserved less than 4 weeks after the last chemotherapy dose resulted in tumour development. To date, all PCR positive tissue resulted in tumours following xenografting (only CML evaluated).

Subsequent lineage characterization with immunohistochemistry is required to verify tumour origin. Examination of organs from mice without obvious tumour development is still ongoing.

Limitations, reasons for caution: Although more mice have been examined for each patient's tissue in the present study (3 per patient) than in the 2 previous studies (1 mouse per patient), insufficient numbers of mice may still be the reason for the negative result in those where no tumour was identified.

Wider implications of the findings: There is a high risk of re-introducing leukaemic cells to patients when grafting cryopreserved ovarian tissue. Other alternatives need to be developed to reduce or eliminate this risk.

Trial registration number: Not applicable

O-082 LH gonadoprotection against ovarian damage induced by alkylating drugs in adult mouse ovaries

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Study question: To evaluate if LH administration protects the follicular pool against gonadotoxic effects of oncologic treatment with alkylating drugs in adult mouse ovaries.

Summary answer: LH treatment prevents follicular depletion induced by Busulfan and Cyclophosphamide in adult mouse ovaries by preserving higher number of follicles, especially primordial and primary populations.

What is known already: Oncologic treatment with high-dose chemotherapy may impose deleterious effects on the ovary. Clinically, impact ranges through partial damage to a complete destruction of the follicular pool. The highest risk is associated to alkylating drugs, while platinum-based compounds like cisplatin associated a medium risk. Several methods are available to preserve fertility in cancer patients but further research is needed to develop new alternatives. Previous studies suggested that luteinizing hormone (LH) prevents the cisplatin-induced apoptosis in oocytes and preserves fertility in prepubertal mouse. We aim to validate the protective effects of LH on the mouse adult ovary against chemotherapy with alkylating agents.

Study design, size, duration: Experimental study, with eleven 6 week-old CD-1 female mice allocated to the following groups: Control (n = 3), Chemotherapy (ChT, n = 4) and ChT+LH (n = 4). Mice in the ChT groups received a dose of alkylating drugs to induce severe ovarian damage on day 0, at the same time with their own treatment (saline or LH, respectively). Animals were maintained during 12 days and then sacrifice to recover ovarian samples for further analysis.

Participants/materials, setting, methods: A single intraperitoneal injection with 12 mg/Kg busulfan and 120 mg/Kg Cyclophosphamide was administered to mice of the ChT treated groups while controls received saline. Simultaneously, animals of the ChT and Ch + LH group also received 100ul of saline or equal volume with 1IU of LH, respectively. A week after, ovarian hyperstimulation was induced, animals mated with fertile males and sacrificed. The ovarian size, follicular growth and populations, ovulation and early embryo development were then evaluated.

Main results and the role of chance: ChT administration reduced the ovarian weight after hyperstimulation (C:45.7 ± 2.0 mg, ChT:14.9 ± 0.9 mg and ChT-LH:14.6 ± 3.2 mg; p = 0.001) and LH administration was not able to recover control values.

When the total amount of follicles was analyzed, a 10-fold decrease was observed after chemotherapy administration (C:1260 ± 168, ChT:182 ± 31; p = 0.03). Nevertheless, LH administration reduced the severity of the follicular depletion (ChT-LH: 264 ± 59; p = 0.04). This improvement was mainly due to a better preservation of the primordial (ChT-LH:42 ± 14 vs. ChT:7 ± 5foll., p = 0.032) and the primary populations (ChT-LH:132 ± 8 vs. ChT:68 ± 12, p = 0.034). However, LH was not able to preserve the late preantral, antral and pre-ovulatory populations already impaired by ChT.

The percentage of quiescent follicles decreased in the ChT group due to the burnout of dormant follicles induced by alkylating agents but LH treatment was able to minimize this effect (p = 0.034) and to maintain values similar to controls (C: 15.7 ± 3.6%, ChT:4.2 ± 3.4% and ChT-LH: 15.8 ± 4.6%).

The amount of ovulated MII-oocytes and 2-cell embryos were seriously impaired in both ChT (MII: 8 ± 5; Embryos:7 ± 5) and ChT-LH treated mice (MII: 10 ± 9; Embryos:7 ± 10), when compared to controls (MII: 17 ± 2; E:10 ± 2). However, LH administration increased the viability of the obtained MII-oocytes (ChT:64.3 ± 27.5% vs. ChT-LH:90.4 ± 11%) and embryos (ChT:63.9 ± 29.3% vs. ChT-LH:95.2 ± 8.2%) although no statistically significant.

Limitations, reasons for caution: This is a preliminary study with a reduced sample size and therefore data should be confirmed in a larger population. Furthermore, LH effects on follicular development potential should be carefully assessed.

Wider implications of the findings: We found that LH administration protects primordial and primary follicles in adult ovaries against the depletion and over activation induced by alkylant agents. However, LH was not able to preserve the already growing populations but allows the surviving follicles to produce oocytes and embryos with increased developmental potential.

Trial registration number: Not applicable.

O-083 Towards an artificial ovary: Grafting preantral follicles on decellularized human ovarian tissue

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Study question: Can decullularized human ovarian tissue support development of follicles and become a biocompatible and biofunctional artificial ovary?.

Summary answer: We have demonstrated an effective protocol for decellularization of human ovarian tissues and successful recellularization with isolated preantral follicles.

What is known already: Decullarization of ovarian tissue entails the removal of cells, including possible malignancies. The physiological extracellular matrix (ECM) left behind offers the complex milieu that facilitates the necessary interaction between ovarian follicles and their surroundings to ensure their growth and development. A bioengineered ovary would thus facilitate the growth and development of reseeded frozen-thawed early stage follicles, free of malignancies. Decellularized tissue (DCT) also benefits from its biocompatibility due to clearance of immunogenic substances. Further research in developing a

bioengineered ovary entails thorough studies into its biocompatibility and biofunctionality.

Study design, size, duration: Donated human ovarian tissue and isolated preantral follicles from women undergoing ovarian tissue cryopreservation for fertility preservation. Biofunctionality of the decellularized human ovarian tissue was evaluated by in vitro and in vivo studies. Furthermore, scaffolds were transplanted to immunocompetent mice and evaluated for inflammatory reactions.

Participants/materials, setting, methods: Ovarian cortical and medullary tissue were decellularized (0.1% SDS for 3, 6, 24 hours; followed by 24 hours DNase (1 mg/mL)). Decellularization and preservation of composition were characterized by DNA and collagen quantification, Periodic Acid-Schiff (PAS) staining, and immunoflourescence for Collagen IA and DNA (4',6-diamidino-2-phenylindole (DAPI)). Human granulosa cells (GCs) were reseeded on the DCT and cultured in vitro. Murine and human preantral follicles were isolated reseeded on the DCT and grafted subcutaneously to immunodeficient mice.

Main results and the role of chance: Incubation in 0.1% SDS for 6-24 hours adequately decellularized both human ovarian cortical and medullary tissue by eliminating all cells and leaving the ECM intact. DNA quantities in the DCT were significantly lower compared to matched native samples. Histological examination using PAS staining confirmed that the cortical and medullary tissues were completely decellularized, and no visible nuclear material was found within the decellularized sections. DCT also stained positive for collagen I and collagen quantities in DCT constituted 88-98% of the individual baselines for native samples (n = 4). Mature human GCs were able to recellularize the DCT in vitro by successfully repopulating and migrating into the scaffold. Xenotransplantation experiments showed that the DCT was able to support survival of isolated human follicles and growth of isolated murine follicles of which several grew to antral stages. The follicular recovery rates after 3 weeks grafting were similar for both human (25%) and murine follicles (26-32.5%). The average diameter of isolated murine follicles increased from 114 ± 26 μm to 195 ± 143 μm following 3 weeks grafting.

Limitations, reasons for caution: Further studies are needed to increase recovery and survival of the reseeded follicles. Furthermore, longer grafting periods should be evaluated for observing development of follicles. Survival of the follicles might be impaired by the lack of stroma cells. This challenge must be overcome to advance the bioprosthetic ovary further.

Wider implications of the findings: This is the first time that isolated human follicles have survived in a decellularized human scaffold. Therefore, this proof-of-concept could be a potential new strategy, to eliminate the risk of malignant cell re-occurrence in former cancer patients having cryopreserved ovarian tissue transplanted for fertility restoration.

Trial registration number: Not applicable.

O-084 Primordial follicle activation in vitro following PTEN inhibition is associated with increased DNA damage of bovine ovarian follicles

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Study question: Does activation of ovarian follicle growth by phosphatase homolog of chromosome-10 (PTEN) inhibition result in altered indices of oocyte and granulosa cell health?

Summary answer: PTEN inhibition leads to increased primordial follicle activation, but increases DNA damage and suppresses the DNA repair capacity of bovine ovarian follicles *in vitro*.

What is known already: Although the PTEN inhibitor bpv(HOpic) has been widely used to activate primordial follicles, it's effects on DNA damage and repair in normal cells, including oocytes and granulosa cells, has not been determined since most of the investigations have used cancer cell lines. PTEN inhibition leads to cytoplasmic sequestration of DNA repair factors BRCA-1 and Rad-51 in breast cancer cells. Notably, unrepaired DNA damage due to impaired DNA repair capacity both in oocytes or granulosa cells is associated with ovarian aging. Meiotic errors are also more likely leading to chromosomal abnormalities in mature oocytes, ultimately impacting on oocyte quality.

Study design, size, duration: This study utilised bovine ovarian cortical fragments (4 × 2×1 mm). Ovarian fragments were cultured for 24 hour in medium with and without bpv(HOpic) then for a further 5 days in control medium without bpv(HOpic). A total of 57 ovarian cortical strips per group and 29,550 follicles from three independent experiments were analysed.

Participants/materials, setting, methods: After 24 hours treatment, tissues were subjected to Akt quantification by Western blotting. At the end of the culture period (total of 6 days) fragments were fixed and analysed histologically and by immunohistochemistry: λH2AX as a marker of DNA damage; RAD-51, BRCA-1 and BRCA-2 as DNA repair factors, and for nuclear exclusion of Foxo3 as a marker of follicle activation.

Main results and the role of chance: There was a significant increase in the proportion of growing follicles cultured with bpv(HOpic) 10 μM (88.6%) compared to 1 μM (76.8%, $P \leq 0.001$) or control (70.5%, $P \leq 0.001$) after 6 days. The proportion of morphologically healthy follicles however, was lower (10 μM bpV (HOpic) 49.4%vs78.3% in control, $p < 0.001$). Akt phosphorylation in bpv (HOpic) exposed tissue was increased ($P \leq 0.001$) and was associated with increased nuclear exclusion of Foxo3 ($P \leq 0.001$). DNA damage in oocytes was higher in bpv(HOpic) groups (1 μM: non-growing, 83%; primary, 78%, secondary, 77%; 10 μM: 74%, 84% and 89%) than control (30%, 59% and 50%) as shown by increased λH2AX expression ($P \leq 0.005$). RAD-51 and BRCA-2 expression were analysed to assess DNA repair proteins involved in homologous recombination. A reduction in RAD-51 expression occurred in bpv (HOpic) treated groups of primary (1 and 10 μM: 34% and 24%) and non-growing follicles (22% and 30%) compared with control (48% and 38%) ($P \leq 0.001$). Higher dose bpv(HOpic) also resulted in lower expression of BRCA-1 compared to control and bpv(HOpic) 1 μM ($P < 0.001$) in non-growing and primary follicles, indicating a reduced response to DNA damage. However, oocytes of primary follicles in 1 μM bpv(HOpic) exhibited a higher level of BRCA-2 expression (36%) compared with control (20%, $P = 0.011$) with a marked decrease in 10 μM (1%, $P \leq 0.001$).

Limitations, reasons for caution: This study focuses on primordial follicle activation after 6 days culture and may not reflect DNA damage and repair capacity in later stages of oocyte and follicle growth.

Wider implications of the findings: Exogenous activation of follicle growth may compromise the bidirectional signalling between oocyte and granulosa cells necessary for optimal follicle health. This large animal model may be useful in assessing follicle activation protocols prior to their use in human ovaries.

Trial registration number: not applicable.

O-085 Can we perform flexible antagonist protocol for luteal phase ovarian stimulation for breast cancer patients seeking fertility preservation?

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Study question: Does the timing of GnRH antagonist initiation during the luteal phase impact the outcome of fertility preservation (FP) cycles?

Summary answer: Flexible GnRH antagonist initiation during luteal phase ovarian stimulation may not impact negatively FP outcome when compared to simultaneous administration with exogenous FSH.

What is known already: Evidence indicates that luteal phase ovarian stimulation may represent an efficient option for women seeking FP, leading to comparable results as those obtained with early follicular phase stimulation. Currently, GnRH antagonists are simultaneously administered with FSH in order to induce early luteolysis and prevent premature LH surge. The feasibility of postponing GnRH antagonist initiation during luteal phase stimulation has not been studied.

Study design, size, duration: This prospective randomized study was conducted between July 2013 and December 2017.

Participants/materials, setting, methods: Sixty-eight women undergoing ovarian stimulation for urgent FP before breast cancer chemotherapy were

randomized into 2 groups according to the beginning of GnRH antagonist administration on stimulation day 1 (S1) or flexibly. In the flexible group, GnRH antagonist was started when the leading follicle reached 12 mm in diameter. Stimulation outcomes were compared between both groups.

Main results and the role of chance: As expected, both groups were comparable in terms of age, body mass index, antral follicle count and AMH levels. Despite different timing in GnRH antagonist initiation, the duration of ovarian stimulation and total amount of gonadotropins were comparable between S1 and flexible groups (11.0 ± 2.5 vs. 11.05 ± 2.2 days and 3530.6 ± 1357.0 vs. 3446.3 ± 1207.7IU, respectively). Finally, the mean number of oocytes recovered and vitrified at metaphase 2 stage were similar in both groups (12.9 ± 10.3 vs. 14.3 ± 8.6 and 10.3 ± 9.9 vs. 10.2 ± 6.9 respectively). The mean day of antagonist introduction in the flexible group were the 6.7 day.

Limitations, reasons for caution: The limited number of patients randomized in each group may represent a limitation for the interpretation of the present results.

Wider implications of the findings: The flexible GnRH antagonist administration during luteal phase stimulation may represent a friendly and cost effective approach. Our preliminary results should be confirmed in a larger population. In addition, the endocrine profile associated with such a protocol and its further impact on oocyte quality will represent important issues.

Trial registration number: not applicable

INVITED SESSION

SESSION 22: CLINICAL UTILITY OF ANTI-MULLERIAN HORMONE MEASUREMENT

Monday 2 July 2018 Room 211 + 212 17:00–18:00

O-086 AMH measurement in fertility treatment

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Anti-Mullerian hormone (AMH) is a dimeric glycoprotein that belongs to the transforming growth factor-beta family. In the female, AMH is solely produced by the granulosa cells of preantral and small antral follicles, and regulates ovarian folliculogenesis. Because of this exclusive source of production in the adult female, AMH is a potentially useful marker of ovarian function, and there have been increasing reports on its clinical utility (Dewailly et al, 2014).

Because of its stable production throughout the menstrual cycle with clinically insignificant intra- and inter-cycle variation and that its serum concentration is not influenced by the use of exogenous hormones (Li et al, 2011a), AMH serves as a useful diagnostic tool in differentiating the different causes of secondary oligo-amenorrhoea, particularly ovarian failure and polycystic ovary syndrome from the other causes, with very good diagnostic accuracy (Li et al, 2011b).

Basal serum AMH concentration is useful in prediction of suboptimal and excessive ovarian responses upon ovarian stimulation for in in-vitro fertilization (IVF) treatment cycles, (Broer et al, 2011). There is also some association between AMH level and fresh cycle live birth rate as well as cumulative live birth rate (Li et al, 2013), although most reports indicated a poor predictive value of AMH on the absolute occurrence of pregnancy or live birth rate from IVF treatment, as reviewed in meta-analyses (Iliodromiti et al, 2014). AMH may be another marker for determination of individualised ovarian stimulation regime and gonadotrophin dosing in IVF treatment programmes (La Marca and Sunkara, 2014).

Since there is yet no international standard for AMH measurement, and the numerical values obtained by the different existing assay methods are not equivalent, the discordance among different assay methods may misclassify a significant proportion of women to an inappropriate gonadotrophin dose (Iliodromiti et al., 2017).

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O-087 Role of AMH in the pathogenesis and diagnosis of ovulatory disorders

INVITED SESSION

SESSION 23: CHROMOTHRYPSIS OR HOW REARRANGING OUR CHROMOSOMES CHANGES EVERYTHING

Monday 2 July 2018 Room 111 + 112 17:00–18:00

O-088 The characteristics and origin of chromothripsis

W. Kloosterman[1]

UMC Utrecht, Genetics, Utrecht, Neth. Antilles

Human genomes are continuously subjected to mutations, which can drive genetic diseases and cancer. An intriguing finding has been the discovery of chromothripsis, a spectacular and complex form of chromosome rearrangement that can occur in the genomes of cancer cells and patients with congenital diseases. Chromothripsis has been described in a large array of human cancers and various types of chromothripsis have appeared, which differ in complexity and genomic hallmarks. From the combined genomic data a consensus hypothesis has been inferred, involving aberrant DNA replication and chromosome shattering as the underlying processes explaining chromothripsis. In addition, recent work has established several cellular models that recapitulate chromothripsis under defined experimental conditions.

We have used long-read nanopore single-molecule sequencing technologies to unravel complex chromosomal rearrangement, including chromothripsis.

We demonstrate the power of long-read sequencing by the analysis of the genomes of two patients with congenital abnormalities. We developed a bioinformatic method to efficiently map genomic structural variants (SVs) from the long-read data. Using this method, we readily detected all *de novo* rearrangements involving multiple chromosomes originating from complex chromothripsis events. Long sequencing reads enabled efficient phasing of genetic variations, allowing the construction of genome-wide maps of phased SVs. We employed read-based phasing to show that all *de novo* chromothripsis breakpoints occurred on paternal chromosomes and we resolved the long-range structure of the chromothripsis. Our work demonstrates the value of long-read sequencing for analysis of complex chromosomal aberrations underlying human developmental disorders.

O-089 Chromothripsis in human reproduction

F. Pellestor

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Chromothripsis and the genesis of complex chromosomal rearrangements in human gametes and embryos.

Franck Pellestor.

Laboratory of Chromosomal Genetics and Chromostem unit, Department of Medical Genetics, Arnaud de Villeneuve Hospital, Montpellier CHRU, Montpellier, France.

INSERM Unit «Plasticity of the Genome and Aging », Institute of Functional Genomics, Montpellier, France.

Over the last 6 years, the discovery of an unexpected type of massive chromosomal rearrangements, called chromothripsis, came revolutionized our view of the genesis of complex chromosomal rearrangement in cancers and congenital disorders.

Chromothripsis is characterized by the shattering of one (or a few) chromosome segment(s) followed by a chaotic reassembly of the fragments generated through a single catastrophic cellular event.

Several features distinguish this phenomenon from other complex structural aberrations. Congenital chromothripsis are characterized by their low copy number changes and their relatively balanced status. This does not signify that unbalanced chromothripsis events don't occur in germline or during post-zygotic divisions, but it strongly emphasizes the effect of selective pressure as a bias factor in the assessment of congenital chromothripsis, since only balanced chromothripsis outcomes compatible with life have been found in individuals to date.

Various mechanisms, involving abortive apoptosis, telomere erosion, mitotic errors, micronuclei formation and p53 inactivation might cause chromothripsis.

The remarkable point is that all these plausible mechanisms have been identified in the field of human reproduction as causal factors for reproductive failures and chromosomal abnormalities genesis.

Specific features of gametogenesis and early embryonic development such as the weakness of cell cycle and mitosis checkpoints and the rapid kinetics of division in germ cells and early cleavage embryos, may contribute to the emergency of chromothripsis. Existing data indicate that constitutional chromothripsis occurs preferentially in the male germline. This confirms the great vulnerability of spermatogenesis to DNA damage and clearly points to spermatogenesis as a critical stage in the genesis of congenital chromothripsis. Also, the possibility of chromothripsis occurrence in female meiosis cannot be neglected because oocytes are particularly vulnerable to environmental attacks and DNA alteration, and can cumulate genomic and chromosomal damages. During the fertilization process, the premature chromosome condensation (PCC) of sperm nucleus may initiate chromothripsis. In preimplantation embryos, the high incidence of chromosome abnormalities and the frequent fragmentations observed support the assumption that chromothripsis may arise frequently during early human embryogenesis.

All the data support the hypothesis that this unanticipated catastrophic phenomenon may arise more frequently than previously thought in both gametogenesis and human embryogenesis, but also confirmed its compatibility with viability and transmission to subsequent generations.

The complexity of chromothripsis and the diversity of its causative mechanisms raise questions about the origin of this phenomenon, its ties to chromosome instability and its implication in human reproduction and congenital disorders formation. Further in-depth analyses on large samples of patients and cells (gametes and blastomeres) are needed to appreciate the contribution of this cataclysmic process to altered human development and the generation of congenital diseases.

INVITED SESSION

SESSION 24: SURGICAL TRAINING: EXPERIENCE FROM EXISTING TRAINING CENTERS FOCUSED ON TEACHING MINIMALLY INVASIVE TECHNIQUES AND POSSIBLE APPLICABILITY OF SIMULATION TRAININGS IN ART CENTERS

Monday 2 July 2018 Room 113 + 114 + 115 17:00–18:00

O-090 The impact of training center activities on the real-life operating room: how to shorten learning curves and to prevent common mistakes

O-091 Robotic surgery vs conventional laparoscopy in the field of infertility

F. Sendag[1]

Ege University, Obstetrics and Gynecology, Bornova - Izmir, Turkey

Abstract text

Robotic surgery vs conventional laparoscopy in the field of infertility.

Sendag, Fatih, M.D., Professor.

Ege University Department of Obstetrics and Gynecology, Bornova, Izmir, Turkey.

Procedures that are technically challenging with the conventional laparoscopy can be performed with robotic assistance. It has advantages of improved visualization and wrist movements allowing precise suturing. This helps to overcome the limitations of laparoscopy, especially in complicated procedures, and may shorten the steep learning curve in minimal invasive surgery.

Robot-assisted laparoscopy seems to be of interest in cases of complex surgical treatment of infertility. Myomectomy, tubal reanastomosis, and deep infiltrating endometriosis appear to be the preferred indications. It has been shown that robotic assistance in reproductive surgery resulted in decreased blood loss, less post-operative pain, shorter hospital stay, and faster convalescence, whereas reproductive outcomes were similar to laparoscopic approaches. The main limitation to the diffusion of this technique remains its cost.

But it will be crucial to have more long-term outcomes regarding fertility: pregnancy rate (natural and IVF), uterine rupture for myomectomy, impact on ovarian reserve, adhesion forming, etc. These results are necessary for showing the real contribution of robot-assisted laparoscopy in the field of infertility.

Randomized controlled trials focusing on long-term outcomes after robotic surgery are needed to determine which patients are likely to benefit from robotic surgery.

SELECTED ORAL COMMUNICATIONS

SESSION 25: CHANGING ETHICS, CHANGING LAWS

Monday 2 July 2018 Room 117 17:00–18:00

O-092 Powering the mitochondrial donation research by donating their powerhouses: Mitochondrial donors for research

M. Choudhary[1], J. Neilson[2], K. Lennox[1], S. Harrop[3], M. Nesbitt[4], L. Hyslop[5], A. Murdoch[6], M. Herbert[7]

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[6]Newcastle Fertility Centre, Biomedicine West Wing, Newcastle upon Tyne, United Kingdom
[7]Wellcome Trust for Mitochondrial Research and Newcastle University, Newcastle Fertiltiy Centre at Life, Newcastle upon Tyne, United Kingdom

Study question: What was the impact of new UK regulations and compensation policy on donor recruitment and egg availability for pre-clinical research in preventing mitochondrial DNA disorders?

Summary answer: Despite the high initial expression of interest, only 5% of those women became mitochondrial donors donating 584 eggs for mitochondrial pre-clinical research.

What is known already: UK became the first country to legalise and permit clinical use of reproductive technologies to prevent transmission of mitochondrial DNA disease following scientific reviews, extensive public consultation, ethical and parliamentary debates. Until 2012, the source of freshly harvested oocytes for use in research in the UK was largely limited to 'egg sharing" schemes in which women undergoing IVF treatment donate half of their oocytes in return for a financial contribution towards the cost of treatment. Following publication of landmark reports from the Nuffield Council on Bioethics and the HFEA, UK implemented a policy permitting reasonable financial compensation to egg donors.

Study design, size, duration: A retrospective analysis of data over 35 month period from the first pilot scheme in UK of mitochondrial donor recruitment for research following implementation of new regulations and compensation policy.

Participants/materials, setting, methods: Ethics and HFEA regulatory approvals were obtained prior to mitochondrial donor recruitment for pre-clinical research aimed at prevention of inheritable maternally derived mitochondrial DNA disorders. The new pilot scheme offered compensation of £500 per donation cycle to mitochondrial donors for research as per approval granted. All women self-injected for ovarian stimulation and underwent oocyte retrieval under sedation. A total of 1290 women expressed interest in donating oocytes for mitochondrial donation research over 35 month period.

Main results and the role of chance: Of 1290 women expressing interest via a webmail contact page, only 223 (33%) attended an initial appointment after receiving further research information. Of these, 135 (61%) met the inclusion criteria for age (18-36 years), BMI (≤30) and ovarian function (antral follicle count >10 and AMH 10-50 pmol/l). Half of the eligible women made an informed decision to not proceed with mitochondrial donation following consultation. Remaining 71 women (mean age 26.5 years ± 4.2) went ahead with ovarian stimulation. Poor response led to 7 cancellations, thus resulting in 64 women successfully donating to research. In all, only 5% (n = 64) of those who initially expressed interest (n = 1290) eventually became mitochondrial donors donating a total of 584 oocytes for research. Following implementation of new regulations and compensation policy, the number of oocytes donated for research has doubled compared to our previous 'egg-sharing for research' scheme.

While the high levels of interest and the increase in oocyte availability for research are encouraging, the high drop-out rate suggests that the financial compensation does not hugely influence decision to donate and the donation procedure itself, rather than the concept of egg donation per se, is a disincentive to the vast majority of women.

Limitations, reasons for caution: As the mitochondrial donor recruitment was for research only, it is not possible to stipulate on the facilitators and barriers for mitochondrial donation for treatment purposes from this study. A qualitative study is indicated to garner insight and explore factors influencing the decision making process for mitochondrial donor recruitment.

Wider implications of the findings: Understanding, and addressing, the barriers to donation for the large fraction of women who initially express interest would help to ensure an adequate supply of oocytes for clinical treatment and for the ongoing underpinning research. Critical to this will be communicating the distinction between conventional egg donation and mitochondrial donation.

Trial registration number: Not applicable.

This study was supported by Wellcome Trust funding (grant number 096919). Thanks to volunteers who participated in the scheme and donated eggs for research.

O-093 The use of animals and human embryos in preclinical safety research: is there an ideal order?

V. Jans[1], W. Dondorp[1], H. Mertes[2], G. Pennings[2], G. De Wert[1]

[1]Maastricht University, Health- Ethics and Society, Maastricht, The Netherlands
[2]Bioethics Institute Ghent, Department of Philosophy and Moral Sciences, Ghent, Belgium

Study question: Should animal studies be preferred to / precede the use of human embryo's in preclinical safety research?

Summary answer: Although regulations suggest that animal studies should be preferred to / precede human embryo research, there are no convincing arguments for this.

What is known already: Legal and regulatory documents suggest that research using human embryos may only be considered when animal studies have either been performed or cannot answer the research question. For instance the British Warnock Report states that human embryos should only be used when research with animal models is not an option. Similarly, ESHRE's Task Force Ethics and Law has suggested an ideal chain of types of preclinical research with animal studies as a first and embryo research as a second step. However, arguments supporting this order are lacking.

Study design, size, duration: A literature study and a focus group interview with key figures in reproductive medicine were performed to identify scientific, legal and/or ethical arguments supporting the 'first animals then human embryos' rule.

Participants/materials, setting, methods: Literature study: articles have been selected from scientific journals focusing on normative aspects of ARTs, animal research and human embryo research. Additionally, relevant legal texts and regulative documents from several countries were studied. Focus group: key figures in reproductive medicine were invited to reflect on the 'first animals then human embryos' rule. Conceptual and ethical analysis: the findings were critically assessed in terms of the consistency of arguments and the normative soundness of conclusions.

Main results and the role of chance: From a scientific perspective, there are no valid arguments supporting the 'first animals then human embryos' rule. In fact, making this a rule stands in the way of doing good research, leading to unnecessary animal studies for questions where it is clear that human embryos are more appropriate research material. From an ethical perspective, welfare and moral status arguments can be distinguished. Welfare arguments seem not to support a moral preference for animal research, apart perhaps in view of concerns about the welfare of oocyte donors. Whereas animals may suffer discomfort and pain, preimplantation embryos are not sentient and would in so far be the preferred research material. Status arguments will not change this: although as 'potential persons' human embryos are thought to have a certain moral status that stands in the way of using them for trivial purposes, this status is relatively low as compared not only to that of human persons but also to that of sentient animals whose interests can be harmed. Therefore, the requirement that human embryos can only be used when the research question cannot be

answered by using animal models should be adjusted and the strict order of preclinical research steps should be loosened.

Limitations, reasons for caution: New developments may affect the reasoning leading to our conclusion. For instance when human embryos may in the future be cultured for research beyond 14 days, then against the background of the widely held view that their moral status increases with further development, this may lead to a different assessment.

Wider implications of the findings: Holding on to the 'first animals then human embryos' rule not only stands in the way of proper science, but is also problematic from an ethical perspective. Sacrificing animals for no good reason is at odds with the acceptance of the 'three Rs' (Replace, Reduce, Refine) in scientific research.

Trial registration number: not applicable.

O-094 National survey on the opinion of French specialists in ART about societal issues to prepare the future revision of the French bioethic law

H. Creux[1], M. Diaz[2], A. Papaxanthos[2], L. Chansel-Debordeaux[3], C. Hocke[2]

[1]Clinique Saint Roch, Reproductive medicine, Montpellier, France
[2]hôpital Pellegrin, Reproductive centre, Bordeaux, France
[3]hôpital Pellegrin, Reproductive laboratory, Bordeaux, France

Study question: National survey on the opinion of French specialists in ART about societal issues to prepare the future revision of the French Bioethics law.

Summary answer: French ART specialists promote social egg freezing, ART in single and homosexual women, genetic screening and financial compensation for donors. Opinions remains divided for surrogacy.

What is known already: The French Bioethics law concerning ART is more restricted than in other countries. Techniques can only be applied in heterosexual couples presenting a documented infertility with a woman under 43 yo. ART treatments are financed by the French Social Security up to 6 IUI and 4 IVF with fresh embryo transfer Donation is anonymous and free, responsible for a lack of donors. Fertility preservation is only allowed for cancer patients or medical reasons. Directed donation, surrogacy, social fertility preservation and genetic screening for maternal age, repeated abortions or implantation failures are forbidden in France.

Study design, size, duration: Descriptive study conducted in June 2017 in a University teaching hospital by using an anonymous questionnary. The questionnary consisted of 15 questions on current issues in ART.

Participants/materials, setting, methods: An anonymous online questionnary was sent by email to 650 French specialists in ART, both clinicians and biologists, with an average response time of 3 minutes.

Main results and the role of chance: After 3 reminders, 408 responses were collected resulting in a participation rate of 62.7%. Participants consisted of 130 men and 271 women; 259 clinicians and 144 biologists. Out of them, 245 were < 50 years old and 159 were ≥ 50 years old.

Concerning the genetic screening, 80% of the physicians were in favour to expand the indications that are presently limited in France to genetic diseases with a particular gravity.

To authorize social fertility preservation was acclaimed by 78.9% of the specialists, but without a social coverage for 69.3% of them and with the possibility to employ unused frozen eggs for the oocyte donation program for 40.3% of them.

Concerning gametes donation, 77.4% of the French specialists in ART were in favor to give a financial compensation to donors, 92% promoted to preserve the anonymity and 80,9% were not in favor to a directed donation.

The ART for single women and for homosexual women were supported by 63.5% and 69.8% of the French specialists, respectively.

The legalisation of surrogacy, all indications confounded (including male homosexuals), was asked by 55.2% of French specialists in ART.

Limitations, reasons for caution: Despite a satisfactory participation rate, some French specialists in ART did not answer the questionnary on line. Moreover, some physicians did not fill in all the questions. That represents a bias of selection.

Wider implications of the findings: This survey provides a support for the French Bioethics comittee to know the opinion of the specialists in ART in their country on the eve of the revised law.

Some situations could be discussed in the French legislative context while others could require the sollicitation of the neighboring European teams.

Trial registration number: Not applicable.

O-095 Outcomes of applications for information about a person related through donor treatment following legislative change removing donor anonymity retrospectively

L. Johnson, K. Bourne, T. Thomson, K. Hammarberg

Victorian Assisted Reproductive Treatment Authority, Victorian Assisted Reproductive Treatment Authority, Melbourne, Australia

Study question: What are the outcomes of retrospective removal of donor anonymity?

Summary answer: Of the donors who were located, most agreed to be contacted by their donor-conceived offspring.

What is known already: In the state of Victoria, Australia, legislation relating to donor conception was introduced in 1988 and has changed over time to increasingly consider the interests of the people born as a result. Since 1998 gamete donors have had to agree to the release of their identifying information if their donor-conceived offspring request it. In March 2017 new legislation retrospectively removed anonymity for pre 1998 donors. Applications for information about related parties are lodged with the Victorian Assisted Reproductive Treatment Authority (VARTA) where donor registries are managed. Donors who are located can stipulate contact preferences, including the preference for no contact.

Study design, size, duration: A prospective study of the outcomes of all applications for information by people related through donor conception from March to December 2017 where donors had consented to the use of their gametes in treatment prior to 1998.

Participants/materials, setting, methods: In the study period VARTA counsellors received 101 applications for information about a related party: 71 by a donor-conceived person and six by a parent of a donor-conceived person for information about the donor, and 24 by a donor for information about their donor offspring. Outcomes of the applications were recorded in a database.

Main results and the role of chance: By 31 January 2018, 65 of the 101 applications for information were still being processed and 36 had a known outcome. Of these 33 were applications for information about a donor (31 from a donor-conceived person and two from a recipient parent) and three were applications from a donor for information about donor-conceived offspring. Of the 33 applications for information about a donor, seven were withdrawn after counselling and two applicants only wanted non-identifying information which did not require the donor to be located. In the remaining 24 applications: 15 donors were located, six were not located or had missing information, and 3 donors were deceased. Of the 15 donors who were located, 11 agreed to be contacted and four lodged a preference for no contact. In the three applications for information about a donor-conceived offspring one agreed to be contacted by the donor.

Limitations, reasons for caution: There is no way of knowing how many donor-conceived people born before 1998 are aware of their donor origins. The findings cannot be generalised to all donor-conceived people or to donors who are not located or for whom no application for information is made.

Wider implications of the findings: Outcomes to date of the retrospective removal of donor anonymity are mixed but indicate that some donors who expected to remain anonymous are willing to be contacted by their donor-conceived offspring if approached.

Trial registration number: Not applicable.

INVITED SESSION

SESSION 26: THE AGEING MAN

Tuesday 3 July 2018 Forum (Auditorium) 08:30–09:30

O-096 Mechanistic basis of ageing effects

J. Aitken[1]

University of Newcastle, Dept. of Biological Sciences, Callaghan- NSW, Australia

Abstract text

Ageing has a significant impact on the reproductive competence of males. When men exceed the age of 35 there is a clear age-dependent impact on the ability of the spermatozoa to initiate a viable pregnancy, resulting in a progressive fall of fertility and a corresponding increase in the incidence of miscarriage. In addition, paternal ageing is associated with genetic changes in the progeny affecting telomere length (telomeres get longer with paternal age) and the incidence of spontaneous genetic and epigenetic mutations (both of which increase linearly with paternal age). All of these age-dependent changes (loss of fertility, increased miscarriage rates, increased mutational/epimutational load carried by the offspring and increased telomere length) can be attributed to two potential underlying causes. Firstly, as males age their reproductive system comes under increasing oxidative stress as reflected by an increase in oxidative damage, particularly oxidative DNA damage in the spermatozoa. Because the precursor germ cells of the testes (particularly spermatogonia) possess significant telomerase activity, they have the capacity to respond to such stress by increasing telomere length before the germ cell differentiates into a spermatozoon. Construction of an animal model for oxidative stress in the male reproductive system via the surgical induction of a transient testicular ischemia, has been found to result in the generation of spermatozoa with extremely long telomeres, which are then passed onto both male and female offspring. At this stage we have no idea what the inheritance of long telomeres from the paternal germ line means in terms of the health trajectory of the offspring. Telomere length aside, oxidative stress would also account for the age-dependent decline in fertility, as a consequence of the induction of lipid peroxidation in the spermatozoa and a consequential loss of sperm function. Antioxidants are clearly important for defending the germ line against oxidative attack since the functional depletion of genes encoding thioredoxin domain-containing proteins (Txndc2, Txndc3) results in accelerated age-dependent disruption of motility and DNA integrity as well as increases in reactive oxygen species generation, enhanced formation of lipid aldehyde-protein adducts, and impaired protamination of the sperm chromatin. The DNA damage seen in the spermatozoa of ageing males could also be responsible for the age-dependent increase in miscarriage rates as well as the increase in mutational load carried by the offspring. The aberrant repair of oxidative DNA damage in the oocyte immediately after fertilization is proposed as a major contributor to the mutational load carried by children, along with spontaneous mutations arising as a result of replication errors in the spermatogonial stem cell population. Together these mechanisms have a major impact on the paternal contribution to offspring health.

O-097 The ageing male germ cell

J. Gromoll[1], **E. Pohl**[2], **K. Redmann**[2], **C. Krallmann**[2], **J.F. Cremers**[2], **K. Czeloth**[3], **S. Kliesch**[2], **M. Zitzmann**[2], **F. Tüttelmann**[4], **B. Horsthemke**[5], **S. Laurentino**[2]

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Abstract text

Parental age has been steadily increasing in Western societies. While in women the effects of age on reproductive function (e.g. decrease in fertility, increase in aneuploidies) are well known, little is known of the effects of ageing on the male germline. On the other hand, it is difficult to distinguish the effects caused by normal ageing from those of associated morbidities. Therefore, the objective of this study was to study molecular parameters of ageing on the germline of a cohort of healthy men.

The study cohort (FAMe – Fertility and Aging in healthy Men) is composed of healthy men (n = 197) between 18 and 84 years of age. All participants went through a thorough selection process which included physical evaluation and andrological assessment and provided sperm and blood samples. DNA was isolated from blood and swim-up (motile) sperm and relative telomere length (rTL) and DNA methylation of *LINE1* transposable elements were analysed. DNA fragmentation index (DFI) was measured by an acridine orange-based flow cytometric assay. In addition WGBS was performed on sperm samples and data validated by deep bisulfite sequencing (DBS).

Relative telomere length in blood decreased with age (ρ=-0,41, $p < 0,001$), while sperm rTL increased ($\rho = 0,39$, $p < 0,001$). DNA methylation of *LINE1* repetitive elements in blood did not show any age-related alterations in this cohort (mean methylation = 79,29 ± 0,14). The mean sperm DFI increased with age ($\rho = 0,49$, $p < 0,001$). WGBS revealed more than 180 age-related DMRs, some of them located in regions associated with genes important for embryonic development, between sperm of young and old men, which were not present in blood DNA. Validation of selected DMRs among all groups of the FAMe study revealed distinct patterns for DMRs with either linearly increasing or decreasing methylation levels.

Telomere attrition is considered a hallmark of ageing and in our cohort we could confirm that in peripheral blood telomeres become shorter with increasing age. In the male germline, however, telomerase has been previously shown to remain active. Our results are in agreement with this, showing sperm rTL increasing with age. Epigenetic drift has been suggested as a consequence of ageing, with some studies showing an overall age-associated decrease of DNA methylation, including in repetitive elements. In this cohort we were unable to detect any changes in *LINE1* DNA methylation with increasing age. Likely *LINE1* methylation is probably affected by co-morbidities associated with ageing but not by the ageing process itself. The effect of ageing on sperm chromatin structure has previously yielded conflicting results, probably due to the heterogeneity of the cohorts analysed. In our cohort, we could detect an increase in the mean DFI and therefore DNA damage with increasing age. The identification of germ cell specific DMRs, which are not present in blood, points to a unique intrinsic ageing process of germ cells. Since it seems highly unlikely that this takes place during spermatogenesis, the testicular stem cells, namely the spermatogonia, seem to be prone to ageing processes.

Taken together healthy ageing affects the DNA integrity of male germ cells and therefore could resemble a potential risk for the progeny of older fathers.

Supported by the DFG (GR1547-19/25)

INVITED SESSION

SESSION 27: IN VITRO POST-IMPLANTATION MODELS

Tuesday 3 July 2018 Room 211 + 212 08:30–09:30

O-098 The use of novel imaging to decipher lineage segregation in the early embryo

N. Plachta

Institute of Molecular and Cell Biology, Singapore, Singapore

O-099 Self-organization of the human embryo in the absence of maternal tissues

INVITED SESSION

SESSION 28: BIG DATA IN ENDOMETRIOSIS – HOPE OR HYPE?

Tuesday 3 July 2018 Room 111 + 112 08:30–09:30

O-100 Big data in understanding disease

O-101 Big data in endometriosis

S. Missmer

Michigan State University, OB/GYN & Reproductive Bio, Grand Rapids, U.S.A.

Abstract text

The excitement and potential surrounding "big data" and it's implications for advancement of precision medicine that will revolutionize care for women and men across the lifecourse is as pertinent to reproductive medicine as it is to other aspects of health and wellbeing. However, there are challenges unique, or at least of greater consequence, to reproductive medicine that must be addressed and for which solutions must be developed. The biologic and sociologic diversity of factors influencing gynecologic and reproductive health is great. This diversity is of benefit to precision medicine advancement if embraced, but this diversity also demands methodologic rigor in approaches to and interpretation of big data discoveries. Reproductive health and wellbeing impacts multiple windows across an individual's life as well as multiple generations within families. Critical elements of multidisciplinary exploration accounting for time-varying variables that assess data validity and reliability will be discussed.

INVITED SESSION

SESSION 29: ASRM EXCHANGE SESSION: MAXIMIZING THE CHANCE OF PREGNANCY FOR EVERY PATIENT: AN INTENTION-TO-TREAT ANALYSIS

Tuesday 3 July 2018 Room 113 + 114 + 115 08:30–09:30

O-102 A rational approach to the management of the older and low response patient undergoing ART

O. Davis

Weill Cornell Medicine, Center for Reproductive Medicine, New York- NY, U.S.A.

Abstract text

This lecture will examine strategies for treating the poor prognosis IVF patient (advanced age, diminished ovarian reserve), while stressing the importance and relevance of an "intention to treat" clinical approach. Ovarian reserve markers and definitions of diminished ovarian reserve will be reviewed, as well as ovarian stimulation strategies for the poor response patient (micro-flare, estrogen priming, minimal stimulation) as well as the evidence supporting the choice of protocol. An intention to treat strategy stresses the maximization of an individual patient's chances for success at the point of entry into treatment, i.e. once presenting for initial consultation. If one defines the denominator for success as the performance of an embryo transfer, it becomes clear that patient attrition can occur at several points before, e.g. through not offering/initiating treatment for the patient with a suboptimal prognosis, cycle cancellation for reduced numbers of developing follicles, prolonging in vitro culture to day 5-6 in the setting of few embryos and/or the performance of PGT-A as standard-operating-procedure. A rationale for transferring a larger number of cleavage stage embryos in women of advanced age (>43) will be presented. The focus of treatment should be on the individual patient's chance for success rather than an IVF clinic's per transfer pregnancy statistics.

O-103 Every last baby out of every last egg: Does PGS really improve the chance of pregnancy for every patient?

R.J. Paulson

University of Southern California, Obstetrics and Gynecology, Los Angeles, U.S.A.

Abstract text

Pre-implantation genetic testing for aneuploidy (PGT-A) is a method of selection of embryos for transfer. It has been shown to increase embryo implantation rates in selected groups of patients. This observation has led some clinics to advise all patients undergoing IVF to include PGT-A as part of their treatment. However, not all groups of patients benefit from this form of embryo selection, and embryo implantation rates are not increased. Furthermore, the testing itself results in the loss of potential implantations that are lost due to false positive test results and due to the trauma of the embryo biopsy. This loss of potential implantations may not be a major problem in women with large numbers of embryos, and those whose embryo implantation rates are already high. However, in women over 40, with low implantation rates and low numbers of embryos, the addition of PGT-A will result in decrease cumulative pregnancy rates on a per-aspiration basis. This lecture will review the approximate magnitude of both cost and benefit of PGT-A and show why women with poor prognosis should not use PGT-A to try and increase embryo implantation rates.

INVITED SESSION

SESSION 30: WHO SESSION: WHO AND FERTILITY CARE: TIME TO ENGAGE?

Tuesday 3 July 2018 Room 117 08:30–09:30

O-104 Introduction: HRP vision and goals regarding infertility

O-105 WHO priorities in infertility and fertility care

T. Matsaseng

WHO Headquarters, RHR, Geneva, Switzerland

Abstract text

Infertility has not been recognized as a public health priority with debates as to whether it's a disease or not. According to the WHO/World Bank report on Disability, infertility following maternal sepsis and unsafe abortion (a critical maternal morbidity) was ranked 8th highest in prevalence as a global serious disability and in the population group under the age of 60, infertility ranked 5th highest as a major global disability behind depression, ocular refractive errors, unintended accidents and alcoholism.

The commonest causes of infertility in the LMIC are, severe male infertility and bilateral tubal disease, that can only be treated by assisted reproductive technologies (ART) and yet these techniques are not widely available and often not affordable. Severe stigmatization, fear, shame and social complications surround vulnerable individuals and couples who are viewed as childless or unable to have children in many developing and transitional countries. Studies have shown that to have a healthy child, couples may accept a 20% risk of death and give up 29% of their income.

Barriers towards treatment access include, lack of infrastructure and/or high unaffordable costs. Where available the treatment is often associated with unsafe practices that results in undesirable complications including ovarian hyperstimulation syndrome (OHSS) and high order multiple pregnancies (HOMP) with inherent poor neonatal outcomes. Furthermore, ethical and

moral issues around third party reproduction (gamete/embryo donation & surrogacy), reproductive tourism and same sex couples (LGBT) have not received adequate attention they deserve.

As we implement strategies to achieve Sustainable Development Goals (SDG no. 3, 5) and the Global Strategy for Women's, Children's and Adolescents 'Health (2016–2030; Survive, Thrive & Transform) agenda, we strongly advocate for inclusion of fertility services within the Reproductive, Maternal, Newborn, Child and Adolescent health, RMNCAH plans in order to achieve gender equality and empower all women and girls. We also wish to promote universal access to Sexual Reproductive Health and Reproductive Rights (SRHR), to ensure that no one is left behind.

Therefore, the aim of the presentation is to highlight WHO engagement in infertility and fertility care and to outline key areas of priority in our agenda as we endeavour to improve access and availability of safe and effective fertility care equally across the globe.

O-106 Estimation of prevalence of infertility from cross-section surveys (DHS) using time-to-pregnancy approach: preliminary results of a pilot study

T. Matsaseng

WHO Headquarters, RHR, Geneva, Switzerland

Abstract text

Infertility is defined as a disease of the reproductive system that results in an inability to become pregnant or bring a pregnancy to term. Primary infertility is predominantly a result of delayed childbearing, complications of childbearing and/or congenital disorders. Secondary infertility is often a result of maternal sepsis (infection following delivery) or of complications associated with unsafe abortions. Both forms of infertility, primary and secondary can result from complications following sexually transmitted infections (STIs), genital tuberculosis (TB) and HIV/AIDS, or following cancer or other diseases and subsequent disease management that proves contrary to either healthy or successful sperm or egg development.

A WHO evaluation of Demographic and Health Surveys (DHS) data from 2004, estimates that more than 186 million ever-married women of reproductive age in developing countries were maintaining an unfulfilled "child wish", translating into one in every four couples between the of age 15–49. A recent analysis by WHO of internationally published references shows that primary infertility rates are not decreasing (and in some countries are increasing)and secondary infertility continues on an increasing global trend from 1990 to 2008 (unpublished, WHO, 2011). Severe stigmatization, fear, shame and social complications surround vulnerable individuals and couples who are viewed as childless or unable to have children in many developing and transitional countries.

Besides lack of infrastructure and/or high unaffordable costs, limitation to access to care is further complicated by underestimation of the disease magnitude particularly in the low middle income countries (LMIC) and to address this issue, obtaining accurate, country-level estimates of infertility prevalence is essential. Demographic approaches and clinical and epidemiological studies do provide more relevant measure to assess the need for services to enhance fertility. However population based methods for shorter durations are limited and estimations approaches vary. While Time-to-pregnancy (TTP) and current duration (CD) approaches have advantages and disadvantages, their role and application still need to be explored in various settings for accurate estimation of disease prevalence.

Therefore, the aim of the presentation is to evaluate and report preliminary results of the pilot study on estimation of infertility prevalence using current duration approach to estimate TTP to data from Demographic and Health Surveys (DHS).

O-107 The landscape of IVF in lower and middle income countries

T. Chiware[1,2], N. Vermeulen[3], K. Blondeel[2,4], R. Farquharson[3], J. Kiarie[2], K. Lundin[3,5], T. Matsaseng[2], W. Ombelet[3], I. Toskin[2]

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Study question: What are currently available and potential in vitro fertilization (IVF) and other assisted reproductive technologies (ART), and services in low- and middle-income countries (LMIC)?

Summary answer: The ART services available in LMIC vary from conventional ART to low cost ART, depending on the region.

What is known already: Infertility affects 186 million people, 8-12% of couples worldwide, with a prevalence estimated at 3.5-16.7% in LMIC, and 30-40% in Sub-Saharan Africa. High costs of IVF and lack of ART services, in addition to cultural, religious, legal, efficacy and safety concerns, have highlighted the need for affordable, accessible and acceptable ART in LMIC. Infertility and childlessness, particularly in LMIC, have devastating consequences, resulting in considerable interest in developing low cost IVF procedures. However, there is a paucity of evidence on the safety, efficiency and ability to replicate techniques, under different field conditions, and integrate low cost ART into existing infrastructures.

Study design, size, duration: A systematic review was performed by searching several databases for articles on low cost ART in LMIC published from January 2010 to June 2017. Two reviewers independently screened the titles and abstracts, then full texts of selected records were reviewed and data extracted.

Participants/materials, setting, methods: The electronic databases searched included PubMed, Popline, EMBASE and Global Index Medicus (regional WHO online databases). An internet search using 'Google" search engine with the terms 'infertility", 'low and middle income countries" and "in vitro fertilization" or 'assisted reproductive technologies" (limited to results published after 2010) was performed. Similar search terms were used for grey literature databases (worldcat.org, greylit.org). In addition, the reference lists of included studies were checked.

Main results and the role of chance: An extensive search of the proposed databases yielded 3443 citations. After review of the titles and abstracts, 180 studies were included. The full text of 180 articles were reviewed and a further 106 articles were excluded. The grey literature search yielded 419 citations of which 334 were excluded after screening of the title, and a further 85 documents after full text assessment due to duplicate entries, not LMIC, not relevant, or no access to the full document. A final total of 74 citations were included as part of the systematic review and separated into regions. The majority of the studies were qualitative and observation studies. In general, ART services are available and described in several LMIC, ranging from advanced ART in China, to introduction of IUI and ART in some African countries. Efforts for low cost ART treatments have been described in feasibility studies, or efficacy studies, most of low to very low quality or providing indirect evidence from developed countries. We found no studies from LMIC reporting the implementation of low cost ART that is efficient, accessible and affordable to the whole population.

Limitations, reasons for caution: To our knowledge, this is the first systematic review of ART in LMIC. Studies published prior to 2010 were excluded. The available studies were heterogeneous making it difficult to extract findings.

Wider implications of the findings: The WHO is in a unique position to provide much needed guidance for infertility management in LMIC. This systematic review provides insight into the landscape of ART in LMIC in various regions. This will guide efforts to improve availability, quality, accessibility and acceptability of infertility care, including ART in LMIC.

Trial registration number: The protocol developed and registered with PROSPERO International prospective register of systematic reviews, number CRD42017064413.

O-108 Developing ART practice in low and middle income countries

O-109 Closing remarks

INVITED SESSION
SESSION 31: NURSES/MIDWIVES INVITED SESSION
Tuesday 3 July 2018 Room 116 08:30–09:30

O-110 Use of ultrasound at embryo transfer - is it necessary?

O-111 Family planning, knowledge about fertility and lifestyle prior to conception; nurses and midwives as key persons in preconception care

T. Tyden[1], L. Salih[2], J. Hultstrand[2], M. Ekstrand[2]

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Abstract text

Preconception care and family planning are identified to be of increasing importance for health in a wider sense as recently highlighted in a series of articles in the Lancet. It is relevant for all women of reproductive age. Slightly half of all pregnancies worldwide are unplanned and of these, 50% ends in abortion, 13% in miscarriage, and 38% in unplanned birth.

In low-income countries many women would benefit from delaying and spacing pregnancies. In high-income countries it is the opposite situation as many women postpone childbearing until ages when their fecundity has decreased. These examples highlight the need for preconception care that also should be tailored for each individual. Furthermore, the most critical period for organ development occurs before many women even know they are pregnant and the first contact with antenatal care is often too late for advice about health-promoting changes in life style.

Lifestyle-related factors such as age, obesity and smoking affect fertility and reproductive outcomes. Studies have shown that people underestimate the importance of age on fertility and overestimate the success rate of in vitro fertilization. Smokers have lower odds for clinical pregnancy per cycle, live birth per cycle, and higher odds for miscarriage, ectopic pregnancy, and babies being small for gestational age and for preterm delivery.Obesity is an increasing health problem globally that also impact reproductive health and reproductive outcomes. These examples emphasize the need for educating women and men about lifestyles that are of importance for their reproduction.

A tool called the Reproductive Life Plan (RLP) is recommended by the Centers for Disease Control and Prevention. It consists of a set of questions that can be used by health care providers to guide a conversation about if and when a person wants to become a parent and how to achieve their reproductive goals. The aim with the RLP is to encourage women and men to reflect on their reproductive intentions, to promote a healthy lifestyle prior to conception and to use appropriate contraceptive methods in line with their RLP. Also the RLP aims to give information on lifestyle prior to conception.

In Sweden, midwives working with contraceptive counseling used the RLP-concept in two randomized controlled studies. The midwives asked questions about the RLP with the women who were allotted to the intervention group (IG). They also handed out a specially designed brochure to women in the IG. The brochure contained information about fertility and life styles of importance prior to a pregnancy. Women in the IG increased their knowledge about fertility and the great majority stated that midwives should continue to discuss the RLP in contraceptive counseling. The results and have been reported in two articles in Human Reproduction. Recently we have used the RLP concept among men attending STI- and sexual health clinics.

I will present lessons learned from our experiences with using the RLP in clinical practice. I will also suggest how nurses and midwives can be important persons in preconception care.

For young women and men Internet as an important source of information. We have therefore developed a mobile friendly website about the RLP www.reproduktivlivsplan.selt has been translated into English and also into some other commonly spoken languages in Sweden.

SELECTED ORAL COMMUNICATIONS
SESSION 32: UPDATES IN EMBRYO MORPHOKINETICS
Tuesday 3 July 2018 Forum (Auditorium) 10:00–11:30

O-112 The effect of oocyte vitrification on subsequent embryo developmental morphokinetics and blastocyst formation: a prospective time-lapse sibling oocyte study

S. De Gheselle, V. Muyshond, K. Tilleman, P. De Sutter

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Study question: Is there a difference in blastocyst formation between fresh and vitrified warmed sibling oocytes and can this difference be attributed to changes in embryo morphokinetics?

Summary answer: There is a delay in morphokinetic embryo development and blastocyst formation between fresh and vitrified sibling oocytes.

What is known already: Oocyte vitrification is often used in medical assisted reproductive treatments e.g. oocyte-donation programs, fertility-preservation for cancer patients, social-freezing but also in IVF cycles where semen production problems occur. The impact of oocyte vitrification on embryo development remains controversial. Morphokinetic analyses on embryo development from vitrified oocytes are usually based on early morphokinetic parameters (De Munck et al., 2015; Chamayou et al.,2015). A recent report by Cobo et al., 2017, showed delayed morphokinetics from vitrified oocytes up to the blastocyst stage. Although this report included a lot of cycles, the study was not performed on sibling oocytes.

Study design, size, duration: In a prospective study between February 2016 and December 2017, morphokinetic development and blastocyst formation was investigated between fresh (FO) and vitrified sibling donor oocytes (VITO). Blastocyts were scored according to the grading system of Gardner and Schoolcraft(1999) and their developmental morphokinetics were analyzed using time-lapse imaging (EmbryoscopeTM). On the day of oocyte retrieval, and after cumulus-oocyte complex denudation, MII oocytes with normal morphology were randomly allocated to fresh ICSI insemination or to vitrification (Cryotop®).

Participants/materials, setting, methods: MII oocytes (n = 438) from 67 donor cycles (36 fresh / 31 warmed) from 27 different healthy anonymous oocyte donors (age 30.3 ± 3.7) were allocated both to a synchronous FO acceptor (n = 215) and to a VITO acceptor (n = 223). Morphokinetic timings were log-transformed and linear-regression analysis (mixed models) was applied (SPSSv24). Fixed effects were determined by comparing FO and VITO. The variable 'donor' was taken into account for random effects ($p < 0.05$).

Main results and the role of chance: Time-lapse analysis of the 16 different time-points and 6 time-intervals showed that there was an overall delay in the cleavage rate from the time of pronuclei disappearance up to the time of blastulation in the VITO compared to their sibling FO. Twelve morphokinetic parameters were statistically significant between the VITO and FO respectively: tPNf [Geometric mean(GM) 26.18 h versus GM 23.87 h; p = 0.000]; t2 [GM 28.51 h versus GM 26.67 h; p = 0.004]; t3 [GM 38.81 h versus GM 36.05 h; p = 0.004]; t4 [GM 42.36 h versus GM 39.17 h; p = 0.001]; t5 [GM 51.52 h versus GM 47.09 h; p = 0.000]; t6 [GM 56.36 h versus GM 51.28 h; p = 0.000]; t7 [GM 60.12 h versus GM 54.20 h; p = 0.003]; t8 [GM 64.57 h versus GM 57.28 h; p = 0.000]; t9+ [GM 76.73 h versus GM 68.39 h; p = 0.000]; tstart compaction [GM 90.57 h versus GM 86.50 h; p = 0.002}; tcompaction [GM 102.09 h versus GM 96.38 h; p = 0.000]; tstart blastulation [GM 100.92 h versus GM 97.95 h; p = 0.013]. These morphokinetic differences resulted in a similar overall

blastocyst formation (expansion status 1-5) in the FO group compared to the VITO group [51.8% (80/155) versus 41.7% (60/144), P = 0.1043], however, there was a statistically higher full (expansion status 3-5) blastocyst rate in the FO group [46.4% (72/155) versus 31.9% (46/144), P = 0.0128].

Limitations, reasons for caution: Sperm parameters were not included in this prospective sibling oocyte study and could have an effect on embryo development.

Wider implications of the findings: Oocyte vitrification has an impact on morphokinetics parameters and embryo development up to the blastocyst stage. This could be explained by effects of vitrification on ultrastructure or biological processes in the oocytes. Diminished mitochondrial activity could also be responsible for a delay in reactivating the cell division machinery after vitrification.

Trial registration number: EC/2017/0086

O-113 Duration of blastomere movement post first cytokinesis is an effective predictor for the clinical pregnancy after fresh cleaved embryo transfer: in a minimal stimulation program

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Study question: Does the duration of blastomere movement post first cytokinesis correlate with clinical pregnancy after fresh cleavage stage embryo transfer in human?

Summary answer: The clinical pregnancy rate after fresh cleaved embryo transfer was significantly lower when the duration of blastomere movement was persisted.

What is known already: Time-lapse incubator systems allow the evaluation of embryos based on their morphokinetic and morphological assessment. Many studies have shown that timings of human embryo divisions correlate with preimplantation development and subsequent pregnancy outcomes after embryo transfer. During monitoring of embryos in time-lapse system, we observed that blastomere movement occurred after first cytokinesis and that there is a variability among embryos in the duration of the blastomere movement. However, the embryo evaluation based on morphological information obtained from time-lapse imaging is still limited. In addition, it is not known how blastomere movement influences embryonic development and pregnancy outcomes.

Study design, size, duration: A total of 781 embryos from 779 patients, 36.4 ± 0.1 year-old, were cultured in EmbryoScope+® time-lapse system and then transferred into patients at cleavage stage from April to July 2017. The data of morphokinetics and morphology were retrospectively analyzed. Blastomere movement (BMov) was observed after first cytokinesis and the time to end the blastomere movement (tBMovE) was annotated. The correlation between the duration of blastomere movement and clinical pregnancy after fresh cleaved embryo transfer was examined.

Participants/materials, setting, methods: Oocytes were retrieved and inseminated either by conventional in vitro fertilization or intracytoplasmic sperm injection. Fertilized oocytes were cultured in EmbryoScope® and the time from insemination to pronuclear (PN) fading (tPNf), 4-cell stage (t4) and tBMovE was annotated. The duration from first cytokinesis to tBMovE was calculated (dBMovE). The embryo quality was evaluated by Veeck's criteria on Day 2.

Main results and the role of chance: The average dBMovE was 6.56 ± 0.36 h [95% confidential interval (CI): 5.86 – 7.26]. dBMovE was significantly correlated with the developmental rate to 4-cell stage on Day 2 (Odds ratio (OR): 0.937 [95% CI: 0.911–0.965], p < 0.01). dBMovE was also associated with the duration between tPNf and t4 (P < 0.01). Furthermore, dBMovE was statically correlated to the morphological grade evaluated by Veeck's criteria (Grade 1: 2.90 ± 0.79; 2: 5.69 ± 0.69; 3: 8.11 ± 0.47; 4: 18.08 ± 3.98, p < 0.01). Logistic regression analysis demonstrated that clinical pregnancy was correlated with dBMovE (OR: 0.966 [95% CI: 0.943-0.986], P < 0.01). Embryos were stratified into 4 groups according to dBMovE (group A: dBMovE < 1.98, B: 1.98 ≤ dBMovE < 3.64, C: 3.64 ≤ dBMovE < 4.59, D: 4.59 ≤ dBMovE). The clinical pregnancy rates after fresh cleaved embryo transfer were 41.0% (50/122), 34.7% (96/277), 27.9% (41/147) and 15.7% (37/235), respectively. There was a significant trend that the clinical pregnancy rates declined with increasing dBMovE (Cochran-Armitage trend test, P < 0.01).

Limitations, reasons for caution: This study is limited by the small sample size, therefore, large scale studies are required. Furthermore, the effect of blastomere movement on the potential of embryos to develop to the blastocyst stage and subsequent pregnancy outcomes after blastocyst transfer should be investigated.

Wider implications of the findings: This is the first report demonstrating the correlation between blastomere movement and pregnancy outcomes. Embryos with extended blastomere movement after the first cytokinesis have a lower competence to initiate a clinical pregnancy after fresh cleaved embryo transfer. Thus blastomere movement could be an effective predictive parameter for selecting embryos.

Trial registration number: None.

O-114 Time Lapse assessment of the occurrence and clinical outcome of direct cleavage in a population of 10,529 embryos cultured to the blastocyst stage

S. Montgomery[1], C. Nelson[2], N. Davis[3], A. Campbell[1], B. Peterson[4], C. Harlow[2], C. Howles[2]

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Study question: Is the occurrence of direct cleavage (DC) in early stage cleavage embryos associated with a decreased chance of clinical pregnancy?

Summary answer: DC in early cleavage stage embryos is associated with a highly significant decreased chance of clinical pregnancy, particularly at the 2 cell stage.

What is known already: DC has been reported to occur at the 2 cell stage in around 14% of embryos. DC is a generic term for early irregular cell divisions which encompasses both an immediate mulitchotomous mitosis (MM) (a cell cleaves into more than 2 daughter cells), and the relatively rapid division of a cell within a short cell cycle (SCC) (</ = 5 hours). For example, with MM, a cell would divide directly from 1 to 3-cells or more, rather than to 2cells; or spend <5 hours before the next mitotic division (SCC). DC can be identified during cleavage stage embryo development.

Study design, size, duration: Anonymised time lapse data was retrospectively reviewed from two sister clinics between August 2014 and January 2017. 10,529 embryos from 1651 consecutive patients using autologous oocytes, were included. Embryos exhibiting a DC (at the 2, 4 or 8 cell cleavage stages) were analysed for ability to reach blastocyst, and their KID (known implantation data) rate was assessed according to maternal age, insemination method (IVF or ICSI), and stage of embryo transfer (cleavage or blastocyst stage).

Participants/materials, setting, methods: Female age ranged from 19 to 46 years. Embryo development parameters were annotated according to strictly defined guidelines using the EmbryoScope® incubator and software (Vitrolife). The population of embryos not displaying DC at 2, 4 and 8-cell cleavage stages provided the control (non-DC). All data was analysed using Chi-square test, Fisher's Exact test and Kruskal-Wallis test with post-hoc testing – Dunn's multiple comparison test to determine statistical significance.

Main results and the role of chance: Overall, embryos exhibiting DC during cleavage stages had a lower incidence of reaching a blastocyst (56%), compared with non-DC embryos which underwent regular divisions (77%). Embryos displaying DC at the 8-cell stage (DC8) had a higher rate (68%) of reaching blastocyst than DC4 (52%) and DC2 (43%) embryos. Overall KID rate of DC embryos (19.4%) was significantly lower than non-DC embryos (38.4%) (p < 0.0001). DC at the 2-cell stage had the most severe impact with the lowest KID rate (7.8%) compared with 15.9% at DC-4 cell and 35.5% at DC-8.

Women of ≥40 years experienced higher incidences of embryos having DC, with the lowest KID rate (2.8%). No statistical difference was found between IVF (n = 431) and ICSI (n = 1303) KID rates, in both non-DC and DC cases. KID rate was significantly lower ($p < 0.0001$) in DC embryos (7.7%), compared with non-DC (24.6%), for day 2 to 4 transfers. For DC8 embryos, KID rate was 36% vs 50% in non-DC8 embryos (not significant) when transferred at the blastocyst stage.

Limitations, reasons for caution: Live birth information was not fully available for all treatment cycles. The study did not consider all patient factors, which may influence cleavage patterns and embryo viability. The study did not distinguish fully between multichotomous mitosis and short cell cycle.

Wider implications of the findings: DC at 2 cell stage is common and had the most severe impact on embryo viability. The adverse effects of DC highlight the need to select against the transfer of such embryos. Future studies may involve tests for centriole function in contribution to this event, and embryo ploidy assessment.

Trial registration number: Not applicable.

O-115 Is there a relation between embryo early cleavage abnormalities and blastulation or implantation rates? A retrospective time-lapse imaging study

R. Naam Ouarezki[1], X. Ferraretto[1], M. Vigan[2], J. Lousqui[1], M.A. Llabador de Royer[1], M. Peigné[3], S. Tubiana[2], S. Epelboin[3], C. Patrat[1]

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Study question: What is the prevalence of atypical dynamic embryo phenotypes identified by time-lapse system and are they associated with poor embryo development and implantation rates?

Summary answer: As abnormal early cleavages are associated with low blastulation and implantation rates, time-lapse and morphokinetic parameters may offer a non-invasive mean for improving embryo selection.

What is known already: Several publications propose morphokinetics as an additional parameter to assist in embryo selection. Most of them demonstrate that embryos exhibiting atypical dynamic phenotypes are highly prevalent in human embryos, and abnormal early cleavages, including Direct cleavage (DC), Rapid cleavage (RaC) or Reverse cleavage (ReC), significantly compromise embryo development. Blastocyst formation rates for these embryos are significantly lower compared with control groups, culminating in poor implantation potential (Rubio and al., 2012; Liu and al., 2014; Wirka and al., 2014; Fan and al., 2016). However, these observations rely on small samples or are not fully explored, especially for ReC.

Study design, size, duration: This research project is a single-center, retrospective cohort study conducted by the Reproductive Department at Bichat hospital (APHP, Paris). We collected the data on all *in vitro fertilization* treatments cycles for which the embryos were observed in the Embryoscope® time-lapse system (Vitrolife Paris, France) from December 2015 to July 2017. The study included 330 consecutive treatment cycles for 256 couples and a total of 1495 embryos. Embryo videos were retrospectively analyzed for dynamic phenotypes.

Participants/materials, setting, methods: Only diploid zygotes were included. Embryo development was evaluated on day 2 (based on cells number, symmetry and percentage of fragmentation) and on day 5-6 (blastocyst formation (Gardner et al., 2000). The data included implantation, issue and clinical factors whether it is factors intrinsic to patients or factors specific to IVF treatment such as: couples' parameters (age, BMI), origin of infertility, type of ovarian stimulation regimen used and insemination method (IVF/ICSI).

Main results and the role of chance: A high prevalence of abnormal early cleavage was observed among embryos: DC 23.5% (349/1486), RaC 25.9% (388/1495) and ReC 7.4% (109/1480). A significant lower percentage of embryos with early atypical dynamic phenotype(s) 5.3% (30/566) had good quality on day 2 (4 or 5 cells, typical character and <30% of fragmentation) as compared to embryos without early cleavage anomalies (42.2% (395/936, $p < 0.001$)). The same observation hold true on good quality on day 5 (≥B3 and ≥BB) (13.8% (65/470) vs 40.6% (197/485) with or without early cleavage defects, respectively <0.001). This significant difference was also observed at day 2 according to the type of anomalies: DC 6.3% (22/349) vs 35.4% (403/1138), RaC 2.6% (10/388) vs 38.3% (415/1083), ReC 9.2% (10/109) vs 30.3% (415/1371), and on day 5: DC 8.2% (24/292) vs 37.1% (240/647), RaC 14.3% (48/336) vs 37.1% (220/593), ReC 9.3% (9/97) vs 30.3% (253/834). Of the 521 embryos that were transferred on day 2 or 5, 104 (19.9%) were identified as abnormal cleavage. Only 14 over 104 affected embryos were known to implant (13.5%) when the implantation rate for embryos with a normal cleavage pattern was 20.1% (84/417) and $p = 0.116$.

Limitations, reasons for caution: The present study is limited by a small sample size of embryos known implantation. Furthermore, we do not know the exact cause of these anomalies. At last, we can question their high prevalence and their impact on the chances of implantation so that many of these embryos can be eliminated?

Wider implications of the findings: The identification of atypical dynamic embryo phenotypes could further refine the process of embryo selection by enabling the deselection of embryos affected, in order to select and transfer the embryo that offers the best chance of pregnancy.

Trial registration number: not applicable.

O-116 Partial embryo compaction: what's behind? Morphokinetic origins and chromosomal status

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Study question: In preimplantation development, how much is partial compaction widespread, does it occur through different morphokinetics mechanisms and is it associated with embryo aneuploidy?

Summary answer: Partial compaction is frequent, is generated by two mechanisms, blastomere exclusion and extrusion, and is associated with embryo characteristics such as maternal age and aneuploidy.

What is known already: Compaction is a well-known developmental stage of the preimplantation embryo, during which blastomeres change dramatically cell shape and establish mutual and extensive cell-to-cell contacts. Overlapping temporally with the specification of the first two embryonic cell lineages and creating a selective barrier between intra- and extra-embryonic environment, compaction is believed to be crucial for further development. Regardless, this stage remains rather obscure and usually is not taken into consideration to assess embryo quality. The Alpha/ESHRE Istanbul consensus on embryo assessment barely mentions that compaction can occur completely or partially, but implications of these two conditions are rather uncertain.

Study design, size, duration: This is a retrospective cohort study using data from 136 PGT-A cycles carried out between May 2013 and July 2017 and whose embryos were cultured in a time-lapse system. Embryos were annotated for morphokinetic characteristics of compaction and analysed at the blastocyst stage for chromosomal constitution. Treatment cycles were divided and analysed comparatively in two age groups, i.e. ≤34 and 35-39 years.

Participants/materials, setting, methods: Three morphokinetic categories of compaction were pre-defined and annotated: i) full compaction, in cases where at the expected time interval all blastomeres appeared with indistinct boundaries and tightly connected; partial compaction in which blastomeres that failed to compact were either ii) excluded from the outset or iii) extruded following an initial involvement in the compaction process. Chromosome analysis was carried out by Array-CGH to determine embryo ploidy status, while statistical analysis with chi-square test.

Main results and the role of chance: Overall, 322 blastocysts were assessed morphokinetically and chromosomally. The total rate of partial compaction was 60.1%. The difference in the rates of partially compacted embryos with excluded or extruded blastomeres was different in the younger age group (68.3% and 31.7%, respectively; $P = 0.00004$), while it was almost identical (50.4% and 49.6%, respectively) in the older age group. Blastomere exclusion was statistically more prevalent in embryos of younger women (68.3% vs.

50.4%, P = 0.02), whereas blastomeres were more frequently extruded in embryos of women aged ≥35 years (31.7% vs. 49.6%, P = 0.02). The overall rate of aneuploidy in blastocysts developed from totally or partially compacted embryos was predictably different in the younger and older age groups (37.8% and 49.7%, respectively; P = 0,037). In both age groups, no differences were observed in the rate of aneuploidy in embryos with excluded or extruded blastomeres. However, even if not statistically significant, partially compacted embryos tend to be less aneuploids compared to totally compacted embryos in younger age group (33,3% vs. 42,9%), whereas this trend was not observed in embryos of older women.

Limitations, reasons for caution: This study is retrospective and based on a data set of limited size. Results should be extended with larger and prospective observations.

Wider implications of the findings: This study sheds novel light on a neglected phase of human preimplantation development. In particular, it indicates that different morphokinetic mechanisms can underlie abnormal compaction. Also, it suggests possible mechanisms of self-correction of aneuploidy that may be compromised in embryos of older women displaying partial compaction.

Trial registration number: Not applicable.

O-117 Time-lapse and NGS evaluation of human embryos cultured in single medium versus sequential media: a prospective randomized pilot study

Z. Yang[1], Y. Kuang[2], X. Zhang[3], Y. Meng[3], L. Tang[4], Q. Lyu[2], L. Su[5], S. Zhang[6], J. Lin[1]

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Study question: Are there differences in morphokinetics, ploidy and implantation potential of human embryos cultured in single medium versus sequential media?

Summary answer: There were no significant differences in key morphokinetic parameters, percentage of euploid blastocysts and overall clinical outcomes between single medium and sequential media.

What is known already: Clinical studies with time-lapse culture concluded that the most predictive morphokinetic parameters (e.g. t5, cc2 and s2) were highly correlated with implantation potential. Various time-lapse studies were performed to compare morphokinetics of embryos cultured in single medium verses sequential media with controversial results. Nevertheless, ploidy of the cultured embryos was not determined in previous studies. There is very limited information about effects of different culture media on ploidy of human embryos as related to implantation potential. Thus, the objective of this study was to evaluate the morphokinetics, ploidy and clinical outcomes of embryos cultured in single medium versus sequential medium.

Study design, size, duration: With an IRB approval, IVF patients (n = 93) at mean age 32.1 ± 3.4 years seeking single embryo transfer (SET) were enrolled in this prospective randomized pilot study in our IVF clinics from June 2016 to June 2017. Sibling MII oocytes (n = 1205) were randomized into two groups after ICSI: 1) Group A: Embryos (n = 603) were cultured in a single-step culture medium (SSC) and 2) Group B: Embryos (n = 602) were cultured in sequential media (G_1/G_2, Vitrolife).

Participants/materials, setting, methods: For both groups, embryos were cultured to blastocyst stage in a time-lapse system (EmbryoScope, VitroLife). Trophectoderm biopsy was performed on day 5 and the blastocysts were vitrified after biopsy. Whole genomic amplification (WGA) and NGS analysis were performed according to manufacturer's instructions (Yikon Genomics, China; Illumina, USA). Single euploid blastocysts with the most predictive morphokenetic parameters available were transferred to individual patients in FET cycles.The statistical analyses were performed using GraphPad InStat (GraphPad Software, USA).

Main results and the role of chance: Time-lapse evaluation showed that there were no significant differences in key morphokinetic parameters between the single medium and sequential media ($p > 0.05$). The time from insemination to initiation of blastulation (tlB) was slightly faster in Group A compared to Group B (96.3 ± 6.5 hpi vs. 97.1 ± 6.7 hpi, respectively, $p > 0.05$). There was no significant difference in fertilization rate between Group A and Group B (84.9% vs. 85.8%, respectively, $p > 0.05$). There was no significant difference in blastocyst rate between Group A and Group B (48.4% vs. 43.1%, respectively, $p > 0.05$). The percentage of biopsied blastocysts in Group A was similar to that of Group B (93.5% vs. 94.6%, respectively, $p > 0.05$). NGS analysis revealed that there were no significant differences in euploid blastocyst rates (d5) between Group A and Group B (43.5% vs. 42.2% respectively, $p > 0.05$). There was no significant difference in clinical pregnancy rate between Group A and Group B (71.1% vs. 70.7% respectively, $p > 0.05$). The observed ongoing pregnancy rate was comparable between Group A and Group B (68.9% vs. 65.8%, respectively, $p > 0.05$). There was no significant difference in miscarriage rate between Group A and Group B (2.2% vs. 4.9%, respectively, $p > 0.05$).

Limitations, reasons for caution: Data are based on observations with embryos from young patients with a good prognosis and may not fully generalize to all patients, especially those at advanced maternal age and with diminished ovarian reserve. Further randomized clinical trials with a larger number of patients are planed to verify these initial findings.

Wider implications of the findings: Our data demonstrate that the single culture medium supports human embryo development from zygote to blastocyst stage and yields similar clinical and ongoing pregnancy rates compared to sequential media. By minimizing exposure of embryos to the ambient atmosphere, non-disrupted culture with the single medium offers additional advantages over sequential media.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 33: INTRAUTERINE INSEMINATION AND FLUSHING

Tuesday 3 July 2018 Room 211 + 212 10:00–11:30

O-118 The effect of intra-uterine slow-release insemination (SRI) on the pregnancy rate in women designated for standard intra-uterine insemination (IUI) – A multicentre randomized, controlled trial

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Study question: To investigate the effect of intra-uterine slow-release insemination (SRI) on pregnancy rates in women with confirmed sub-/infertility who are eligible for standard intra-uterine insemination (IUI).

Summary answer: In women under 35 years of age the pregnancy rate is improved by SRI compared to standard bolus IUI.

What is known already: Only a few studies have dealt with the actual IUI technique, or have questioned the application method. A modified IUI application technique is slow release insemination (SRI), which was first described in 1992 (Muharib et al, 1992). The authors hypothesized that a persistent low concentration of spermatozoa might prolong the period of potential fertilization and thereby mimic physiological sperm transportation into the fallopian tube. The present authors have recently published data from two pilot randomized, controlled cross-over studies that indicate a statistically significant advantage of SRI over conventional bolus IUI.

Study design, size, duration: Multicentre, randomized, controlled cross-over trial: 82 women were randomized into two treatment arms between 2012

and 2017. A computerised randomisation programme was used to assign women to standard IUI tor SRI. Women who failed to conceive in this first treatment were offered the alternative method in the second course. Sample size was calculated using a minimum clinically significant effect defined as a relative risk of 2.0 in favour of SRI.

Participants/materials, setting, methods: Inclusion criteria: primary or secondary infertility after six months of unprotected sexual intercourse; age 20–40 years; tubal patency; infertility due to anovulation and/or endometriosis and/or male partner with a minimum of >10 million of motile sperm cells per sample and/or unexplained infertility. Women underwent either the standard bolus IUI treatment or the SRI method. For SRI an EVIE device was used for a four-hour continuous slow release injection of spermatozoa into the uterus.

Main results and the role of chance: The overall observed pregnancy rate for SRI was 13.2%, compared to 10.0% for IUI (relative risk [RR] = 1.32); however, this difference was not statistically significant (95% CI 0.69–2.53; p = 0.202). For all women aged under 35 years undergoing SRI, the observed RR for pregnancy was 2.33, with 17% of the SRI procedures resulting in pregnancy compared to just over 7% of the IUI procedures. This difference was statistically significant (p = 0.032). There were no significant differences with respect to female and male characteristics for patients aged under 35 years in the IUI and SRI subgroups. Pregnancy rates were not different with clomiphene citrate, rFSH or progesterone in either group, neither in the whole population nor in the group of patients aged under 35 years. The pregnancy rate in patients aged under 35 years using clomiphene citrate and SRI was, however, exceptionally high (24.1%). There were no significant differences in the use of medications in women who successfully became pregnant after SRI or IUI.

Limitations, reasons for caution: We are aware that the crossover design might be seen as a limitation of this study. Some authors reject the utilization of this study design in infertility trials; however, others claim this an efficient and pragmatic design, particularly as only one cycle of each treatment is given to each woman.

Wider implications of the findings: These data lend support to the hypothesis that the pregnancy rate might be improved by using SRI rather than IUI, especially in women aged under 35 years. Additional, larger, clinical trials are required to fully prove this hypothesis, especially if an economic benefit of SRI is also to be demonstrated.

Trial registration number: Current Controlled Trials Register (registration number NCT02315040).

O-119 What is in unexplained subfertility the impact of gonadotropins or clomiphene citrate on endometrial thickness: a secondary analysis of a trial on intrauterine insemination

M.H. Mochtar[1], N. Danhof[1], R. Van Eekelen[1], B. Mol[2], F. Van der Veen[1], M. Van Wely[1]

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Study question: What is in unexplained subfertility the impact of follicle stimulating hormone (FSH) or clomiphene citrate (CC) in intrauterine insemination (IUI) on endometrial thickness (EMT), and is EMT related to pregnancy?

Summary answer: The mean EMT was significant thicker in women treated with FSH compared to treated with CC. EMT is not independently associated with ongoing pregnancy rate.

What is known already: CC is associated with thinner EMT compared to FSH in ovarian stimulation IUI. Whether a thin EMT has an effect on ongoing pregnancy is unknown. We recently performed a RCT comparing ovarian stimulation with follicle stimulating hormone (FSH) to CC in couples with unexplained subfertility undergoing IUI that showed no significant difference in ongoing pregnancy rates and live birth rates.

Study design, size, duration: This is a pre-planned secondary analysis on the effect of EMT in terms of pregnancy outcome of a multicentre randomised controlled superiority trial; the SUPER study, in which we investigated 738 couples diagnosed with unexplained or mild male subfertility from 24 clinics positioned in the Dutch Consortium for Healthcare Evaluation in Obstetrics and Gynaecology, undergoing ovarian stimulation with FSH or CC in IUI.

Participants/materials, setting, methods: 738 women received 1944 cycles. We used EMT per cycle. We measured changes over consecutive cycles per woman. We determined difference in EMT between women randomised to FSH and CC over all cycles using an independent samples t-test. We subsequently investigated the association between EMT and ongoing pregnancy using logistic regression, with precision measures adjusted for women receiving multiple cycles. Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation.

Main results and the role of chance: The 1944 IUI cycles resulted in 226 (11.6%) ongoing pregnancies. The mean EMT was 8.9 mm (±2.1) in women treated with FSH and 7.5 mm (±2.2) in women treated with CC (mean difference: 1.4 mm, p < 0.001, 95%CI 1.2-1.6). EMT was not significantly associated with ongoing pregnancy rate (odds ratio: 1.02 per 1 mm thicker, p = 0.59, 95% CI 0.96-1.08). Also after correcting for allocated stimulating agent, or after looking at nonlinear associations between EMT and pregnancy outcome, we found a similar result.

Limitations, reasons for caution: Secondary analyses, commonly used to investigate whether the conclusions are relevant for a particular sub population, are prone to false positive findings. Since this analysis was pre-planned, the risk of reporting bias is low.

Wider implications of the findings: We confirmed that CC result in thinner endometrium compared to FSH. Nonetheless, we found no association between EMT and chances in ongoing pregnancy rate. So EMT should not be used in the decision to continue CC or to switch to FSH in women undergoing IUI with ovarian stimulation.

Trial registration number: SUPER study is registered at NTR 4057

Acknowledgement: SUPER study group.

O-120 A cost-effectiveness analysis of ovarian stimulation with follicle stimulating hormone (FSH) compared with clomiphene citrate (CC) in intra uterine insemination (IUI)

N. Danhof, M. Van Wely, F. Van der Veen, M. Mochtar

AMC, Center for Reproductive Medicine, Amsterdam, The Netherlands

Study question: Is FSH or CC the most cost-effective stimulation regimen in couples with unexplained or mild male subfertility undergoing IUI with adherence to strict cancellation criteria?

Summary answer: The additional costs to achieve one additional ongoing pregnancy with FSH compared with CC were €10556,87. CC is the most cost-effective stimulation regimen in IUI.

What is known already: IUI with ovarian stimulation is the first line treatment in couples with unexplained or mild male subfertility. Almost 176000 IUI cycles are performed in Europe each year. A Cochrane review advises to use FSH for ovarian stimulation in IUI, but we have recently completed a multicentre RCT, in which we found that ovarian stimulation with FSH is not superior to CC in terms of ongoing pregnancies and live births. In using strict cancelation criteria, we found a comparable but low multiple pregnancy rate for FSH and CC.

Study design, size, duration: A cost-effectiveness analysis from a healthcare perspective alongside the multicentre randomised controlled superiority trial; the SUPER study. We investigated 738 couples diagnosed with unexplained or mild male subfertility from 24 clinics positioned in the Dutch Consortium for Healthcare Evaluation in Obstetrics and Gynaecology, undergoing ovarian stimulation with FSH or CC in IUI with adherence to strict cancellation criteria.

Participants/materials, setting, methods: We estimated costs from public sources and published literature. We assessed incremental cost-effectiveness ratios (ICERs) for 75 IE FSH and for 100 mg CC for costs per ongoing pregnancy.

Main results and the role of chance: The mean costs per couple were € 1 472,40 for FSH and € 1 014,65 for CC. The effectiveness of FSH was 4% higher compared with CC. The additional costs to achieve one additional ongoing pregnancy with FSH compared with CC were € 10 556,87. For a willingness-to-pay of € 15 000 for an additional ongoing pregnancy, there is 67% chance that FSH is cost-effective compared with CC. For a willingness-to-pay of € 45 000

for an ongoing pregnancy, there is a 91% chance that FSH is cost-effective compared with CC.

Limitations, reasons for caution: We performed the analysis from a healthcare perspective. We focussed on direct medical costs, but we find it unlikely that including indirect costs of the ovarian stimulation in IUI and indirect costs from a social perspective would change our conclusion, since the impact of these costs is small.

Wider implications of the findings: We could save more than €27 million in Europe on a yearly basis using CC instead of FSH in couples with unexplained or mild male subfertility undergoing IUI with adherence to strict cancellation criteria. Whether FSH should be used depends on society's willingness to pay for an additional ongoing pregnancy.

Trial registration number: Inapplicable.

Acknowledgement: thanks to the SUPER study group.

O-121 Is intrauterine insemination with ovarian stimulation associated with higher chances of ongoing pregnancy compared to expectant management in couples with unexplained subfertility?

N. Van Geloven[1], R. Van Eekelen[2,3], M. Van Wely[2], D.J. McLernon[4], F. Mol[2], I.M. Custers[2], P. Steures[5], S. Bhattacharya[6], B.W. Mol[7], F. Van der Veen[2], M.J.C. Eijkemans[3]

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Study question: Does starting IUI-OS within 1.5 years after completion of the fertility workup increase ongoing pregnancy rates compared to expectant management in couples with unexplained subfertility?

Summary answer: IUI-OS is associated with higher chances of ongoing pregnancy compared to expectant management in unexplained subfertile couples, especially those with poorer prognoses of natural conception.

What is known already: IUI-OS is often the first-line treatment for couples with unexplained subfertility. Two randomised controlled trials compared IUI-OS to expectant management but found conflicting results. In the absence of a consensus from these trials, a cohort of couples with unexplained subfertility exposed to expectant management and IUI-OS offers an opportunity to determine the chances of conception under both conditions. In addition, a large cohort allows evaluating whether the effect of IUI-OS depends on a couple's prognosis of natural conception, which might explain the discrepancy between trial results.

Study design, size, duration: A prospective cohort study on couples with unexplained or mild male subfertility who could start IUI-OS at any point after completion of the fertility workup in 7 Dutch centres between January 2002 and February 2004. Couples with bilateral tubal occlusion, anovulation, or a total motile sperm count $<1 \times 10^6$ were excluded. Follow up was censored at the start of IVF or at last contact and truncated at a maximum of 1.5 years after the fertility workup.

Participants/materials, setting, methods: The endpoint was time to ongoing pregnancy. We used the sequential Cox approach comparing in each month ongoing pregnancy rates over the next 6 months of couples who started IUI-OS to couples who did not. We calculated the prognosis of natural conception for individual couples and updated this over consecutive failed cycles, then evaluated whether prognosis modified the effect of starting IUI-OS. We corrected for known predictors of conception using inverse probability weighting.

Main results and the role of chance: Data from 1896 couples were available. 800 couples had at least one IUI-OS cycle within 1.5 years post fertility workup of whom 142 couples conceived (rate: 0.50 per couple per year, median follow up 4 months). The median period between fertility workup completion and starting IUI-OS was 6.5 months. Of 1096 couples who remained untreated, 386 conceived naturally (rate: 0.31 per couple per year, median follow up 7 months).

Starting IUI-OS was associated with a higher chance of ongoing pregnancy by a pooled, overall hazard ratio of 1.96 (95%CI: 1.47-2.62) compared to expectant management. The effect of treatment was modified by a couple's prognosis of achieving natural conception ($p = 0.01$), with poorer prognoses or additional failed natural cycles being associated with higher relative chances after starting treatment. The predicted 6-month ongoing pregnancy rate for a couple with a prognosis of 25% over the next 6 cycles (approximately 40% over one year) was 25% (95%CI: 21-28%) for expectant management and 24% (95%CI: 15-31%) when starting IUI-OS right after the fertility workup. For a couple with the cohort-average prognosis of 12% (20% over one year), these predicted rates were 12% (95%CI: 10-14%) for expectant management and 24% (95%CI: 17-30%) for starting IUI-OS.

Limitations, reasons for caution: The effect estimates are based on a prospective cohort. Although we balanced known predictors of pregnancy between treated and untreated couples using inverse probability weighting, observational data may still be subject to residual confounding. The results need to be confirmed by large randomised controlled trials.

Wider implications of the findings: Starting IUI-OS is effective in couples with unexplained subfertility who have a poor prognosis for natural conception and should therefore remain their first-line treatment. Our results may explain the discrepancies between trials that compared IUI-OS to expectant management, but further studies are required to confirm these findings.

Trial registration number: Not applicable.

O-122 Tubal flushing with oil- or water-based contrast medium at hysterosalpingography for infertility: 3-year outcome of a randomized clinical trial

J. Van Rijswijk[1], N. Van Welie[1], K. Dreyer[1], H. Verhoeve[2], A. Hoek[3], J.P. De Bruin[4], A. Nap[5], M. Van Hooff[6], M. Goddijn[7], A. Hooker[8], N. Lambalk[1], P. Hompes[1], H. Zafarmand[7], V. Mijatovic[1], B.W. Mol[9]

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Study question: What is the impact of the use of oil- or water-based contrast medium at hysterosalpingography (HSG) on fertility outcomes 3 years after randomization?

Summary answer: Women who had their tubes flushed with oil-based contrast had 3-year cumulative ongoing pregnancy rates of 83.2% versus 80.7% after the use of water-based contrast.

What is known already: For decades it has been suggested that tubal flushing with oil-based contrast (Lipiodol) at HSG improved pregnancy

rates (Mohiyiddeen L, et al. 2015). We recently showed in a landmark trial published in the New England Journal of Medicine that in infertile women tubal flushing during HSG with oil-based contrast resulted in more ongoing pregnancies than HSG with the use of water-based contrast at 6 months after randomization(39.7% versus 29.1% (RR 1.37, 95%CI 1.16-1.61, p < 0.001)) (Dreyer et al. 2017). It is unclear whether this difference continues beyond 6 months.

Study design, size, duration: This was a follow-up study of the H2Oil trial, a multi-center randomized controlled trial in the Netherlands in which 1,119 women were included and randomized to hysterosalpingography with oil-based contrast (n = 557) or water-based contrast (n = 562). Here, we present the fertility outcomes at three years after randomization.

Participants/materials, setting, methods: All 1,119 participants were contacted 3-5 years after randomization, and were asked to fill in questionnaires about the fertility treatments and pregnancies. Additionally, we reviewed the medical files to record all pregnancies and fertility treatments. Primary outcome was the first ongoing pregnancy within 36 months after randomization. We also compared naturally conceived pregnancies, pregnancies conceived after IUI and IVF and time to ongoing pregnancy.

Main results and the role of chance: In total, 383 ongoing pregnancies were accomplished in the oil-based contrast group versus 344 ongoing pregnancies in the water-based contrast group. The cumulative ongoing pregnancy rate 3 years after randomization was 83.2% (95%CI:79.3-87.1%) in the oil-based contrast group versus 80.7% (95%CI:76.4-85.0%) in the water-based contrast group. The Kaplan-Meier survival curves for time to ongoing pregnancy were significantly different in favor of the oil-based contrast group (median time to ongoing pregnancy 10.00 versus 14.83 months, p-value log-rank test 0.001).

Of the 383 ongoing pregnancies in the oil-based contrast group, 207 (54.3%) were naturally conceived, 114 (29.8%) after IUI and 60 (15.7%) after IVF/ICSI. Of the 344 ongoing pregnancies in the water-based contrast group, 174 (51.2 %) were naturally conceived, 93 (27.0%) after IUI and 73 (21.2%) after IVF/ICSI. Conception of 2 versus 4 pregnancies was unknown.

When follow-up is censored for starting IVF/ICSI treatment, the cumulative ongoing pregnancy rate was 74.2% (95%CI:68.1-80.3%) in the oil-based contrast group versus 70.1% (95%CI:63.0-77.2%) in the water-based contrast group, with the time to ongoing pregnancy significantly shorter in favor of the oil-based contrast group (median time to ongoing pregnancy 10.16 versus 17.07 months, p-value log-rank test <0.001).

Limitations, reasons for caution: This study was limited to women at low risk for tubal pathology, <39 years and without known endocrinological diseases, the findings should therefore not be generalized. Here, we only report ongoing pregnancies and not live birth.

Wider implications of the findings: Tubal flushing with oil-based contrast does not only result in higher 6-months ongoing pregnancy rates, but also in higher 3-year cumulative ongoing pregnancy rates. Time to ongoing pregnancy was significantly shorter in the oil-based contrast group. In our opinion, tubal flushing with oil-based contrast should be offered to infertile women.

Trial registration number: Dutch Trial Register, NTR 6577.

O-123 Does tubal flushing with oil- versus water-based contrast at hysterosalpingography increase the chance of a second child; long term follow-up of the H2Oil RCT

N. Van Welie[1], J. Van Rijswijk[1], H. Verhoeve[2], H. Annemieke[3], J.P. De Bruin[4], A. Nap[5], M. Van Hooff[6], M. Goddijn[7], A. Hooker[8], N. Lambalk[1], P. Hompes[1], V. Mijatovic[1], H. Zafarmand[7], B.W. Mol[9], K. Dreyer[1]

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Study question: Does tubal flushing with oil- versus water-based contrast at hysterosalpingography (HSG) increase the chance of a second child?

Summary answer: There is no clear effect of a single procedure of tubal flushing with oil- versus water-based contrast on the chance of a second child.

What is known already: For decades it has been suggested that tubal flushing with oil-based contrast(Lipiodol) at HSG improved pregnancy rates (Mohiyiddeen et al. 2015). We recently showed in a landmark trial published in the New England Journal of Medicine that in infertile women undergoing HSG tubal flushing with oil-based contrast resulted in more ongoing pregnancies than with the use of water-based contrast at 6 months after randomisation(39.7% versus 29.1% (RR 1.37, 95%CI 1.16-1.61))(Dreyer et al. 2017). While the effect of oil-flushing on conception of the first child is established, it is unclear whether tubal flushing with oil-based contrast increases the chances of subsequent children.

Study design, size, duration: This was a follow-up study of the H2Oil trial, a multi-center randomized controlled trial in the Netherlands in which 1,119 women were randomized to HSG with oil-based contrast (n = 557) or water-based contrast (n = 562). Participating women were approached 3-5 years after randomization with a questionnaire about their fertility. Additionally, we reviewed the medical files to record all pregnancies and fertility treatments. Here, we present the fertility outcome in terms of a second ongoing pregnancy.

Participants/materials, setting, methods: The outcome of this follow-up study was a second ongoing pregnancy (after the birth of a first child). We also assessed mode of conception and time to pregnancy.

Main results and the role of chance: There were 1,119 women randomized to oil-based contrast(n = 557) or water-based contrast(n = 562). Cumulative ongoing pregnancy rates were 83.2% versus 80.7% after oil and water flushing, respectively. In the oil-based contrast group 142(25.6%) couples had a renewed child wish, versus 129(23.1%) in the water-based contrast group.

In total, 97 women (17.5%) in the oil-based contrast group had a second ongoing pregnancy versus 91(16.3%) women in the water-based contrast group (p-value 0.60).

Of the 97 second ongoing pregnancies in the oil-based contrast group, 67 (69.1%) occurred naturally, 14(14.4%) after IUI and 12(12.4%) after IVF/ICSI. Of the 91 second ongoing pregnancies in the water-based contrast group, 58 (63.7%) were naturally conceived, 9(9.9%) after IUI, and 21(23.1%) after IVF/ICSI. Mode of conception of 4 versus 3 pregnancies was unknown.

The Kaplan-Meier curves for time to ongoing pregnancy showed no statistical difference for the use of oil- or water-based contrast(median time to ongoing pregnancy 50.97 versus 51.37 months respectively, p-value log-rank test 0.693). When time to ongoing pregnancy was censored at the start of IVF/ICSI, the Kaplan-Meier curves for time to ongoing pregnancy showed no statistical difference for the use of oil- or water-based contrast(median time to ongoing pregnancy 46.47 versus 51.37 months respectively, p-value log-rank test 0.717).

Limitations, reasons for caution: This study was limited to women with a low risk of tubal pathology, <39 years and without known endocrinological diseases. Furthermore the time of follow-up is relatively short, despite the large sample size.

Wider implications of the findings: Tubal flushing with oil-based contrast does not increase the chance of a second child. However, in the oil-based

contrast group the need for IVF/ICSI to establish this goal was reduced with almost 50%.

Trial registration number: Dutch Trial Register, NTR 6577

INVITED SESSION

SESSION 34: LAUNCH OF THE INTERNATIONAL EVIDENCE-BASED GUIDELINE FOR THE ASSESSMENT AND MANAGEMENT OF POLYCYSTIC OVARY SYNDROME (PCOS)

Tuesday 3 July 2018 Room 111 + 112 10:00–11:30

O-124 Building an international PCOS guideline: Initiative, process and translation

O-125 International guideline on PCOS: Diagnosis and treatment

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Abstract text

PCOS is common and heterogeneous with reproductive, metabolic and psychological features. Diagnosis delays are common internationally, with patient dissatisfaction with care and inadequate provided information and resources. There are gaps in clinician knowledge and inconsistencies in care, underpinned by a lack of quality evidence based guidelines and inadequate translation of evidence into practice. The International Evidence based Guideline for the assessment and treatment of polycystic ovary syndrome (PCOS) addresses health professional and consumer priorities. It integrates the best available evidence with international, multidisciplinary clinical expertise and consumer preferences to provide health professionals, consumers and policy makers with guidance on the assessment and treatment of PCOS. The guideline aims to promote accurate diagnosis, optimal consistent care, prevention of complications and improved patient experience and health outcomes for the one in ten women worldwide with PCOS. Extensive international health professional and patient engagement informed the need, priorities and core outcomes for the guideline. International Society-nominated panels and co-opted experts included women with PCOS, paediatricians, endocrinologists, gynaecologists, primary care physicians, reproductive endocrinologists, psychiatrists, psychologists, dermatologists, dieticians, exercise physiologists, public health experts, researchers, and a project management, evidence synthesis and translation team developed the guideline.

Evidence-based guideline development followed international best practice, involving 60 systematic and narrative reviews and applying the full Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework to reflect quality of evidence, and consider feasibility, acceptability, cost, implementation and ultimately the strength of recommendations. Governance included an international advisory board from six continents, a project board, five guideline development groups with 8-10 members each, advisors and a translation committee. Special Interest groups of world experts were formulated to review and provide feedback on the guidelines. Guideline development groups and special interest groups/ experts were nominated by the partner and collaborator organisations. The Australian Centre for Research Excellence in PCOS, funded by the National Health and Medical Research Council (NHMRC), partnered with the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) to fund and complete the guideline. Four project board and 15 guideline development group face to face meetings occurred across Europe, USA and Australia over 15 months, and enabled training, guideline development and informed translation. Sixty prioritized clinical questions were addressed with 40 systematic and 20 narrative reviews, generating 170 recommendations and practice points. Feedback from our over 40 partner and collaborator special interest groups was sought during consultation to inform the final guideline. The guidelines which cover diagnosis, emotional well-being, lifestyle, medical therapy and infertility treatment will be launched and presented at ESHRE.

O-126 Guideline recommendations: Treatment, emotional wellbeing and pregnancy

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Abstract text

PCOS is common and heterogeneous with reproductive, metabolic and psychological features. Diagnosis delays are common internationally, with patient dissatisfaction with care and inadequate provided information and resources. There are gaps in clinician knowledge and inconsistencies in care, underpinned by a lack of quality evidence based guidelines and inadequate translation of evidence into practice.

The International Evidence based Guideline for the assessment and treatment of polycystic ovary syndrome (PCOS) addresses health professional and consumer priorities. It integrates the best available evidence with international, multidisciplinary clinical expertise and consumer preferences to provide health professionals, consumers and policy makers with guidance on the assessment and treatment of PCOS. The guideline aims to promote accurate diagnosis, optimal consistent care, prevention of complications and improved patient experience and health outcomes for the one in ten women worldwide with PCOS.Extensive international health professional and patient engagement informed the need, priorities and core outcomes for the guideline. International Society-nominated panels and co-opted experts included women with PCOS, paediatricians, endocrinologists, gynaecologists, primary care physicians, reproductive endocrinologists, psychiatrists, psychologists, dermatologists, dieticians, exercise physiologists, public health experts, researchers, and a project management, evidence synthesis and translation team developed the guideline.

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project board and 15 guideline development group face to face meetings occurred across Europe, USA and Australia over 15 months, and enabled training, guideline development and informed translation. Sixty prioritised clinical questions were addressed with 40 systematic and 20 narrative reviews, generating 170 recommendations and practice points. Feedback from our over 40 partner and collaborator special interest groups was sought during consultation to inform the final guideline. The guidelines which cover diagnosis, emotional well-being, lifestyle, medical therapy and infertility treatment will be launched and presented at ESHRE.

SELECTED ORAL COMMUNICATIONS
SESSION 35: GENETIC CAUSES OF MALE INFERTILITY

Tuesday 3 July 2018 Room 113 + 114 + 115 10:00–11:30

O-127 Analysis of the chromosome territories dynamic in male germ cells and its association to male infertility

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[4]Cytocell Ltd, Technopark, Cambridge, United Kingdom

Study question: Is there a specific three-dimensional distribution pattern of chromosomes in the different spermatogenic cells?

Summary answer: Chromosomes are arranged in a specific manner along the different stages of spermatogenesis.

What is known already: In somatic cells, chromosomes occupy specific nuclear regions called chromosome territories (CTs) that are arranged in cell-type specific non-random patterns according to chromosome parameters such as gene density and chromosome size. Moreover, CTs have a functional effect in the regulation of the genome providing additional mechanisms to modulate gene expression. Therefore, it has been suggested that CTs in meiotic cells may be crucial for the proper development of spermatogenesis and early embryo development. Actually, an altered distribution of some CTs has been observed in infertile men. However, the CTs distribution along the spermatogenesis has been poorly explored.

Study design, size, duration: Experiments were performed by using testes from 5 C57BL6/6J mice. The method includes the following steps: i) Optimized cell fixation to preserve the three-dimensionality cell morphology, ii) Fluorescence in situ hybridization of all mouse chromosomes, iii) Three-dimensional image captures of a minimum of 100 nuclei per cell type by confocal microscope, iv) Cell type identification by immunofluorescence, v) Image analysis and data extraction by a numerical computing software, vi) Data analysis by using statistics software's.

Participants/materials, setting, methods: Testicular tissue was disaggregated and cells were spread out on polylysine-coated slides. After cell fixation, three FISH rounds were carried out using the Mice OctoChrome kit. Protein SYCP3 and histone H1T were identified by immunofluorescence to distinguish between spermatogenic cells. Serial optical sections were obtained with a confocal microscope and images were processed by ImageJ. Matlab scripts were used to normalize images and determine chromosome volume and proportion, chromosome radial position and chromosome relative position.

Main results and the role of chance: The application of the developed methodology allowed determining the territoriality of chromosomes in male germ cells. Results showed that there is a specific three-dimensional distribution pattern of chromosomes throughout the different spermatogenic stages. On the one hand, the analysis of the chromosomes radial position revealed that chromosomes are arranged in a specific manner from the center to the periphery of the nucleus. Moreover, it has been observed preference in radial positioning according to some chromosomal parameters such as chromosome size, gene density or the percentage of guanine and cytosine contain. On the other hand, in terms of chromosomes vicinity, results showed that there is a systematic relative position of specific chromosomes pairs, showing preference in proximity or non-proximity.

Limitations, reasons for caution: The methodology applied in this study is complex and time consuming because of requiring three consecutive FISH rounds and one extra round for immunofluorescence.

Wider implications of the findings: Our results will provide the new basis of future studies to find out the relationship between chromosome positioning, genome regulation and male fertility.

Trial registration number: not applicable.

O-128 The altered testicular environment in patients with Klinefelter Syndrome

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[2]Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Brussel, Belgium
[3]Universitair Ziekenhuis Brussel, Pediatric Endocrinology, Brussel, Belgium
[4]UZ Brussel, Pediatric Endocrinology, Brussel, Belgium
[5]UZ Brussel, Centre for Reproductive Medicine, UZ Brussel, Belgium
[6]Vrije Universiteit Brussel, Biology of the Testis, Brussel, Belgium

Study question: Is testicular fibrosis in Klinefelter syndrome (KS) related to the altered structure of the tubular wall and/or altered vascularization?

Summary answer: An altered expression pattern in adult patients was observed for collagen I and IV. An increase in vessel density was observed in prepubertal KS testis.

What is known already: Patients with KS are infertile due to a massive germ cell loss. By the time of diagnosis, adult KS testes often show extensive fibrosis, hyalinization of the seminiferous tubules and hyperplasia of the interstitium. The architecture of the tubular wall, composed of peritubular myoid cells (PTMCs), extracellular matrix proteins (ECM) and fibroblasts, is altered in men with impaired spermatogenesis and could be related to tubular fibrosis by increased deposits of ECM.

In a mouse model for KS, an altered vascularisation of the KS testes was suggested. Angiogenesis (the formation of new blood vessels) is also related to fibrotic processes.

Study design, size, duration: In this study, we want to characterize the testicular environment in testicular biopsies from adult KS patients and compare it with the testicular environment in testicular tissue from fertile controls and Sertoli Cell Only patients. SCO patients also lack germ cells but do not show the typical fibrotic appearance as seen in testes from KS patients.

Participants/materials, setting, methods: Immunohistochemical analysis of testicular tissue sections from adult control (n = 6), KS (n = 20) and SCO (n = 20) patients was performed using markers for PTMCs (ACTA2), ECM (laminin, fibronectin, collagen I and IV) and blood vessels (VWF). The expression of tubular wall markers was characterized by identifying abnormal staining patterns. The mean blood vessel area and the vessel density was calculated in VWF stained testicular sections from adult (n = 25), pre- (n = 4) and peripubertal (n = 15) KS patients.

Main results and the role of chance: Altered expression for ACTA2 was observed in KS as well as in SCO patients. In SCO patients, the tubular wall often showed two layers of ACTA+ cells interrupted by a negative ring (probably ECM deposits). However, in KS patients, the same pattern was observed but the ring of ACTA2 staining was often interrupted indicating loss of ACTA2 staining. No statistical difference was observed in the expression patterns of fibronectin and laminin between SCO and KS patients. In KS patients, more tubules lacking collagen I expression were observed. An altered expression pattern was also observed for collagen IV. No difference in blood vessel area and vessel density was observed in adult testicular sections from controls, SCO and KS patients and in peripubertal sections from KS patients. However, a higher vessel density was observed in prepubertal KS patients. When vessels were

divided according to their size, more small blood vessels were present in these prepubertal patients.

Limitations, reasons for caution: These data are descriptive. It is not possible to draw causal conclusions from the stainings.

Wider implications of the findings: More research is necessary to identify causes of testicular fibrosis in KS patients. If the mechanism behind this fibrotic process could be identified, this process could be inhibited to increase chances of fertility in KS patients.

Trial registration number: not applicable.

O-129 A heterozygous DNAJB13 mutation induces multiple morphological and structural abnormalities to the sperm flagella (MMSAF) and may be a cause of idiopathic asthenozoospermia

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Study question: Does a novel heterozygous *DNAJB13* mutation, identified in 9 out of 92 idiopathic asthenozoospermia patients affect multiple male infertility-related genes and cause low sperm activity?

Summary answer: A heterozygous mutation c.106 T>C(p.Ser36Pro) of *DNAJB13*was demonstrated to exhibit defects in sperm motility and affect multiple morphological and structural abnormalities to the sperm flagella (MMSAF).

What is known already: *Dnajb13*, which is upregulated in cryptorchidism mouse models and is highly expressed in the testis, encodes a member of the heat shock protein 40 co-chaperone family (types II). Homozygous mutant mice died before sexual maturation. However, no mutations of *DNAJB13* (NM_153614.2) have been reported in patients with idiopathic asthenozoospermia.

Study design, size, duration: This genetic study used Medical Exome microarrays and Sanger sequencing to discover related-genes mutations, and was followed by a series of studies to investigate mutant protein function. Altogether, 92 idiopathic asthenozoospermia patients and 200 fertile men from the Hunan human sperm bank from 2010-2013 in China were screened for *DNAJB13* mutations.

Participants/materials, setting, methods: Patients and fertile men were recruited from our hospital in China. Genomic DNA samples from the individuals were extracted from peripheral blood. A proband was subjected to Medical Exome microarrays to identify mutations. Mutational analysis in the 92 idiopathic asthenozoospermia patients and 200 fertile men were performed. Identified *DNAJB13* mutations were further investigated using bioinformatics, indirect immunofluorescence assay, papanicolaou staining, CASA, immunoelectron microscopy, Co-IP assays, transmission electron microscopy, iTRAQ, and multiple reaction monitoring studies.

Main results and the role of chance: We identified a heterozygous mutation c.106 T>C, p.Ser36Pro in *DNAJB13* in 9 (10%) idiopathic asthenozoospermia patients. We failed to find this mutation in the 200 fertile control men. Furthermore, using papanicolaou staining, CASA analysis and sperm functional tests, we found that spermatozoa with *DNAJB13* mutations had more deformities, i.e. vacuolus in the head and excessive residual cytoplasm, inflexible midpiece and asymmetric beating flagellum, and abnormal sperm acrosome reaction compared with the control group and patients without the mutation. Immuno-electron microscopy was carried out to observe ultrastructural multiple morphological and structural abnormalities of the sperm flagella (MMSAF) due to *DNAJB13* mutations. iTRAQ and multiple reaction monitoring studies were used to analyze the changes of multiple asthenozoospermia related proteins, spermatozoa structural proteins—NME5, RSPH9, sperm-egg binding and fusion proteins—SPESP1, and energy metabolism proteins—HK1 proteins. These proteins were significantly decreased in spermatozoa of idiopathic asthenozoospermia patients with *DNAJB13* mutations.

This study indicates that *DNAJB13* may be involved in spermatogenesis and sperm motility and a cause of idiopathic asthenozoospermia.

Limitations, reasons for caution: Although our *in vitro* assays demonstrated the effect of the *DNAJB13* mutation on sperm motility in structure and function level, further studies are needed to validate the *in vivo* effects of DNAJB13 mutation.

Wider implications of the findings: This finding further expands the spectrum of known genetic defects associated with asthenozoospermia and provides researchers and clinicians a better understanding of the etiology and molecular mechanism of asthenozoospermia.

Trial registration number: Not applicable.

O-130 Defective H3K9 demethylation of the male genome and abnormal first mitosis observed in mouse oocytes injected with human round spermatids

Y. Kai[1], K. Nakata[2], J. Ito[3], N. Kashiwazaki[3], N. Yamashita[2], Y. Mio[1]

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[2]*Yamashita Shonan Yume Clinic, Reproductive Medicine Research Center-, Fujisawa, Japan*
[3]*Azabu University, Department of Animal Science & Biotechnology School of Veterinary Medicine, Sagamihara, Japan*

Study question: Does round spermatid injection (ROSI) contribute to normal embryo development in assisted reproductive technology?

Summary answer: ROSI tended to cause failure of H3K9 demethylation of the male genome, formation of male pronuclei, and chromosome segregation at the first mitosis.

What is known already: ROSI-derived embryos are prone to exhibit abnormal chromosome segregation and epigenetic abnormalities in animals. Indeed, developmental efficiency is significantly lower with ROSI than with intracytoplasmic sperm injection (ICSI), not only in animals, but also in humans. Despite views to the contrary, ROSI is still an option for assisted fertilization in animals and humans.

Study design, size, duration: This study was approved by the ethical committee of JISART (Japanese Institution for Standardizing Assisted Reproductive Technology) and was registered with the Japan Society of Obstetrics and Gynecology. ROSI and ICSI were conducted by injection of human round spermatids or spermatozoa into mouse MII oocytes. DNA/histone methylation status, pronuclear formation and chromosome segregation were analyzed statically and/or dynamically from injection to the first cleavage by immunofluorescence and fluorescence live cell imaging analysis.

Participants/materials, setting, methods: Human male gametes were obtained after donation and ethical approval. Round spermatids were obtained from 13 patients with non-obstructive azoospermia after testicular sperm extraction. MII oocytes were collected from 8-week-old B6D2F1 mice and pre-activated by CZB medium containing 7.5 mM $SrCl_2$ without Ca^{2+} for ROSI. Immunofluorescence was performed using antibodies against 5mC, 5hmC and H3K9me3. Live cell imaging was performed using TagGFP2-H2B mRNA and fluorescent-labeled Fab313, which can react with H3S10ph next to H3K9me3.

Main results and the role of chance: Firstly, immunofluorescence was performed using ROSI- and ICSI-derived two-pronuclear (2PN) zygotes in order to analyze the DNA/histone methylation status. Analysis of DNA methylation status targeting 5mC and 5hmC revealed that all ROSI-derived zygotes (n = 10) showed conversion of 5mC to 5hmC in the male pronucleus, as in ICSI-derived zygotes (n = 5), but analysis of histone methylation status targeting H3K9me3 revealed that all ROSI-derived zygotes (n = 10) retained H3K9me3 in the male pronucleus, unlike ICSI-derived zygotes (n = 7). Fluorescence live cell imaging analysis visualizing histone H2B and H3S10ph also revealed that the male genome was positive for H3K9me3 in oocytes after ROSI (n = 89), but not after

ICSI (n = 17). Failure of male pronucleus formation was observed in 84.3% of oocytes after ROSI. The 2PN fertilization rate of ROSI was low in comparison with ICSI (15.7% vs. 82.4%) and 35.7% of ROSI-derived 2PN zygotes showed distorted male pronuclei not seen with ICSI. Furthermore, half of the ROSI-derived 2PN zygotes showed aberrant chromosome congression at the first mitosis leading to aberrant chromosome segregation.

Limitations, reasons for caution: Our results came from cross-species fertilization and from the limited period until first mitosis, for ethical reasons. Although we have confirmed that oocyte pre-activation does not differ with SrCl2 or phospholipase C-zeta cRNA injection in mouse ROSI, phospholipase C-zeta cRNA injection was not tested in this study.

Wider implications of the findings: Defective demethylation of H3K9 in the male genome and poor developmental ability in ROSI might arise from incomplete protamine-histone replacement. Histone modification could be a key factor to utilize round spermatids in the clinical setting.

Trial registration number: Not applicable.

O-131 SPANX A/D subfamily plays a key role in nuclear organisation, metabolism and flagellar-motility of human spermatozoa

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Study question: Which is the physiological function of endogenous SPANX A/D subfamily in human spermatozoa?

Summary answer: Quantitative proteomics reveals that SPANX A/D may play a role in nuclear organisation, mitochondrial metabolism and cilium-movement of human spermatozoa by interacting with 307 proteins.

What is known already: SPANX (Sperm Protein Associated with the Nucleus mapped to the X chromosome) multigene protein family is considered a Cancer Testis Antigen (CTA) whose expression is limited to the testis and spermatozoa in normal tissues, but also to a wide variety of tumors in non-gametic cells. This family includes two subfamilies, SPANX N and SPANX A/D. Although, the physiological function of the whole protein family is completely unknown, the low expression of SPANX A/D subfamily is related with asthenozoospermia and globozoospermia, and hence with a lower fertility in human spermatozoa.

Study design, size, duration: We followed a label free quantitative proteomics approach, in quadruplicate, to uncover the interactome of SPANX A/D subfamily in human spermatozoa. Additionally, a number of techniques in molecular biology were used to analyse the expression and subcellular localization of SPANX A/D in human spermatozoa.

Participants/materials, setting, methods: We used human normozoospermic seminal samples coming from the Quirón Bilbao fertility clinic. MS-based interactome study was carried out by immunoprecipitating endogenous SPANX A/D and subsequently, analyzing by a Q Exactive Mass spectrometer at the University of Southern Denmark (Odense, DK). MS raw files were processed with MaxQuant software v1.3.0.7, and output data was analyzed in Perseus bioinformatics analysis program. The same SPANX antibody was used to perform Western Blotting and immunoflurescence experiments.

Main results and the role of chance: We have described the presence of SPANX A/D subfamily in normozoospermic human spermatozoa, in both soluble and insoluble protein fractions, by immunobloting approaches. Immunofluoresce assays also show a strong immunoreactivity of SPANX A/D in the middle section, tail, acrosome and nuclear craters of human spermatozoa. Indeed, label-free proteomics data confirms that SPANX isoforms A, B, C and D are expressed in human male germ cells. Additionally, the MS-based interactome study carried out reveals a list of 307 potential interactors of endogenous SPANX A/D. According to gene ontology analysis, these interacting partners are involved in a wide range of key functions for the human spermatozoa, including the nuclear pore organization, the cilium- or flagellum-dependent motility, the assembly of the axonemal dynein complex, the mitochondrial electron transport and oxidative phosphorylation. This outcome is in line with the data obtained by immunocytochemistry indicating that SPANX is localized in different compartments of the cell.

Limitations, reasons for caution: Further studies are needed to validate the most intense SPANX interactors by accomplishing different molecular techniques. This information could help us to ratify the physiological role of SPANX A-D family in human spermatozoa.

Wider implications of the findings: A better understanding of the physiological function of SPANX in fertility may be useful to detect new therapeutic targets for treating infertility, as well as to develop safer polyvalent sperm contraceptives. Moreover, it could be essential to have a better understanding of the physiopathological mechanisms of this CTA in cancer.

Trial registration number: CEISH/61/2011.

O-132 Copr5is associated with Miwi and modulated the piRNA pathway, a possible mechanism involved in the human teratozoospermia sperm phenotype

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Study question: Is the Prmt5-associated factor Copr5 involved in the piRNA pathway during spermatogenesis?

Summary answer: Copr5 silencing in mice affects spermatogenesis and low level of Copr5 mRNA correlates with human teratozoospermia sperm.

What is known already: Protein arginine methyl transferase 5 (Prmt5) was implicated in genome defense in primordial germ cells (PGCs) via a PRMT5-dependent methylation of the Piwi protein family that participates within the piRNA pathway in promoting transposon silencing. However, the role of the Prmt-5 and histone-associated protein Copr5 in the regulation of the piRNA pathway remains unknown.

Study design, size, duration: Testes from Copr5 Knock-out (KO) mice and wild type (WT) (n = 3 per group) were used for immunoprecipitation, western blot analyses, immunhistochemistry (IHC) and RTqPCR experiments. Ten human sperm samples were collected from patients with normozoospermia (n = 3) and teratozoospermia (n = 7) for RTqPCR experiments.

Participants/materials, setting, methods: Proteins, total RNA and paraffin-embedded testis sections were extracted and/or prepared from the Copr5 KO and WT mice. 200 µl of each semen were used for total RNA extraction using the miRNeasy serum/plasma kit (Qiagen).

Main results and the role of chance: Prmt5- and histone-associated protein Copr5 is highly expressed intestis and its depletion impacted on the maturation of spermatogonia to spermatids, although Copr5 KO animals were fertile. Extinction of Copr5 in mice testis leads to a down-regulation of the level of Miwi protein, as confirmed by western blot analyses and immunohistochemistry of WT and KO mice testis whole cell extracts and paraffin-embedded testis sections, respectively. In addition, the mRNA level of three pre-pachytene piRNAs, prepiR1, prepiR2 and prepiR3 was decreased in KO Copr5 compared to WTmice, whereas in KO mice the expression level of the LINE1 mRNA, the most abundant retro-transposons that was usedas a read out of deregulation of the piRNA pathway, was increased. Using both human GEO data and patients with teratozoospermia, the present study reports a correlation between a low level of Copr5 mRNA and teratozoospermia, a human pathology characterized by abnormally shaped sperm that can negatively affect fertility.

Limitations, reasons for caution: Further investigation is needed to know whetherthe absence of Copr5 in mice might molecularly impact more strongly

on the piRNA pathway over generation and render mice unfertile, thus phenocopying the human pathology.

Wider implications of the findings: Copr5 KO in mice perturbed some genome surveillance mechanisms in germ cells by an alteration of the expression level of two major components present in the Piwi-interacting RNA (piRNA) pathway, the Miwi and the LINE1.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 36: IVF LABORATORY TECHNIQUES AND MANAGEMENT

Tuesday 3 July 2018 Room 117 10:00–11:30

O-133 A two-step protocol for oocyte In Vitro Maturation in PCO/PCOS patients increases the yield and quality of usable embryos and results in ongoing pregnancies

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[3]University of Medicine and Pharmacy at Ho Chi Minh City, Department of Obstetrics and Gynecology, Ho Chi Minh City, Vietnam

Study question: What is the effectiveness of a two-step IVM protocol (CAPA-IVM) involving a pre-maturation culture with C-type natriuretic peptide (CNP) versus Standard IVM in PCO/PCOS patients?

Summary answer: Pre-maturation with CNP followed by IVM (CAPA-IVM) increased the yield and quality of usable embryos resulting in a high ongoing pregnancy rate from first transfer.

What is known already: Oocyte In Vitro Maturation (IVM) is a valuable woman-friendly assisted reproductive technology, especially suited for patients presenting polycystic ovaries or Polycystic ovary syndrome (PCO/PCOS). Since IVM makes use of oocytes obtained from small antral follicles (2-12 mm diameter), embryological and clinical outcomes were proven to be reasonably inferior to conventional stimulated IVF (ICSI) techniques.

A novel two-step IVM system (termed CAPA-IVM) using FSH priming but omitting hCG administration has recently been translated from mouse to human in a pilot study using sibling oocytes, indicating superior results.

This study further explores the embryological and clinical advantages of using CAPA-IVM in PCO/PCOS patients.

Study design, size, duration: This prospective randomised controlled pilot study includes PCO/PCOS patients (Rotterdam criteria, 2004), 24-36 years old, BMI ≤30, without previous history of recurrent failure of ART (including recurrent miscarriages). Enrolments took place between March and June 2017. Patients were randomly allocated to two protocols: CAPA-IVM and Standard IVM, via block randomization with block size of 4. Sample size was 40 (20 each group).

Routine hormonal analyses included AMH, FSH, LH, estradiol and progesterone.

Participants/materials, setting, methods: Patients received 3 doses of rFSH 150IU daily, starting on day 1 or 3 after bleeding. Oocyte retrieval was planned on day 5 to 8 without a prior hCG trigger.

The pre-maturation culture lasted 24 h whereas IVM lasted 30-32 h. The primary outcome was the yield of usable (vitrified) Day 3 embryos, per patient. Secondary outcomes were oocyte maturation, fertilization and good quality embryo rates. A maximum of 2 embryos were transferred in a deferred cycle.

Main results and the role of chance: Mean age (28.9), BMI (21.2) and AMH (10.8 µg/L) were not different among patients allocated to any of the IVM protocols. Baseline values for FSH and LH were 5.82 ± 1.58 IU/L and 12.65 ± 6.12 IU/L, respectively.

The MII (maturation) rate was significantly higher in CAPA-IVM vs Standard IVM (60.4 ± 15.3% vs 50.8 ± 20.8%; $P = 0.0454$). Rates of fertilization per mature oocyte and usable embryos per fertilized oocyte were similar among the groups. However, there was a significant increase (1.8-fold higher) in the mean number of usable embryos (per patient), in CAPA-IVM vs Standard IVM (3.9 ± 2.6 vs 2.2 ± 1; $P = 0.0061$).

Moreover, the rates of good quality embryos (grades 1 and 2) per fertilized oocyte, mature oocyte or cumulus-oocyte-complex (COC), were significantly higher in CAPA-IVM vs Standard IVM (48.9 ± 22.8 vs 36.2 ± 23.2%, $P = 0.0248$; 43.2 ± 23.4% vs 30.8 ± 22.6%, $P = 0.0257$; and 27.2 ± 15.3 vs 15.1 ± 12.9%, $P = 0.002$; respectively).

The ongoing pregnancy rate (per transfer) is currently 52.6% and 36.8% (CAPA-IVM and Standard IVM, respectively). No significant differences have been yet recorded, since ongoing pregnancies are still in follow-up.

Limitations, reasons for caution: Although patients were randomly assigned to either of the IVM protocols, the patient population is still small. Nevertheless, the results with CAPA-IVM in Vietnamese patients are concordant with those previously reported in Belgian patients and published earlier, showing transferability of the CAPA-IVM method.

Wider implications of the findings: This study reports the first pregnancies of CAPA-IVM. The significant increase in oocyte maturation, number of usable embryos and embryo quality by the two-step IVM protocol in this prospective randomized controlled pilot study suggests that IVM could be positioned as one of the routine assisted reproductive techniques for PCOS patients.

Trial registration number: not applicable.

O-134 Assisted oocyte activation significantly increases fertilization and pregnancy rates resulting in healthy live births in patients with oocyte activation deficiencies

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Study question: To what extent does assisted oocyte activation (AOA) improve fertilization and pregnancy outcome in patients diagnosed with oocyte activation deficiencies (OADs)?

Summary answer: AOA significantly increased fertilization and pregnancy rates and resulted in healthy live births in patients with a history of low or failed fertilization after ICSI.

What is known already: Whether AOA is effective in couples with low or failed fertilization after ICSI remains controversial. This is mainly due to the fact that most clinics applying AOA do not investigate whether the origin of OAD is attributed to sperm- or oocyte-related factors; information that is important for counseling couples about future treatment options. The Mouse Oocyte Activation Test (MOAT) is one diagnostic method to discern the gamete origin of failed fertilization. To date, large-scale studies assessing the efficiency of AOA in patients experiencing failed fertilization after ICSI and undergoing a diagnostic test to reveal the origin of OAD, are lacking.

Study design, size, duration: A retrospective analysis was performed, involving 167 AOA cycles (April 2001 to July 2017) of 105 men with low or failed fertilization after ICSI for whom sperm activation capacity was examined by MOAT prior to the subsequent AOA cycle(s). The AOA efficiency in terms of fertilization and pregnancy rate and neonatal outcome was investigated according to the MOAT result and compared to 207 previous conventional ICSI cycles of these couples.

Participants/materials, setting, methods: For MOAT, mature mouse oocytes (B6D2F1, 7-12 weeks) were injected with patients' spermatozoa. Depending on the percentage of activated oocytes next day, patients were categorized in MOAT group 1 (0-20% activation), 2 (21-84% activation) or 3 (85-100% activation) and compared to a positive fertile control. For AOA treatment, mature patients' oocytes were injected with patients' spermatozoa along with $CaCl_2$ (0.1 M), followed by a two-fold exposure to ionomycin (10 µM, 10 min) with a 30 minute time interval.

Main results and the role of chance: MOAT revealed 18 patients with a sperm-related OAD (MOAT group 1), 49 patients with a diminished oocyte

activation capacity of the sperm (MOAT group 2) and 38 patients with a presumably oocyte-related OAD (MOAT group 3). AOA treatment significantly improved fertilization rates: MOAT group 1: 69.69% for 34 AOA cycles versus 10.53% in 19 prior ICSI cycles, MOAT group 2: 62.54% (76 cycles) versus 14.21% (123 cycles), MOAT group 3: 58.26% (57 cycles) versus 17.18% (65 cycles) ($p < 0.001$). Moreover, significantly higher pregnancy rates were observed after AOA treatment compared to previous ICSI cycles, in MOAT group 1 (46.94% versus 0.00%), 2 (34.88% versus 6.35%), and 3 (29.03% versus 6.25%) patients ($p < 0.05$). Interestingly, AOA results for MOAT group 1 tended to be better than for MOAT group 3 in terms of fertilization rates (69.69% versus 58.26% respectively, $p < 0.05$) and pregnancy rates (46.94% versus 29.03% respectively, $p = 0.05$). In total, 52 healthy children were born (28 boys and 24 girls): 19 singletons in MOAT group 1, 13 singletons and 3 twins in MOAT group 2, and 12 singletons and 1 twin in MOAT group 3.

Limitations, reasons for caution: As AOA technology is still considered experimental, its application is thus best reserved for those cases where there is a clear indication of an OAD. To date, our results demonstrate that AOA seems to be a safe and effective technique. However, further follow-up of children born after AOA is warranted.

Wider implications of the findings: Our large cohort study shows that in cases with OAD, AOA restores fertilization and pregnancy rates to a satisfactory level. More sensitive tests (e.g. calcium pattern analysis) will aid to further distinguish those cases that are likely to benefit from AOA, and additionally guide further clinical management of these patients.

Trial registration number: not applicable.

O-135 Three babies born after pronuclear transplantation in young women with unexplained infertility and repeated implantation failure of euploid embryos

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Study question: Can pronuclear transplantation (PNT) be applied to women with unexplained infertility and repeated implantation failure?

Summary answer: Three healthy babies born after PNT in a group of five women <37 years old, who were unable to conceive using repeated cycles of ART.

What is known already: Using nuclear transplantation or whole cytoplasm replacement in humans is an experimental approach which avoids transmission of mitochondrial disease. Concerns have been raised about these procedures and particularly about later expression of potentially affected mitochondria and the potential of nuclear-cytoplasmic incompatibility. PNT resulted in a single ongoing twin pregnancy in 2003 for a patient with recurrent embryo arrest, but this did not result in live birth. Maternal spindle transfer for prevention of Leigh syndrome resulted in the first delivery of a healthy boy in 2016.

Study design, size, duration: The study period was from September 2015 to January 2018. Patients were informed and consent to possible risks and the experimental protocol was approved by ethics committee of Ukrainian association of reproductive medicine. Inclusion criteria were: (1) no less than two failed previous IVF attempts with embryo arrest, (2) low blastulation rates, (3) recurrent implantation failure of euploid embryos; (4) age ≤ 37 years, normal ovarian reserve, absence of endometrial or uterus factor of infertility.

Participants/materials, setting, methods: Oocyte retrievals for five infertile women (from 29 to 34 years of age) were synchronized with donors; thawed donor oocytes were used in a case of patient's oocytes excess. ICSI with patient's partner's sperm had been performed simultaneously for all oocytes. PNT, assisted by HVJ-E cell fusion kit, was applied at ~12 h after fertilization. Reconstituted zygotes were cultured until blastocyst stage in time-lapse incubator and biopsied for aCGH or NGS analysis.

Main results and the role of chance: Overall, 36 zygotes from 43 patient's zygotes (84%) were successfully reconstituted, that resulted in 19 blastocysts (53%); 14 were euploid (74%). Elective single embryo transfer (eSET) of thawed embryos was done for each patient. Positive hCG levels (> 100 mIU/mL) were confirmed in 4/5 patients after the first try. In two patients amniocentesis was performed at 17 weeks of gestation to confirm successful cytoplasm replacement by an independent laboratory. The third patient refused amniocentesis; however, donated umbilical cord blood after she gave birth. For two cases the result was: "None of the variants of the biological mother and all mitochondrial variants of the donor were detected in the fetus" and for the third one it was: "The majority of mitochondrial variants detected in the child are shared with the mitochondrial donor and not with its biological mother". First baby girl was born on 5th of January 2017 through vaginal delivery and a second baby boy was born on 19th of February 2017; third baby boy was born on 15th of October 2017 by Caesarean section. The three children performed well in pediatric analyses. The fourth case was at an early stage of development at the moment of abstract submission.

Limitations, reasons for caution: Since, nuclear transplantation in human is still experimental and highly controversial; further investigations and long term follow up of children is required. The number of patients included into study is low; any broad conclusions may be premature. Patients above 37 years of age may not benefit from PNT.

Wider implications of the findings: Not only mitochondrial diseases, but a rare condition named preimplantation embryonic lethality (embryo arrest) could possibly be overcome by pronuclear transplantation. This experimental technique may be used as an assessment tool to study nuclear-cytoplasmic interactions.

Trial registration number: Not applicable.

O-136 Blastocysts collapse as an embryo marker of low implantation potential a time-lapse study

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Study question: To validate the relationship between blastocyst collapse and implantation potential in *in vitro* human embryo culture using Time-Lapse technology.

Summary answer: Blastocysts showing a severe collapse during development are less likely to implant and generate a viable pregnancy compared with embryos which do not show collapse.

What is known already: The process of hatching in mammalian embryo, involving the escape of the blastocyst from the zona pellucida (ZP) was first observed by Lewis and Gregory in 1929. Whether blastocyst collapse could be a novel marker of embryo quality, and correlated with outcome of ICSI/IVF cycles, should be demonstrated in an independent dataset of embryos. This study investigated the correlation between blastocyst collapse and pregnancy and implantation rates following elective single transfer at blastocyst stage (eSET).

Study design, size, duration: This is a retrospective multicenter analysis, carried out between January 2016 and February 2017. Four IVF units participate to the study: EFREC, RIE, Edinburgh, UK, IVI Valencia, IVI Barcelona and IVI Zaragoza in Spain. Blastocyst were analysed by measuring the maximum volume reduction during development and defined as having collapsed if there was ≥50% of the surface of the TE was separated from the ZP.

Participants/materials, setting, methods: The study included 1297 ICSI/IVF cycles having an eSET on day 5. Embryos were cultured individually in an EmbryoSlide™, using an EmbryoScope™ imaging system in 6% CO2, 5% O2, 89% N2. Morphological assessment was made by examining a video of development using the associated EmbryoViewer software. Following embryo transfer, blastocyst was retrospectively allocated to one of two groups (collapsed or not collapsed). Pregnancy and implantation rates were analysed.

Main results and the role of chance: A total of 1297 cycles were analyzed. 259 blastocysts collapsed once or more during development (19.9%) and the

remaining 1038 either contracted minimally or neither contracted nor collapsed during development (80.1%). A significantly higher ongoing pregnancy rate of 51.9% (CI95% 48.9%-59.9%) was observed when blastocysts which had not collapsed during development were replaced compared to cycles in which collapsed blastocysts were transferred 37.5% (CI95% 31.6%-43.4%). A multivariable logistic regression analysis revealed an ongoing pregnancy 1.78 (1.33-2.40) OR, and the stratified study demonstrated that blastocyst collapse is mainly affecting implantation potential on good quality embryos.

Limitations, reasons for caution: Live birth rates should also be investigated to see if there is a difference in final outcome between the groups.

Wider implications of the findings: The pattern of blastocyst collapse could improve IVF/ICSI outcome following eSET at blastocyst stage. Therefore, it could be used as a negative marker for embryo selection.

Trial registration number: not applicable.

O-137 The impact of dry incubation on osmolality of media in time-lapse culture dishes

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Study question: Does continuous culture in a dry incubator lead to an increase in culture medium osmolality that is affected by the type of time-lapse dish used?

Summary answer: Osmolality increased for all tested time-lapse dishes, with a greater effect for Primovision, Miri-TL and standard microdrop culture compared to Embryoscope dishes.

What is known already: Osmolality of culture media is critical for optimal embryo development with high osmolality having adverse effects. Osmolality of culture media is affected by factors such as humidity, temperature, dish preparation method and media droplet volume. Compared to humidified incubation, negative effects of dry incubation on human embryo development have been observed. However, the use of dry incubators is becoming increasingly common due to advantages of time-lapse continuous culture. Different time-lapse systems use different dishes with various media to oil surface ratios, which may alter the effect of dry incubation on osmolality.

Study design, size, duration: Changes in osmolality were measured in four culture dishes over 7 days in a dry incubator (Miri-TL). The cultures dishes were: Miri-TL culture coin (MTL), Vitrolife EmbryoSlide (ES), and Primovision dish (PV), with a Vitrolife Micro-Droplet dish (MD) as the control. The study was repeated three times. Osmolality was determined in duplicate at days 0, 3, 5 and 7 of culture. The impact of oil depth on osmolality was also studied for standard MD dishes.

Participants/materials, setting, methods: Dishes were prepared with protein supplemented media and oil. For MD dishes, the impact of 3, 5 or 7 mL oil overlay was determined over 5 days. The time-lapse dishes were prepared as per manufacturer. MD dishes had 20 uL microdroplets under 5 mL of oil. For each condition, osmolality was tested on a 10 uL sample using a VAPRO osmometer. Relative changes in osmolality were assessed (ANOVA) and means were compared using Tukey's HSD.

Main results and the role of chance: Culture media osmolality was affected by oil depth, with a 3 mL oil overlay resulting in the highest osmolality compared to either 5 or 7 mL. For time-lapse dishes, osmolality increased as the culture continued over 7 days in the dry incubator. From a starting osmolality of 258 mOsm, media osmolality in MD, MTL and PV increased similarly over 7 days of culture, but for ES dishes, the increase in osmolality occurred to a lesser extent, showing that the ES condition was less prone to increasing osmolality during a 7 day dry incubation. For MD, MTL, PV and ES dishes, osmolality increased, respectively, by 26.9, 23.7, 20.3 and 12.2 mOsm on day 3, 42.3, 42.5, 38.5 and 27.7 mOsm on day 5, and 53.5, 50.4, 57.3 and 33.2 mOsm on day 7. ES osmolality was lower than the control MD dish on day 3 ($p < 0.05$), both MD and MTL on day 5 ($p < 0.05$) and all dishes on day 7 ($p < 0.01$), showing that the ES condition had the greatest ability to prevent media evaporation, resulting in slower osmolality rise over 7 days of culture during dry incubation.

Limitations, reasons for caution: This study was performed using a MiriTL bench top incubator; therefore the results may not be applicable to other dry incubators. Osmolality increased in all dishes and the impact of these increases on human embryo development is unknown.

Wider implications of the findings: The osmolality of culture media increased with dry incubation despite oil overlay in various dishes, an increase that was dish dependent. The ES dish, which uses deep culture wells that results in the lowest ratio of culture medium surface area to oil, had the lowest amount of media evaporation.

Trial registration number: Not applicable.

O-138 Utility of the Vienna consensus on ART Laboratory Performance Indicators for managing multiple IVF laboratories performance

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Study question: Is the Vienna consensus on Laboratory Performance Indicators useful in standardising performance and setting benchmarks across a commercial group of IVF clinics?

Summary answer: The Vienna consensus facilitates a uniform reporting platform, however, with the focus on extended embryo culture Day 2 embryo development rates are less relevant.

What is known already: Laboratory performance indicators are critical for managing laboratory quality control. Ensuring reproducible laboratory outcomes across a growing commercial group of IVF clinics is essential, with agreed outcomes and benchmarks conventionally set in house. The Vienna consensus, sets 19 indicators including 12 key performance indicators (KPIs), five performance indicators (PIs) and two reference indicators (RIs) with internationally agreed definitions, competence levels and aspirational benchmark values.

Study design, size, duration: Prospective cohort study of seven IVF clinic performing 2418 fresh and 1300 frozen cycles between 1 January to 30 June and nine IVF clinics performing 1799 fresh cycles and 1005 frozen transfer from 1 July to 31 October 2017.

Participants/materials, setting, methods: Nine IVF clinics in the United Kingdom and Poland. Comprehensive data collection using IDEAS software platform, with centralised analysis of clinic performance in accordance with the 19 laboratory indicators and associated competence and aspirational benchmark values described in the Vienna consensus.

Main results and the role of chance: Of the 19 indicators, 18 were routinely recorded with day 2 embryo development rate not collected by any centre due to extended embryo culture systems. For the 18 different measures, different laboratories were able to achieve each of the predetermined competence values with only blastocyst survival (competence >90%) attained by all laboratories (range 91.2 to 100%). However, no laboratory achieved all competency values. Despite overall high clinical pregnancy rates exceeding national averages (47.2% per embryo transfer for fresh and 58.2% for frozen), the highest performing laboratory only achieved 12 of the 18 competency values. The clinic with the lowest compliance attained only four competencies, despite achieving clinical pregnancy and live birth rates in accordance with the Human Fertilisation and Embryology Authority limits. The aspirational benchmarks for ICSI fertilisation rate (≥80%), IVF fertilisation rate (≥75%) and blastocyst development rates (≥35%) were not achieved by any clinics with the highest observed rates being 78.0%, 73.7% and 59.5% respectively.

Limitations, reasons for caution: Patient characteristics were not taken into account with summary data used for each clinic, adjustment for confounders is likely to enable more accurate identification of clinical and laboratory issues. The aspirational benchmarks may not be achievable using summary data for a broad patient population.

Wider implications of the findings: The Vienna consensus provides an agreed standard for clinical and laboratory reporting, with each competency value potentially attainable by every clinic. UK regulator data on clinic outcomes potentially provides false reassurance, with external benchmarking and group performance providing a more accurate estimate of achievable performance.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 37: ENDOMETRIOSIS AND ENDOMETRIUM: CLINICAL RESEARCH

Tuesday 3 July 2018 Room 116 10:00–11:30

O-139 Endometrial scratching by pipelle biopsy in IVF (the PIP study): A pragmatic randomised controlled trial

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[8]*University College London Hospital, Reproductive Medicine Unit, London, United Kingdom*
[9]*Homerton University Hospital, Homerton Fertility Centre, London, United Kingdom*
[10]*Melbourne IVF, Melbourne IVF, Melbourne, Australia*
[11]*Fertility Associates, Fertility Associates, Christchurch, New Zealand*
[12]*Fertility Associates, Fertility Associates, Wellington, New Zealand*

Study question:: Does endometrial scratching delivered by an endometrial pipelle biopsy, increase the probability of live-birth in women undergoing IVF/embryo transfer?

Summary answer: Endometrial scratching was not associated with any improvement in live birth rate.

What is known already: Endometrial scratching has been suggested to improve the probability of embryo implantation, and therefore pregnancy, in women undergoing IVF. It is proposed that the mechanical disruption to the endometrium results in a favourable inflammatory response, increasing the endometrial receptivity. Pooled results from randomised trials suggest benefit from endometrial scratching prior to an IVF cycle, especially in women with previous implantation failure. However, many of the studies had a high risk of bias secondary to study design, such as exposure of controls to endometrial disruption and lack of allocation concealment. Therefore, there is uncertainty about the validity of a beneficial effect.

Study design, size, duration: A pragmatic, multi-centre, open-label, randomised trial was conducted between June 2014 and June 2017 in 13 centres across five countries. Women were randomised 1:1 to either endometrial scratching or no procedure, using an online trial-specific database which ensured allocation concealment. Sample-size was calculated separately in women with recurrent implantation failure (≥2 unsuccessful embryo transfers, 15% increase live-birth rate anticipated) and without (8% increase). At 80% power and a 5% significance level, 1300 women were required.

Participants/materials, setting, methods: Eligible women were undergoing embryo transfer (fresh or frozen) using their own oocytes, with no recent exposure to disruptive intrauterine instrumentation. Women in the endometrial scratching arm underwent a pipelle biopsy between day 3 of the preceding cycle and day 3 of the IVF/embryo transfer cycle. The primary outcome was live-birth using an intention-to-treat approach. Risk ratio and 95% confidence intervals were calculated, and logistic regression was used to test for subgroup differences.

Main results and the role of chance: A total of 1364 women were randomised: 690 to endometrial scratching and 674 to control. Baseline and cycle characteristics were similar between the two groups. Endometrial scratching was not associated with any improvement in live birth rate 26.1% (180/690) vs 26.1% (176/674), odds ratio =1.00 (0.78 to 1.27). The effect remained similar after adjusting for protocol deviations and the observation that fewer women in the control arm underwent an embryo transfer. There was no difference in the rate of biochemical pregnancy, ectopic pregnancy, ongoing pregnancy, clinical pregnancy or multiple pregnancy between the two groups. Subgroup analysis did not identify any subpopulations that may benefit from endometrial scratching; there was no evidence of a benefit in women: with recurrent implantation failure, undergoing fresh or frozen cycles, or depending on the timing of the scratch in relation to the embryo transfer. The median pain score from endometrial scratching was 3.5/10 (IQR 1.9–6.0). There were 14 adverse events related to endometrial scratching: 7 vasovagal reactions, 2 excessive bleeding and 5 excessive pain.

Limitations, reasons for caution: Although a higher proportion of women in the endometrial scratching arm underwent embryo transfer, this did not impact the results. The definition of recurrent implantation failure was two or more previous unsuccessful embryo transfers, with no consideration for the stage or quality of the previously transferred embryos.

Wider implications of the findings: This was a large trial and the pragmatic design increases the generalisability of the results. As the beneficial effects reported previously were not confirmed by this trial, and the procedure caused a moderate amount of pain and bleeding, the current use of endometrial scratching in fertility clinics should be abandoned.

Study funding/competing interest(s) Research grants were provided by (New Zealand): A+ Trust, Auckland District Health Board; The Nurture Foundation for Reproductive Research; and Maurice & Phyllis Paykel Trust. There are no competing interests.

Trial registration number: ACTRN12614000626662

O-140 The impact of non-cavity distorting intramural fibroids on IVF outcomes: A systematic review and meta- analysis

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Study question: To identify the impact of non- cavity distorting uterine fibroid on IVF outcomes.

Summary answer: Presence of non-cavity distorting IM fibroids with or without subserosal fibroids will reduce LBR CPR, and MR but not IR.

What is known already: Uterine fibroids (UF) including. those non-cavity distorting variant can cause infertility. When these women require IVF treatment, controversies whether its presence will negatively impact on their IVF outcomes has been continually debated. The heterogenous nature of UF has given inconsistence result in many published studies. Available meta analysis on this subject however is dated and has significant heterogeneity. Moreover, IVF techniques and protocol have also been rapidly evolved since the last review. An updated review and Meta-Analysis is needed to give informed choice to patient and IVF practitioner.

Study design, size, duration: Systematic review and meta-analysis.

Participants/materials, setting, methods: We performed a systematic review and meta-analysis on extracted data according to PRISMA. Relevant articles were selected from literature search carried out using Medline, Embase, and Web-of-science. Randomised and non-randomised controlled studies were included. Primary outcomes are live birth rate (LBR) and clinical pregnancy rate (CPR) and secondary outcomes are implantation rate (IR) and miscarriage rate (MR). Qualities of included studies were scored using Newcastle-Ottawa Quality Assessment Scales and meta-analysis conducted by using RevMan 5.3.

Main results and the role of chance: We found 2482 articles but only included 26 articles that satisfy our inclusion and exclusion criteria. All are non-randomised studies with a total of 7733 IVF cycles. Most (26/27) are high qulity studies. Fourteen (n = 14/27) studies included IM with SS and only (n = 12/26) reported IM only. Our meta-analysis revealed non-cavity distorting IM fibroids with or without subserosal fibroids, significantly reduced CPR (RR = 0.83, 95% CI = 0.63–0.99, P < 0.000, I^2 = 42%, 2955 IVF cycles, 23 studies), LBR (RR = 0.80, 95% CI = 0.73-0.87, P = 0.000, I^2 = 40%, 1815 cycles, 16 studies) and MR (RR = 1.26, 95% CI = 1.06-1.50, P = 0.009, I^2 = 21%, 509 cycles, 16 studies). Where as IR (RR = 0.79, 95% CI = 0.81–1.01, P = 0.07, I^2 = 33%, 1379 IVF cycles, 10 studies) is not significanltly

affected. Our subgroup analysis on non cavity distorting IM only, revealed significantly reduced CPR (RR = 0.84, 95% CI = 0.77-0.92, P = 0.000, I^2 = 18%, 1871 IVF cycles, 12 studies), LBR (RR = 0.83, 95% CI = 0.73–0.94, P = 0.005, I^2 = 32%, 799 IVF cycles, 6 studies) and MR (RR = 0.84, 95% CI = 0.77-0.92, P = 0.000, I^2 = 18%, 1871 IVF cycles, 12 studies). However, IR (RR = 0.71, 95% CI = 0.49 – 1.02, P = 0.07, I^2 = 81%, 1511 IVF cycles, 5 studies).

Limitations, reasons for caution: included studies are non-randomised studies. The result of this study is still subjected to confounders relating to clinical heterogeneity.

Wider implications of the findings: This finding will facilitate clinician on the decision of need for performing myomectomy in non cavity distorting uterine fibroid.

Trial registration number: NA.

O-141 Supplementation in adolescent girls with endometriosis (SAGE): A double blind, randomized, placebo-controlled trial

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Study question: Does adjuvant supplementation with omega-3 fatty acids or vitamin D remediate pain and improve quality of life in adolescents with surgically-confirmed endometriosis?

Summary answer: All groups–vitamin D, omega-3 fatty acids, and placebo improved at three and six months in pelvic pain and mental and physical quality of life.

What is known already: Adolescents with endometriosis are an underserved population who struggle with chronic pain, missed school, poor social relationships, and concerns about future infertility. Endometriosis is a pro-inflammatory condition characterized by elevated levels of cytokines and growth factors, decreased cell apoptosis, and increased angiogenesis. Vitamin D and omega-3 fatty acids may decrease inflammatory factor proliferation, which could reduce progression of endometriosis and endometriosis-associated pain. Several articles and books exist in the lay press regarding the use of diet and supplementation to manage endometriosis, but there are no published data demonstrating an association between nutritional supplementation and modification of endometriosis symptoms.

Study design, size, duration: Double blind, randomized, placebo-controlled trial in which 69 adolescent girls and young women were randomized to receive vitamin D3 (2000 IU), fish oil (1000 mg), or placebo daily for 6 months – administered as identical white capsules. An independent biostatistician developed a permuted blocks randomization schema that the research pharmacy implemented to allocate the blinded study medications. No member of the study team nor the participants were privy to treatment assignment.

Participants/materials, setting, methods: 59 girls and young women (age 12-25) recruited from a pediatric gynecology clinic completed all study visits. 19 were allocated to placebo, 23 to vitamin D, and 17 to fish oil. All participants had surgically-confirmed endometriosis and a minimum visual analog scale (VAS) pain score ≥ 3 out of 10 for their worst pain in the month preceding study enrollment. There were no differences in severity of endometriosis or pelvic pain at baseline.

Main results and the role of chance: 59 participants completed all study visits; there were no differences in characteristics between completers and non-completers. The majority of participants had rASRM stage 1 endometriosis at surgical diagnosis. From baseline to 6-months, a significant increase in serum 25(OH)D was observed only in the vitamin D arm; likewise, an increase in total omega-3 fatty acids, eicosapentaenoic acid(EPA) and docosahexaenoic acid (DHA) was observed only among those randomized to fish oil. Participants in all 3 arms demonstrated improvement in VAS pain; mean(SD) worst pain in the past month improved from baseline to 6 months in placebo [6.0 (1.9) to 4.4 (3.2), P_{trend} = 0.06], vitamin D [7.0 (2.2) to 5.5 (3.0), P_{trend} = 0.04], and fish oil [5.9 (2.9) to 5.3 (3.5), P_{trend} = 0.40] arms ($P_{heterogeneity}$ = 0.64). The mean number of hours that pelvic pain usually lasted improved similarly from baseline to 6-months among participants in all 3 arms. Three participants from each arm required a clinical visit for pain at the 6-month time point, which was similar to baseline. Average use of non-narcotic pain medication did not change from baseline in any study group. Quality of life similarly improved among the 3 arms for both mean SF-12 mental component and physical component scores ($P_{heterogeneity}$ = 0.38 and 0.60 respectively).

Limitations, reasons for caution: The small sample size was powered to detect a minimum difference in change of 1.6 in VAS pain, requiring 19 subjects per arm, assuming standard deviation = 1.7, 80% power, and alpha-error = 0.05. Enrolled from one clinic, the experience of this population of adolescents may not generalize to all young women.

Wider implications of the findings: The strong placebo effect evident in multiple outcome measures demonstrates the possibility that enhanced clinical interaction and active study involvement improves pain associated with endometriosis. Vitamin D and omega-3 fatty acids may not reduce pelvic pain, pain medication usage, or improve quality of life independent of this effect.

Trial registration number: NCT02387931

O-142 The Endometriosis Fertility Index (EFI) can be estimated accurately prior to surgical treatment of endometriosis

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Study question: Can the final EFI-score be estimated before surgical endometriosis treatment, from pre-operative non-surgical findings only (type-A estimation) or pre-operative plus diagnostic laparoscopy data (type-B estimation)?

Summary answer: The final EFI can be estimated well before completion of operative laparoscopy for endometriosis, based on non-surgical data with or without surgical diagnostic laparoscopic data.

What is known already: The EFI is a thoroughly validated clinical instrument to predict non-ART clinical pregnancy rate after endometriosis surgery, but as the EFI-score is calculated at the end of an operative laparoscopy – i.e. after all endometriosis lesions have been removed – it currently is not clear if this score can be also estimated prior to surgical treatment. If this would be possible, then such estimated EFI-score could be used to triage patients with endometriosis-associated infertility to treatment with surgery or with ART (assisted reproductive technologies).

Study design, size, duration: Prospective single center cohort study on 82 patients, undergoing laparoscopic endometriosis surgery between June and December 2016. Although the study was exploratory, the sample size (minimum 71) was appropriate to confirm an agreement of minimally 95% between estimated and final EFI (one-sided test, alpha 0.05, beta 0.8). Two definitions were used for agreement: clinical (scores within the same EFI-score range: 0-4, 5-6, 7-10) and numerical agreement (maximally allowed difference of 1 EFI-point, irrespective of range).

Participants/materials, setting, methods: All patients underwent complete CO_2-laser-laparoscopic excision of any rASRM-stage endometriosis in a tertiary referral center. A coded clinical research file (CRF) consisted of 3 parts: type-A contained only pre-surgical information, type-B contained type-A information plus information of the diagnostic phase of laparoscopy, and type-C included all pre-, intra- and post-operative information. An algorithm was created to translate pre-surgical clinical/imaging information for EFI type-A calculation. Scoring was done by one person, with an appropriate time interval.

Main results and the role of chance: For the comparison between the estimated EFI type-A and the final EFI, the rate of agreement was high, with narrow

95% confidence intervals (CI): 91.5% (95%CI 83.2%-96.5%) for clinical agreement, and 87.8 (95%CI 78.7%-94.0%) for numerical agreement. For the comparison between estimated EFI type-B and the final EFI, agreement rates were even higher: 98.8% (95%CI 93.4%-100%) and 96.3% (95%CI 89.7%-99.2%) for clinical and numerical agreement respectively. Taken together, these results confirm that the final EFI-score (calculated after operative laparoscopy) can be accurately estimated by pre-operative non-surgical findings only (EFI type-A estimation), and the accuracy of estimation is increased when the information from the diagnostic phase of the laparoscopy was added to the pre-operative non-surgical findings (EFI type-B estimation). It is also noteworthy that estimation of the rASRM stage was far less accurate, especially when based on pre-operative non-surgical findings only (agreement between type-A and final rASRM stage 68.3% (95%CI 57.1%-78.1%) and type-B and final 90.2% (81.7%-95.7%).

Limitations, reasons for caution: Rating was done by one person, so it remains to be confirmed whether others can repeat the estimations with similar accuracy. The high quality of the ultrasound (and report) performed by experts - to calculate EFI-type-A –, may render implementation in general clinical practice outside endometriosis referral centers less feasible.

Wider implications of the findings: If other groups confirm our accurate pre-surgical estimation of the final EFI, this will revolutionize the possibilities of individualizing the management of endometriosis-associated infertility. Choice of first-line treatment with laparoscopic surgery or medically assisted reproduction could be based on the estimated EFI, while taking pain symptoms into account.

Trial registration number: Institutional study registration number: S59221.

O-143 The effect of different IVF protocols for women with endometriosis on their IVF outcomes: A Systematic review and Meta-Analysis

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Study question: What is optimal IVF protocol for women with endometriosis and the effects of different protocols on the outcomes of Live Birth (LBR), Clinical Pregnancy (CPR), oocyte retrieval (MOR) and miscarriage.

Summary answer: The ultra-long protocol should be the recommended protocol for women with endometriosis and/or endometrioma. The antagonist protocol has given non-inferiority effect to the protocol.

What is known already: Infertile women with endometriosis may require IVF/ICSI to conceive. Women with endometrioma during IVF treatment yield fewer oocytes during oocyte retrieval procedure despite requiring more stimulation drugs. It is also established that the embryos from women with endometriosis and/or endometrioma is poorer compared to women with no endometriosis, and the exact mechanism is still left unexplained. Various treatment strategies have been investigated to improve the outcomes. Several protocols are utilised including long protocol, ultra-long protocol and antagonist protocol intended to improve the IVF outcomes, but to date, a suitable protocol exclusive to women with endometrioma is yet to be found.

Study design, size, duration: This is a Meta-Analysis and systematic review. Electronic search through various Databases including Pubmed, ScienceDirect, OVID, Cochrane, Medline and Google Scholar was performed to search for both published and unpublished data.

Randomized Controlled Trials (RCT), cohort studies, retrospective studies as well as prospective studies from 1980 up to 2017 were reviewed.

Participants/materials, setting, methods: A total of 2667 papers were identified through electronic search and after screening, 15 studies were eligible to be included for data synthesis. The quality of each paper was assessed and scored according to Newcastle-Ottawa Assessment scale. All suitable data were extracted and analysed using Review Manager 5 software.

Main results and the role of chance: We retrieved 2667 articles looking into these protocols however after careful scrutiny we found several studies comparing ultralong vs. long protocol (n = 9), long vs. antagonist (n = 4) Short vs. antagonist (n = 1), ultralong vs. short agonists (n = 2). From the meta-analysed data, there are significantly higher CBR in women with ultralong protocol (OR CI 95% (1.23, 4.10) 853 cycles, 9 studies, I2 = 70%) as reported by the previous meta-analysis.

There was no significant difference in the miscarriage rate [OR CI 95% 1.19 (0.55,2.58) 361 cycles, 4 studies] and little difference in the meiosis II oocyte retrieval rate [OR CI 95% -0.30 (-0.48,-0.12)].

Limitations, reasons for caution: Due to the observational nature of the included studies, the result of this study is subjected to confounders relating to clinical heterogeneity.

The relatively small number of studies and sample population within each study may increase the risk of bias.

Wider implications of the findings: The ultra-long protocol is thought to render the endometrioma dormant, thus improving the chance of retrieving high-quality oocytes and embryos. However due to potential side effects, patients can be pretreated with Dienogest (Visanne) which has been proven to reduce symptoms and prevent recurrence, and may reduce the size of endometrioma.

Trial registration number: not applicable.

O-144 Nationwide cohort study of the risk of major adverse cerebrovascular and cardiovascular events in the Asian women with endometriosis

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Study question: Based upon positive correlation of anti-mullerian hormone (AMH) to cardiovascular disease in recent studies, we investigated the association between endometriosis and major adverse cerebro-/cardio-vascular events (MACCE) in an Asian population.

Summary answer: The risk of MACCE including major CVD or CVA increases in the Asian women with endometriosis at reproductive age.

What is known already: Endometriosis has a negative impact on ovarian reserve. AMH, an ovarian reserve marker, declines with increasing cardiovascular risk in women.

Study design, size, duration: Taiwan National Health Insurance Research Database (NHIRD) was utilized to answer the question. A retrospective population-based cohort study was held through 1997 to 2013.

Participants/materials, setting, methods: A total of 17,543 patients with endometriosis and age between 18 and 50 years were identified from 1,000,000 general populations after excluding initially concomitant diagnoses of MACCE. The MACCE was defined as major cardiovascular disease and cerebrovascular accident. The comparison group (n = 70,172) without endometriosis was selected matching in a 1:4 ratio. We compared the demographic data, frequency of comorbidities, incidence of and risk of MACCE between the disease and non-disease groups.

Main results and the role of chance: With a median follow-up of 9.2 years, the endometriosis patients had significantly higher frequency and cumulative incidence of MACCE than non-endometriosis counterparts (2.76% vs. 2.18%, $p < 0.0001$). After adjusting with multivariate analysis, the patients with endometriosis had 1.17-fold greater risk for future development of MACCE (95% CI 1.05-1.29, $p = 0.0053$). In further, the adjusted hazard ratios of endometriosis for major CVD and CVA were 1.19 and 1.16, respectively (all $p < 0.04$). Apart from endometriosis, older age, lower urbanization level, hypertension and diabetes were identified as risk factors for MACCE occurrence as well.

Limitations, reasons for caution: This is a retrospective study to analyze the diagnosis of endometriosis and MACCE in a nationwide population data.

Further investigation will be needed to clarify the trajectories of serum AMH and associated incidence of MACCE in endometriosis patients.

Wider implications of the findings: Early awareness of deteriorating ovarian reserve via checking serum AMH is important to prevent following MACCE in endometriosis patients. Early interventions, such as surgery or medication, would be helpful to break the vicious circle among endometriosis, deteriorating ovarian reserve, and MACCE.

Trial registration number: not applicable.

INVITED SESSION

SESSION 38: EUROPEAN AND GLOBAL ART MONITORING

Tuesday 3 July 2018 Room 211 + 212 11:45–12:45

O-145 European IVF-monitoring of ART and development of a strategy for vigilance

O-146 ICMART World Report 2014

G.D. Adamson[1], F. Zegers-Hochschild[2], S. Dyer[3], G. Chambers[4], O. Ishihara[5], R. Mansour[6], M. Banker[7], J. De Mouzon[8]

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International Committee for Monitoring Assisted Reproductive Technologies (ICMART) World Report on ART, 2014 and The International Glossary on Infertility and Fertility Care, 2017.

Study question: In 2014 what was global utilization, effectiveness and safety of ART, and what is status of The International Glossary on Infertility and Fertility Care, 2017.

Summary answer: Globally, ART utilization and data collection continue to increase but with wide variations in utilization, effectiveness and safety. The significantly expanded, revised Glossary was published.

What is known already: ICMART began ART global data collection in 1991. Utilization, effectiveness and safety have continuously improved with more cycles, higher pregnancy rates and lower multiple birth rates, the latter coupled with an increase of frozen embryo transfer (FET) cycles; however, wide variations exist globally. Over 7 million ART babies have been born. ICMART has helped develop African registries in association with the African Network and Registry for Assisted Reproductive Technology (ANARA). Data collection and quality remain challenging. The Glossary is an important document created through ICMART's leadership with a partnership of all the major global reproductive medicine organizations and other stakeholders.

Study design, size, duration: Countries and regions annually collect ART data, some prospectively and others retrospectively. ICMART retrospectively requested these data from all known global sources for 2014, analyzed them collaborating with Uppsala Clinical Research (UCR) at the University of Uppsala, Sweden, and presents preliminary results. The Glossary was created through a global partnership of major professional organizations and other stakeholders. ICMART's Data Collection System (DCS) first used for 2014 data represents years of collaboration between ICMART and UCR.

Participants/materials, setting, methods: The European IVF Monitoring Consortium (EIM), Society for Assisted Reproductive Technology (SART), Latin American Network of Assisted Reproduction (REDLARA), Australian/ New Zealand Registry, ANARA and other countries, totaling 70, contributed national data through standardized formats to the DCS. Data were reviewed, corrected, analyzed and summarized by ICMART working with UCR using standard statistics. ICMART led a partnership of international stakeholders who revised the ICMART/WHO 2009 Glossary. ICMART and UCR collaborated to create the ICMART DCS.

Main results and the role of chance: Data collection and analysis are ongoing. Preliminary results are presented at ESHRE. The number of ART cycles continues to increase, but utilization is still very influenced by affordable access to ART which is related to insurance or public funding. Regional differences persist in the age of the population treated, number of embryos transferred, rate of multiple births, and other factors.

The International Glossary on Infertility and Fertility Care, 2017, is significantly expanded and has revised many commonly used terms. The new ICMART DCS is functional.

The role of chance is minimal. Actual global ART results are limited to reporting countries and clinics representing approximately 2/3 of global cycles. However, this is a very large sample size from which imputation of total global results is performed. Many countries have limited data validation and ICMART can perform only minimal verification of submitted data.

Limitations, reasons for caution: Actual reported data represent 2/3 of total performed cycles. Data validation is not universal or standardized. Routine use of Glossary terms takes time and translation into multiple languages. The ICMART DCS needs to have its functionality continuously assessed and improved so that data quality can continue to expand and improve.

Wider implications of the findings: ICMART World Reports standardize data, track trends, enable comparisons, stimulate questions and improve ART quality. Better understanding of ART increases societal acceptance and support for ART research and clinical access. The International Glossary on Infertility and Fertility Care, 2017 represents a unique stakeholder partnership that harmonizes ART terminology globally.

O-147 Gender inequality and utilisation of assisted reproductive technology: an international analysis

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[4]Fertility Physicians of Northern California, Palo Alto Medical Foundation, Palo Alto-California, U.S.A.

Study question: Is there an association between gender inequality and utilisation of assisted reproductive technology (ART) treatment globally?

Summary answer: Countries with less gender inequality have higher rates of ART utilisation, regardless of the country's level of economic and human development.

What is already known: ART utilisation varies enormously worldwide. Previous research has indicated that consumer affordability, access to clinics, and normative cultural acceptance are important factors in ART utilisation.

Study design, size, duration: A longitudinal cross-national analysis of ART utilisation in 69 countries, with the outcome defined as the total number of ART cycles per million population. The Gender Inequality Index (GII), Human Development Index (HDI), and Education Index (EI) developed by the United

Nations Development Programme (UNDP), were used as composites measures of gender inequality, human development and education respectively. Data is drawn from several sources (ICMART, UNDP, World Bank).

Participants/materials, setting, methods: Our sample includes data from 69 countries for the years 2002–2012. Following a descriptive graphical and bivariate correlation analysis, mixed and fixed effect regression methods served to establish the cross-sectional and longitudinal relationship between the predictor variables and the number of ART treatments per million population in a country.

Main results and the role of chance: A one standard deviation movement in the GII towards greater gender equality is associated with an increase in ART utilisation by a factor of 2.8 (95% CI: 1.5–5.2), when controlling for a country's level of economic and human development. There were differences in the strengths of association of different components of gender equality and human development with ART utilisation. Increases in a country's female parliamentary representation, level of male and female education and wealth had the strongest longitudinal relationships with ART utilisation. Preliminary findings indicate the following: an increase of 10% in female parliamentary representation saw a county's ART utilisation increase by a factor of 1.3 (95% CI: 1.1–1.6). A one standard-deviation increase in the EI (equivalent to 2.5 additional mean years of schooling) was associated with ART utilisation increasing by a factor of 1.9 (95% CI: 1.0–3.5). A one-unit increase in log gross national income per capita (2011 PPP US$) was associated with ART utilisation increasing by a factor of 2.6 (95% CI: 1.5–4.3).

Limitations, reasons for caution: Multiple imputation was used to impute missing covariate data for 13% of observations. Countries, predominantly in the Middle East and North Africa, with low ART clinic reporting rates were excluded from the analysis. As the analysis was cross-national, inferences to the individual level are not possible.

Wider implications of the findings: Access to infertility care is central to women's sexual and reproductive health, to women's rights, and to human rights. Our analysis demonstrates that as gender equality improves, so does utilisation of assisted reproductive technologies. Progress towards global, equitable access to infertility care can hence be expected with global improvement of women's status within societies.

Study funding/competing interest(s): None.

Trial registration number: n/a

INVITED SESSION

SESSION 39: NEW ASPECTS IN POLYCYSTIC OVARY SYNDROME

Tuesday 3 July 2018 Room 111 + 112 11:45–12:45

O-148 First line treatment in PCOS: what to do?

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Abstract text

There are several guidelines on first line treatment in PCOS. The most relevant one's are the ESHRE/ASRM consensus, the NICE guideline and the WHO's global guideline which is under development. (1,2,3) The common denominator of these guidelines is that they all -broadly speaking- recommend lifestyle modification, followed by or combined with medical ovulation. With respect to the latter there are some variations: ESHRE/ASRM recommend Clomiphene Citrate (CC) as first line treatment, while NICE recommends CC, metformin or a combination of the two, while WHO advises CC or Letrozole. The explanation for these differences is probably the accumulation of evidence which has taken place in between the dates of publication of the various guidelines.

The evidence on the commonly used drugs has been summarized in conventional but authoritative Cochrane reviews, which provide the data on pairwise meta analysis. (4,5,6) On clomiphene citrate the evidence suggests that it improves the chance of a clinical pregnancy compared with placebo without any differences between different anti-oestrogens (low quality evidence) On aromatase inhibitors, specifically Letrozole, the evidence suggests that Letrozole improves live birth and pregnancy rates compared with CC. (low quality evidence) On insulin sensitisers, specifically metformin, the evidence is complex, but suggests that metformin alone may be beneficial over placebo for live birth, and that any superiority of metformin over CC and vice versa is inconclusive, although there may be interaction with body mass index. (very low to low quality evidence) Combined therapy with metformin and clomiphene citrate improved clinical pregnancy compared with clomiphene citrate alone it is at present unknown whether this translates into increased live births and gastrointestinal side effects are considerable (low to moderate quality evidence).

This traditional pairwise meta-analyses only allow the comparison of two interventions for ovulation induction. Since many of these medications have not been compared directly it is still not clear what the most effective treatment option would be. A recent network meta-analysis has studied all possible combinations and has provided interesting data, which can be summarized as follows: all interventions are effective, Letrozole improves live birthrate compared to CC. Combined therapy with CC and metformin is superior to CC and to metformin in terms of pregnancy rates.

The conclusion, taking all available evidence into account, is that at present letrozole and combined treatment with CC and metformin are the drugs of first choice.

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O-149 Does race and ethnicity matter for metabolic risks in PCOS?

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Abstract Text

The phenotypic appearance of polycystic ovary syndrome (PCOS) varies across geography, ethnicity and race. The genotypic variation is not as marked comparing Asian and Caucasian populations, suggesting that environmental factors may play a major role in the presentation of PCOS.

Over the past few decades, it has been recognised that hyperinsulinism, insulin resistance, diabetes mellitus and hyperlipdemias are more common in people with PCOS when compared to controls of the same body mass index. Early studies indicated marked differences in metabolic profiles between immigrants in their recently arrived country, people of the same ethnicity who remained in their original country and other population groups of different races.

Significant differences are found in the metabolism in PCOS between Hispanics, African Americans and those of European decent in North America.

These have been compared with Europeans residing in Europe with the same ethnic background. There are also marked differences between Asians, depending on whether they are East Asian or South Western Asian, with major discrepancies in body mass index, diabetes mellitus and insulin resistance.

Recent studies in the Indigenous Australian population have shown the marked change that diet and lifestyle has induced over the past thirty years as people moved from a high exercise and traditional diet lifestyle to more western food and sedentary practices.

The increasing recognition of metabolic dysfunction in patients with PCOS makes the diagnosis, investigation and management of the condition even more important, affirming the importance of international guidelines for PCOS. Awareness of individual group risks will also lead to superior treatment for metabolic and fertility dysfunction.

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INVITED SESSION

SESSION 40: ERRORS IN IVF: DO THEY OCCUR AND HOW MANY?

Tuesday 3 July 2018 Room 113 + 114 + 115 11:45–12:45

O-150 To err is human: even in IVF

D. De Ziegler[1], P. Pirtea[2], M. Poulain[3], R. Fanchin[3], J.M. Ayoubi[3]

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The seminal publication of Kohn et al. (To Err is Human: Building a Safer Health System. Kohn LT, Corrigan JM, Donaldson MS, editors. Washington (DC): National Academies Press (US); 2000.), has made us aware of the fact that safety systems need to acknowledge that we, humans, make and will continue to make mistakes. IVF is no exception. Considering that IVF is performed in basically healthy people, avoiding errors of possible catastrophic consequences is of paramount importance.

The first step for minimizing the consequences of our errors is to understand the context in which they occur. Two distinct factors that define this context need to be clearly apprehended. First, there are the existing hazards, which are potential sources danger inherent to any procedure. In mountain climbing for example, one typical hazard is height. Second, there are the prevailing risks which are defined as the possibility that something unpleasant happens – an adverse event. Risks are inherently affected by existing hazards. In our mountain climbing example, slipping on top of a mountain can obviously have more dreadful consequences than if the same happens in low land. While hazards cannot be altered – height is inherently linked to mountains – risks can be mitigated. In mountain climbing, roping will prevent the possibility of dreadful consequences from slipping. In medicine – in ART in particular – we need to develop safety systems that adequately prevent adverse events from occurring in spite of the hazards and risk existing and errors occurring.

For being effective, safety systems imply an in-depth knowledge and understanding of the existing risks and possible consequences or adverse events. For this, we commonly plot adverse events emanating from prevailing risks on a frequency vs. severity diagram. Certain minor adverse events are frequent and of menial consequences, which can be considered as acceptable. Other adverse events may have serious – possibly fatal – consequences and are therefore unacceptable. Only a proper understanding of the dynamic of each risk can permit to develop and efficient risk averting system.

In order to avert adverse events from occurring as a result existing risks, one needs to develop protective measures or defenses. The choice of defenses must result from a careful assessment of the risks existing and root cause analyses of the possible adverse events that can emanate from these risks. Improper and poorly designed and/or too-complex defenses that impede proper functioning will not properly avert risks and may actually constitute a risk factor of its own. One should beware of the too often encountered 'let's write another procedure attitude' instead of conducting a proper root cause analysis of any encountered adverse event.

Accepting that errors are made is a crucial step in the design of any safe operation, and ART is no exception. A safety system that acknowledges that it is human to err – in IVF included – will bank on enacting a no-blame safety culture that integrates and share incident reports for averting the occurrence of more severe adverse events. Return on experience of 'near misses' in a positive no-blame culture is a crucial step for enacting safety systems that correct procedure based on minor deviations before these result in catastrophes when left unheeded.

O-151 This is how many errors occur in the IVF lab: a true analysis

M. J. De Los Santos

IVI Valencia, IVF Laboratory, Valencia, Spain

In ART as in the rest of areas of application of tissues and cells there are situations that may trigger the incidence of adverse reactions (harm to a donor, harm to a recipient, harm to a foetus or offspring) and adverse events (risk of harm despite no harm actually occur).

Many IVF laboratories are very complex in terms of not only the technology and equipment being used but in the introduction of overprocesses adding many steps during procurement, processing and distribution. These and other factors, all contribute in creating the perfect culture media facilitating the errors to happen, and despite hazards can be prevented from causing serious unwanted effect by the implementation of barriers, incidents still may occurs due to the inadvertent weakness of each barrier.

This talk focuses on identifying the steps where errors may take place, analyzing the root causes in the IVF laboratory and how implement tools to measure the error rate, decrease it and control it.

There are different strategic policies for risk management that help in assessing and prioritizing the possible existing hazards, so the probability of an adverse event occurring will be kept to a minimum. Strategies such as "Failure mode, effects and critical analysis" (FMECA) are very useful of tools in ART. Such approaches help to create lists of relevant steps or processes under risk, make an estimation of any identified hazard to happen based on the severity of the error, its frequency as well as its probability of detection. All together will create a risk priority index which helps in ranking the risks and prioritize the actions to take.

As some of the causes leading to error in the IVF laboratory are due to human mistakes, the reasons of human errors will be also addressed. Among the factors to blame on, are: complexity of the activity, lack of education and training, perception that the procedures were not appropriate or efficient or just lack of attention to well-defined procedures. A list of key factors that facilitate the incidence of human errors has been compiled by Toft and coworkers. Some of them can be conscious automaticity, involuntary automaticity, ambiguous accountability and stress. All of them are considered especially relevant when considering aspect in IVF related to loosing traceability leading to sample

mismatch that are undesirable reaction that can occur in an IVF laboratory with important consequences for both patients and healthcare professionals.

SELECTED ORAL COMMUNICATIONS
SESSION 41: FOCUS ON BLASTOCYST STAGE
Tuesday 3 July 2018 Room 117 11:45–12:45

O-152 Predicting blastocyst formation rate using a hierarchical and data mining-based statistical model

J. Yao Serna[1], R. Milewski[2], D. Bodri[1], T. Sugimoto[1], R. Kato[1], T. Matsumoto[1], S. Kawachiya[1]

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Study question: Is it possible to establish equally efficient predictive algorithms for blastocyst (BT) formation using two different statistical methodologies?

Summary answer: Combining several cleavage-stage morphokinetic variables through data mining-based statistical algorithm achieved higher predictive power than the hierarchical model which included only the two strongest predictors.

What is known already: In recent years increasing number of time-lapse monitoring (TLM) studies focused on finding morphokinetic variables that could be predictors of blastocyst formation, successfully identifying several (early) cleavage-stage variables that were consistently associated with it. However, only a recent study (Motato et al, 2016) has managed to establish a four-category hierarchical score (including tM=time to morula and s3=t8-t5) predicting blastocyst development which showed a good discrimination ability (AUC: 0.849). It is arguable however, whether including tM is really useful if a blastocyst prediction model would be used in a clinical setting to select day-3 embryos for prolonged culture.

Study design, size, duration: All consecutive infertile patients from a single centre whose cleavage-stage embryos were submitted to prolonged culture (n = 1133) in a TLM incubator (EmbryoScope, Vitrolife) between 2012 and 2014 were included in this retrospective analysis. Patients (38.2 ± 3.9 years) underwent minimal ovarian stimulation, prolonged embryo culture and elective vitrification of expanded blastocysts. Cleavage-stage, static (tPNf, t2-t8), and interval morphokinetic parameters (cc2a-b, s2-3) were scored according to recently published consensus criteria and standardized to time of pronuclear fading (tPNf).

Participants/materials, setting, methods: A first, hierarchical model - including the two strongest BT formation predictors selected by multivariate analysis - and a second, more robust one - based on combination of principal component analysis (PCA) and logistic regression methods - were created. The PCA technique is a data-mining method that relies on transformation of an initial correlated set of features into new uncorrelated variables called principal components. For both models the area-under-the-curve values (AUC) with 95%CI were calculated.

Main results and the role of chance: The average rate of expanded blastocyst formation was 44% (501/1133). The examination of detailed histograms revealed that areas with a high, medium and low BT formation rates existed for all, eleven cleavage-stage morphokinetic variables thus permitting their conversion from continuous (hours) to categorical ones. Following multivariate analysis combining the two strongest morphokinetic predictors (t2 and s3) resulted in a simple, five-category (from 'E" to 'A") hierarchical model, representing 9, 15, 23, 34, and 19% of the entire cohort of cultured embryos, respectively. The model effectively distinguished between embryos of increasing BT development potential (18, 22, 40, 60 and 70% from category 'E" through 'A", $p < 0.0001$). For the second model quartiles of the created Sc parameter (Q1-Q4) had increasing BT formation rates (Q1: 14%, 'Q2": 39%, 'Q3": 61%, 'Q4": 81%, $p < 0.0001$). The AUC value of the data mining-based model was significantly higher compared to the hierarchical one (0.794, 95CI%: 0.77-0.82 versus 0.702, 95CI%: 0.67-0.73).

Limitations, reasons for caution: Our unselected cohort was biased towards advanced-aged, poor-prognosis patients undergoing mild IVF treatment coupled with single vitrified-warmed blastocyst transfer. This might limit generalizability to other less infertile populations or to centres that use different treatment protocols.

Wider implications of the findings: Both models were similarly successful in stratifying cleavage-stage embryos of different BT formation potential and could be further tested in everyday clinical practice. However, the more robust PCA-based methodology originally was developed in a fundamentally different setting from ours with similarly high predictive capabilities (Milewski, 2015) suggesting wider between-center applicability.

Trial registration number: not applicable.

O-153 Blastocyst morphology grading: what is the most important predictor of live birth when accounting for endometrial receptivity? A multicentre analysis of 2477 single blastocyst transfers

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Study question: What is the most important embryo morphology parameter associated with live birth (LB) rate after controlling for female age and endometrial receptivity?

Summary answer: Developmental stage (DevSt) of an embryo, followed by trophectoderm (TE) grading are the most important morphology parameters independently associated with LB rate.

What is known already: It is well known that transferring better quality embryos results in a higher chance of achieving a pregnancy. The move towards single blastocyst transfers (SBT) has placed more importance on the ability to select an embryo with the highest implantation potential, yet it remains unclear which morphology parameters should be prioritised. Previous studies investigating the impact of embryo morphology parameters on pregnancy outcome in SBT cycles have shown conflicting results. Most importantly, none of these studies have controlled for differences in endometrial receptivity, which plays a critical role in the establishment of a successful pregnancy and is a major confounder.

Study design, size, duration: This was a retrospective study (n = 2477 fresh SBTs from 2183 couples) of cycles performed between January 2012 and April 2015 at three different clinics. The embryo morphology parameters that were assessed on day of embryo transfer (day 5/6) by experienced embryologists included DevSt (morula (M), early blastocyst (EB), full blastocyst (BL), expanded blastocyst (XBL), hatching blastocyst (HgBL)), inner cell mass (ICM: A, B, C) and TE (A, B, C).

Participants/materials, setting, methods: Fresh IVF/ICSI SBT cycles where the value of serum progesterone on the day of human chorionic gonadotrophin (P4dhCG) was available were included. P4dhCG was used as it is considered one of the best markers of endometrial receptivity after ovarian stimulation. Generalized estimating equation regression models were used to evaluate the independent effect of DevSt, ICM grade and TE grade on LB rates, while controlling for female age, P4dhCG and the non-independence of data.

Main results and the role of chance: The mean age of the population was 36.0 years (95% CI: 35.8-36.2) and the mean P4dhCG was 2.95 (95% CI: 2.89-3.00). In most cycles XBL (27.5%) were transferred, followed by BL (22.2%) and HgBL (14.2%). DevStage was strongly associated with the probability of live birth ($p < 0.001$) independently of female age (OR: 0.92, 95% CI: 0.90-0.94) and P4dhCG (OR: 0.80, 95% CI: 0.74-0.87), both of which were also strong predictors of LB. The predicted LB according to the DevStage was a) M: 5.3% b) EB: 13.5% c) BL: 22.8% d) XBL: 31.1% e) HgBL: 35.2%. In BL, XBL and HgBL, the addition of ICM and TE grading in the multivariable analysis suggested that besides female age (OR: 0.92, 95% CI: 0.90-0.94) and P4dhCG (OR: 0.80, 95% CI: 0.73-0.88), only DevStage ($p = 0.003$) and TE quality ($p = 0.020$) were independent predictors of LB while the predictive capacity of ICM was no longer significant ($p = 0.296$). The mean probability of LB was higher for AA

embryos (34.8%, 95% CI: 31.6-38.0), followed by BA embryos (30.3%, 95% CI: 25.1-35.6), AB embryos (27.9%, 95% CI: 23.7-32.1) and BB embryos (23.9%, 95% CI: 19.3-28.6). This order of implantation potential persisted regardless of developmental stage (BL vs XBL vs HgBL).

Limitations, reasons for caution: This study is limited by its retrospective nature as well as the subjective nature of embryo scoring. Furthermore, limited numbers of embryos with poor quality ICM or TE (C grade) were transferred and hence the estimates around their predictive role are not as robust.

Wider implications of the findings: This is a large study analysing for the first time the independent role of blastocyst morphology in predicting LB after SBTs while controlling for both female age and P4dhCG. Its findings suggest that DevStage and then TE grade should be prioritised over ICM grade when selecting an embryo for transfer.

Trial registration number: N/A.

O-154 Predicting live birth by combining cleavage- and blastocyst-stage time-lapse variables using a hierarchical and a data mining-based statistical model

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Study question: Is it possible to establish highly predictive algorithms for live birth (LB) by combining cleavage- and blastocyst-stage morphokinetic variables using two different, robust statistical models?

Summary answer: The combination of cleavage- and blastocyst-stage morphokinetic variables through hierarchical or data mining-based statistical algorithms could be successfully used to predict live birth.

What is known already: Most of published predictive algorithms focused on cleavage-stage morphokinetic variables only, despite the fact the prolonged embryo culture is increasingly used as a way of improving pregnancy rates, especially in the context of single embryo transfer. So far, only a handful of studies examined the relation between implantation potential and blastocyst-stage morphokinetic parameters (Kirkegaard 2013, Campbell 2013, Motato 2016). Despite these efforts time-lapse parameters extracted from later stages of embryo development (morula, early and expanded blastocyst) are still under investigation, although it was suggested that adding these variables to existing cleavage-stage models could significantly improve their predictive potential.

Study design, size, duration: All 326 single blastocyst transfers (SBT) from all consecutive patients whose embryos were submitted to time-lapse monitoring (TLM) between 2012 and 2014 were retrospectively analysed. Patients (38.2 ± 3.9 years) underwent minimal ovarian stimulation, prolonged embryo culture in TLM incubator, elective vitrification and vitrified-warmed SBT. All pregnancies were followed-up until live birth. Early (PNf, t2-t9), late (tM, tearlyB, tfullB, texpB_{1-2}) and interval morphokinetic parameters (cc2a-b, s2-3) were scored according to published consensus criteria and standardized to tPNf.

Participants/materials, setting, methods: A first, hierarchical model - including the two strongest LB predictors (t2 and texpB_2) - and a second, more robust one - based on combination of principal component analysis (PCA) and logistic regression methods - were created. The PCA technique is a data-mining method that relies on transformation of an initial correlated set of features into new uncorrelated variables called principal components. For both models the area-under-the-curve values (AUC) with 95% confidence intervals were calculated.

Main results and the role of chance: The overall LB rate per SBT was 30% (97/326). The examination of detailed histograms revealed that areas with a significantly higher proportion of LBs ('optimal LB ranges") existed for six cleavage-stage (t2, t3, t4, t6, cc2a, and cc2b) and four blastocyst-stage variables (tearlyB, tBfull, texpB_1 and texpB_2) thus permitting the conversion of these morphokinetic variables from continuous (hours) to categorical ones. Combining two of the strongest variables (t2 and texpB_2) resulted in a simple, four-category ('A" to 'D") hierarchical model. The model effectively distinguished between blastocysts with increasing live birth potential (categories 'D": 9%, 'C": 27%, 'B": 39%, 'A": 54%, $p < 0.0001$). For the second model quartiles of the created Sc parameter (Q1-Q4) had increasing LB rates (Q1: 9%, 'Q2": 15%, 'Q3": 34%, 'Q4": 42%, $p < 0.0001$). AUC values for both models were equally high (0.722, 95CI%: 0.66-0.78 versus 0.723, 95CI%: 0.67-0.78 for the data mining-based and hierarchical models, respectively).

Limitations, reasons for caution: Our unselected patient cohort was biased towards advanced-aged, poor-prognosis patients who undergone mild IVF treatment coupled with single vitrified-warmed blastocyst transfer exclusively. This might limit generalizability to other less infertile populations or to centres which use different treatment protocols.

Wider implications of the findings: Although we have created two different statistical models their predictive power was similar and higher than most previously published algorithms. The more robust, PCA-based model was originally developed in a fundamentally different setting from ours, yet its predictive power was similarly high in both datasets suggesting a wider, between-center applicability.

Trial registration number: not applicable.

O-155 Day 5 versus Day 6 fresh and frozen blastocyst transfer cycle outcomes: a systematic review and meta-analysis

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Study question: Is there a difference in clinical pregnancy and live birth rates between day 5 and day 6 blastocysts following fresh and frozen transfers?

Summary answer: Day 5 (D5) blastocyst transfers present higher clinical pregnancy and live birth rates than day 6 (D6) in both fresh and frozen transfers.

What is known already: Blastocyst transfer (BT) is increasingly popular in assisted reproductive technology (ART) centers today. Numerous studies described conflicting results in clinical outcomes following D5 and D6 BT. To our knowledge, no meta-analysis has focused on clinical outcomes in both fresh and frozen BT. Concerning frozen blastocysts, one meta-analysis found no significant difference in pregnancy outcomes between D5 and D6 blastocysts (Sunkara *et al.*, 2010). Yet since 2010, more articles comparing D5 and D6 frozen embryo transfer cycles have been published, and ART practices have evolved particularly with the wide use of vitrification.

Study design, size, duration: Systematic review and meta-analysis of published controlled studies. Searches were conducted on MEDLINE, EMBASE, Cochrane Library, Eudract and clinicaltrials.gov from 2005 to May 2017 using the following search terms: Blastocyst; Day 5; Day 6; Pregnancy; Implantation; live birth; embryo transfer.

Participants/materials, setting, methods: Forty-seven full-text articles were preselected from 808 references, based on title and abstract and assessed utilizing the Newcastle-Ottowa Quality Assessment Scales. Study selection and data extraction were carried out by 2 independent reviewers according to Cochrane methods. Random-effect meta-analysis was performed using Review Manager software on all data (overall analysis) followed by subgroup analysis (fresh, vitrified/warmed, slow frozen/thawed). Risk ratio (RR) and 95% confidence interval (CI) were estimated using Mantel-Haenszel method. Sensitivity analyses were performed.

Main results and the role of chance: Data from 28 relevant articles were extracted and integrated in the meta-analysis totaling 12,932 transfers involving fresh and frozen D5 or D6 embryos. Meta-analysis of all transfers showed overall significantly higher clinical pregnancy rates (CPR) following D5 BT (overall RR = 1.26, 95% CI:1.14-1.40, $p < 0.001$), and remained unchanged after adjustment for subgroups (RR = 1.27, 95% CI:1.15-1.40).

For CPR, calculated subgroup RRs were: 2.29, 95% CI:1.69-3.09, $p < 0.001$ for fresh BT; 1.27, 95% CI:1.16-1.40, $p < 0.001$ for vitrified/warmed BT and 1.15, 95% CI: 0.93-1.41, p = 0.2 for slow frozen/thawed BT.

Live birth rate (LBR) was also significantly higher after D5 BT (overall RR = 1.46, 95% CI:1.25-1.70, $p < 0.001$), and remained unchanged after adjustment for subgroups. The LBR calculated RRs for subgroups were: 1.65, 95% CI:1.23-2.21, $p < 0.001$ for fresh BT; 1.37, 95% CI:1.19-1.59, $p < 0.001$ for vitrified/warmed BT and 1.44, 95% CI: 0.70-2.96, p = 0.32 for slow frozen/thawed BT.

Sensitivity analysis led to similar results and conclusions: CPR and LBR were significantly higher following D5 compared to D6 BT.

Limitations, reasons for caution: The validity of meta-analysis results depends mainly on the quality and the number of the published studies available. Indeed, this meta-analysis included no randomized controlled trial (RCT). Slow frozen/thawed subgroups showed substantial heterogeneity.

Wider implications of the findings: In regards to results of this original meta-analysis, ART practitioners should preferably transfer D5 rather than D6 blastocysts in both fresh and frozen cycles. Further RCTs are needed to address the question of whether D6 embryos should be transferred in a fresh or a frozen cycle.

Trial registration number: Prospero CRD42018080151

SELECTED ORAL COMMUNICATIONS
SESSION 42: NURSING AND MIDWIFERY
Tuesday 3 July 2018 Room 116 11:45–12:45

O-156 Facilitators and barriers affecting help seeking of infertile women in the United States: A systematic review

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Study question: What are facilitators and barriers contributing to help seeking behaviors in women who experience infertility?

Summary answer: After conducting a systematic review, the literature does not provide a clear understanding of facilitators and barriers due to limitations in study designs.

What is known already: In the United States it is estimated that 7.4 million women in childbearing ages 15-44 have used some type of infertility services for family building, however this value may not reflect all women who experience infertility. It is estimated that upwards to 50% of women who meet criteria for infertility do not seek treatment.

Study design, size, duration: A systematic review was conducted and reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guide.

Participants/materials, setting, methods: A systematic review of studies published between 2000 and 2017 was undertaken using the following databases: PubMed, SocIndex, CINAHL, PsychInfo, ScienceDirect, and Google Scholar. Primary search terms were infertility, subfertility, female, patient acceptance of health care, help-seeking, health seeking, fertility awareness, social support, culture, and treatment-seeking behaviors. The studies were organized using the matrix method and critiqued using Chrisman's Health Seeking Model.

Main results and the role of chance: A total of 12 articles examined facilitators and barriers that contributed to help seeking behaviors in women who experienced infertility. In studies that reported race/ethnicity, samples were majority Caucasian American women. Nearly half of the studies were secondary analyses of self-reported data from national surveys. There were 29 unique measurement tools used among the articles. Positive help seeking facilitators were perceived symptom salience, positive self-esteem, life satisfaction, and increased importance of parenthood, and fertility awareness. The most noted barrier was lifetime diagnosis of depression and depressive symptoms. Findings from these studies suggest there are multi-factorial barriers and facilitators for help seeking. There were inconsistencies in reporting common demographic factors especially race and ethnicity. A more uniform tool is needed to comprehensively assess mediating factors affecting help seeking.

Limitations, reasons for caution: Search, abstraction, and analysis were all conducted by one author. Varying use of help seeking, treatment seeking, and health seeking may have limited results. However, all 12 studies are highly reflective of the main aim of this review to understand the facilitators and barriers of women who sought fertility treatment.

Wider implications of the findings: Further exploration is needed to understand these factors in other ethnicities. The use of theoretically guided frameworks of help seeking may limit variations in measurement tool selection. More understanding could lead to practice guidelines that encourage facilitators and remove barriers to allow earlier and more consistent access to available care.

Trial registration number: not applicable.

O-157 A feasibility and acceptability study of a novel self-help coping intervention for recurrent miscarriage

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[6]*London Women's Clinic, London Women's Clinic, London, United Kingdom*

Study question: Is a multi-centre RCT to test the effectiveness of the Positive Reappraisal Coping Intervention (PRCI) in women who have experienced recurrent miscarriage, viable?

Summary answer: Feasibility findings ascertained that study design was successful, there was a sizeable population willing to participate who engaged with the intervention and found it acceptable.

What is known already: The early waiting period of a new pregnancy following recurrent miscarriage (RM) is associated with high levels of uncertainty and anxiety for the affected woman, yet there is limited support available and many women are left to cope alone with managing these distressing emotions. The PRCI, a self-administered supportive technique is effective at promoting positive feelings and sustaining ability to cope in fertility patients awaiting the outcome of in vitro fertilisation and seen as helpful in the first three weeks of pregnancy following RM, suggesting the PRCI benefits could be sustained for the waiting period before the twelve week scan.

Study design, size, duration: A two-centre RCT feasibility study established the viability of conducting a multi-centre definitive RCT to test the hypothesis that the PRCI can improve psychological wellbeing of women during the initial experience of pregnancy following recurrent miscarriage. Nested within the RCT was a qualitative process evaluation that explored participant's subjective experience of study methods and the intervention.

Recruitment (n = 75 feasibility RCT, n = 14 qualitative process evaluation) took place over a two-year period.

Participants/materials, setting, methods: Participants were recruited from Recurrent Miscarriage Clinics in two major hospitals in the United Kingdom.

Participants were randomised, received the intervention and weekly questionnaires to assess psychological wellbeing up until twelve weeks of pregnancy, or to simply complete the same weekly questionnaires. Descriptive statistics

explored the feasibility of study procedures and were utilised to make an informal assessment of any impact of the intervention.

Study participants accepted an invitation to participate in qualitative process evaluation.

Main results and the role of chance: This study successfully gathered knowledge about the feasibility aspects of conducting a future multi-centre definitive study to determine the effects of the PRCI on the psychological well-being of women with RM during the challenging waiting period of a new pregnancy.

Main themes identified included:

- The components of the protocol worked well together.
- There was a sizeable and appropriate population willing to participate in the study.
- Participants engaged with the PRCI and found it an acceptable intervention to use.
- The PRCI appeared to convey benefits to participants with an indication anxiety levels were lower in intervention group.

Results showed that an effectiveness RCT of the PRCI is possible but with modifications to take in to account some specific issues. These included the need to:

- Introduce strategies to ensure more robust estimates of potential participants in study sites.
- Pay careful consideration to potential barriers and facilitators to recruitment.
- Introduce additional measures to aid monitoring of use of the intervention.
- Introduce additional measurements of coping.
- Address the reactivity to one of the study questionnaires.

Importantly, study data provided conclusive evidence of the need for an intervention such as the PRCI to address the psychological needs of women during this time.

Limitations, reasons for caution: While every effort was made to support the recruitment of a diverse sample to the feasibility study, the UK setting may limit extrapolation to other national and cultural contexts.

Wider implications of the findings: If a future, definitive study of the PRCI proves it to be an effective intervention, then this model of care has the potential to be made more widely available as a safe, low cost, convenient and easily deliverable intervention to provide much needed support to a vulnerable patient group.

Trial registration number: This study is funded by the National Institute of Health Research, UK (Award reference number CDRF-2012-03-004).

It is registered with the ISCTRN registry, registration number: ISRCTN43571276

O-158 A retrospective observational study to compare the success rate of embryo transfer between Doctors and Nurse Practitioners in a nurse-led fertility centre

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Study question: Is the success rate of embryo transfer between Doctors and Nurse Practitioners comparable?

Summary answer: The rate of success of embryo transfer was significantly better from the Nurse Practitioner group, indicating that professional role does have an impact on success.

What is known already: The number of embryos transferred in the UK in 2015 was 85,134 and according to HFEA data the average clinical pregnancy rate in the same year for a couple undergoing IVF/ICSI using the woman's own eggs was 31%. In the North West of England alone, 3979 embryo transfers took place in 6 different centres between July 2015 and June 2016, resulting in a clinical pregnancy rate ranging from 13-36%. Due to the variation in treatment outcomes between UK centres and the fact that there are very few nurse-led fertility units, it was important to identify if professional role influences success.

Study design, size, duration: This was a retrospective observational study based on data collected between 1st October 2015 and 31st October 2017 (25 months). Data were collected from 5,182 embryo transfers facilitated by 11 nurses and 14 doctors. The study focused around couples undergoing IVF/ICSI using the woman's own eggs and excluded fertility preservation, PGS, egg sharing, surrogacy and donated gamete or embryo cycles.

Participants/materials, setting, methods: The study focused on the results per embryo transfer of 11 nurses with experience ranging from 6 months to 12 years and 14 doctors with experience ranging from 6 months to 16 years, working within a large NHS fertility centre in the UK. The data were collected from the electronic fertility database (IDEAS). Outcomes were measured for IVF/ICSI cycles using the woman's own eggs by positive pregnancy test and clinical pregnancy rate.

Main results and the role of chance: The cumulative success rates for positive pregnancy determination test (PPDT) and clinical pregnancy (CPR) within the nursing group were PPDT 48.93%, CPR 37.23% respectively, and within the doctors group were PPDT 43.24%, CPR 31.35% respectively. The nurse practitioner group were significantly better in both categories ($P = 0.001/0.001$ respectively). The significant difference in PPDT and CPR following embryo transfer by a Nurse Practitioner as compared with a doctor from these data was achieved from a large number of cycles (>5000), indicating that the higher PPDT and CPR achieved following transfer with a Nurse Practitioner were not chance findings.

These findings conclude that success rates following embryo transfer by Nurse Practitioners are not only comparable but significantly improved on those achieved by their medical colleagues, demonstrating that Nurse Practitioners can positively and significantly influence success. Experienced nurses can be trained and with sufficient exposure to embryo transfers can become an essential part of a successful fertility team. Fertility healthcare providers may wish to consider the findings of this study when planning future workforce requirements as the use of Nurse Practitioners can provide a sustainable and cost effective option to a medically dominated service in which experienced fertility doctors are often in short supply.

Limitations, reasons for caution: Quality of embryos was not included in this data analysis.

Quality of the endometrial environment was not assessed.

Frozen versus fresh embryo transfer was not measured.

Number of embryo transfers per practitioner varied.

The degree of difficulty of individual cases was not taken into consideration.

Wider implications of the findings: Nurses can be trained to successfully transfer embryos.

Trained nurses performing embryo transfer can positively influence the chance of a successful pregnancy. A larger scale, matched cohort study would help to measure the cost/benefit and help to identify if this model should underpin workforce development.

Trial registration number: N/A.

O-159 The importance of a genetic link between siblings after oocyte donation

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Study question: How do (aspiring) parents perceive and experience the importance of a genetic link between siblings born after oocyte donation?

Summary answer: Because other factors co-determined the fulfilment of the child wish, being able to use the same donor over time was less prominent throughout the interviews.

What is known already: In case of sperm donation, we know that the opportunity to use the same donor for subsequent conceptions seems important for (aspiring) parents (Somers et al., in preparation). In case of oocyte donation, we see at the clinic that couples starting treatment focus

less on this topic. Scarce literature is available on the importance of a genetic link between siblings after oocyte donation (Stuart-Smith et al., 2012). The strength of this study is that we carried out couple interviews instead of individual interviews and that we address both anonymous and known oocyte donation.

Study design, size, duration: For this study, 19 heterosexual couples were recruited at the Department of Reproductive Medicine of a University Hospital. They consented for an in-depth, semi-structured couple interview. One couple was interviewed separately, for two couples only the woman participated. The interviews were conducted between June 2013 and May 2014. Data were analysed through step-by-step inductive thematic analysis. Approval of the clinic's Ethics Committee was obtained.

Participants/materials, setting, methods: 6 couples became parents after known sister-to-sister oocyte donation and 8 couples opted for anonymous oocyte donation. Nine of them gave birth to one child or twins, 3 couples had consecutive children from a different donor and 2 couples had children from the same donor. We also included aspiring parents (5 couples) who were either already in treatment with anonymous oocyte donation or about to start.

Main results and the role of chance: When the heterosexual couples in our study talked about their (future) family, being able to use the same oocyte donor over time did not seem very important. The number of children in the family depended on several external or uncontrollable factors such as: (1) the number of oocytes retrieved per donation: a child wish could remain unfulfilled because no more oocytes were left, (2) whether the recipients wanted to go through the uncertain and intense treatment phase again, (3) the donor: if she wanted to donate again and/or if the recipients dared to ask her to donate again or the quest for a new donor and (4) practical reasons such as financial situation, professional career and housing.

Four couples talked about their preference of using the same oocyte donor. This was perceived as positive because the children would be full siblings. However, they mentioned that they could not decide on the same donor when they opted for anonymous donation. Most aspiring parents focussed on having a first child. They also talked about other options to give meaning to their life such as completing an adoption procedure and childlessness.

Limitations, reasons for caution: A specific question about being able to use the same oocyte donor over time could not always be asked because the participants co-determined the flow of the interview. Future research could focus on lesbian parents and aspiring parents in the trajectory of intra-partner oocyte donation.

Wider implications of the findings: Similar to the counselling of couples starting treatment with sperm donation, couples considering oocyte donation should be asked how they see their future family. The number of desired children should be discussed as it can influence the type of requested oocyte donation.

Trial registration number: NA.

INVITED SESSION

SESSION 43: MHR SYMPOSIUM: FIRST TRIMESTER BIOMARKERS OF IMPAIRED PLACENTATION

Tuesday 3 July 2018 Room 211 + 212 14:00–15:00

O-160 Replication of Cytomegalovirus and Zika virus in first-trimester human placentas, mechanisms of viral pathogenesis and host cell defense

L. Pereira

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Abstract text

Human cytomegalovirus (CMV), a *Herpesvirus*, is a leading viral cause of congenital infection and birth defects, including microcephaly, neuromotor deficits, and hearing and vision loss. Likewise, Zika virus (ZIKV), a *Flavivirus*, responsible for the recent American pandemic, causes a spectrum of congenital malformations rarely seen with other neurotropic viruses. How these viruses disseminate from maternal circulation to the fetus is poorly understood. For almost two decades, we have investigated the biology of CMV infection in developing human placentas and deciphered mechanisms that result in pathology observed in placentas from newborns with congenital disease. We recently completed studies of ZIKV infection in cells isolated from human placentas and amniochorionic membranes and reported patterns of viral proteins in explants of chorionic villi from first-trimester placentas (Tabata, *et al.* Cell Host & Microbe, 2016; Tabata, *et. al.* Journal of Infectious Diseases, 2017). Comparisons of CMV and ZIKV infection and between prototype and pathogenic strains revealed impaired replication and functional defects in infected cells. Host cell factors were identified that modulate viral replication and prolong viremia *ex vivo*. Our studies suggest novel strategies to strengthen natural protection and reduce virus transmission at the uterine-placental interface.

O-161 Cell-free fetal hemoglobin as a biomarker of impaired place

S. Hansson

Institute of Clinical Science Lund- Lund university, Department of Obstetrics and Gynecology-, Lund, Sweden

Abstract text

Preeclampsia (PE) is a severe pregnancy-related syndrome that annually affects at least 8.5 million women worldwide. It is a leading cause of maternal and perinatal morbidity and mortality, responsible for about 18% of all maternal deaths and up to 40% of neonatal deaths. With its aetiology still largely unknown, diagnosis of PE is based on maternal clinical symptoms; high blood pressure (BP) and proteinuria manifesting after 20 weeks of gestation. Currently, delivery is the only known cure. In addition, early disease diagnosis is challenging and there are still no reliable biomarkers for clinical prediction or diagnosis.

We have shown elevated levels of ***free fetal hemoglobin*** (HbF) in the maternal blood circulation in PE as early as the first trimester and we have shown the levels to correlate with the BP, i.e. the severity of the disease in late pregnancy. Extracellular haemoglobin and its hydrophobic metabolite heme are highly toxic with pro-inflammatory, pro-oxidative, tissue damaging as well as vasoconstrictive effects.

By using the well-established dual-placenta perfusion model to study the human placenta *ex vivo*, we have shown that free HbF causes damage to the placenta barrier and endothelial cells by inducing oxidative stress and inflammation. Furthermore, cell-free HbF has also been shown to increased the microcirculatory resistance. In different animal models, cell-free HbF has been shown to cause kidney and placental damage similar to that seen in PE, i.e. glomerular endotheliosis and reduction of the podocyte-specific protein, nephrin.

To prevent toxicity of cell-free hemoglobin as well as its degradation metabolites heme and free iron, several scavenger proteins and degradation enzymes protect the body. ***Haptoglobin*** (Hp) binds free hemoglobin and transports it to macrophages and hepatocytes where the uptake is facilitated by CD163 receptor-mediated endocytosis. Hemoglobin is degraded to heme and then further catabolized by the rate limiting enzyme ***heme oxygenase 1*** (HO-1). ***Alpha-1-microglobulin*** (A1M), a lipocalin protein, that in addition to heme also binds radicals and has enzymatic reducing capacity. We have recently shown that A1M is up regulated in early and late onset PE. ***Hemopexin*** (Hx) is a circulating plasma glycoprotein, mainly synthesized in the liver, that binds free heme with high affinity. Hemopexin also has enzyme activity, which has been suggested to regulate the vascular responsiveness to angiotensin II.

In a series of studies we have deciphered how maternal plasma HbF and heme impact the scavenger enzyme systems Hx and HO-1 in patients with PE and studied how these proteins can be used as biomarkers for PE. Logistic regression analysis with ROC-curve analysis was performed to evaluate them as biomarkers. In PE plasma, significantly higher levels of HbF and heme in combination with significantly lower Hx activity, Hx and HO-1 levels was shown compared to normal pregnancies. The Hx activity was inversely correlated to the diastolic blood pressure and the HO-1 concentration was inversely correlated to both the systolic and diastolic blood pressure. The ROC-curve analysis showed a combined detection rate for these biomarkers of 84% at 10% false positive rate in late pregnancy.

Studies on ***fetal growth restriction*** (FGR) have shown increased levels of cell-free HbF, whilst elevation of its ratio with HO-1 suggests failure of the catabolic compensation. Placental endothelial cells, primed with cell-free HbF,

developed a pro-inflammatory phenotype and disruption of endothelial cell flow-alignment.

Conclusions: There is an important aetiological role for cell-free HbF in both fetal growth restrictions and in preeclampsia.

By measuring components in the HbF metabolism as potential biomarkers, they can be clinically useful to support the PE diagnosis. Future studies are needed to evaluate the role of these biomarkers as first trimester predictive biomarkers for PE, particularly in late-onset PE and neonatal outcome.

INVITED SESSION

SESSION 44: LIVE SURGERY

Tuesday 3 July 2018 Room 111 + 112 14:00–17:00

INVITED SESSION

SESSION 45: MANAGING EMBRYO STRESS IN PREIMPLANTATION DEVELOPMENT

Tuesday 3 July 2018 Room 113 + 114 + 115 14:00–15:00

O-162 Nuclear reprogramming and chromatin functions in oocytes and preimplantation embryos

K. E. Latham

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Nuclear Reprogramming and Chromatin Functions in Oocytes and Preimplantation Embryos.

Reprogramming of the genome is an essential part of creating each new life. The genomes of two highly differentiated gamete genomes must be reprogrammed to create an embryonic genome capable of development. This process begins with reprogramming the genome for meiosis, continues in the early cleaving embryo, and continues throughout preimplantation development until the earliest lineages are established. Meiotic reprogramming, including transcriptional silencing, histone deacetylation, and chromatin condensation, is essential to create a chromatin state that is compatible with correct spindle formation and faithful chromosome segregation. Further reprogramming is associated with preparing and activating the embryonic genome, first creating transcriptional competence, and then creating a repressive chromatin environment that allows gene regulation. As the embryo cleaves, further reprogramming occurs, culminating in the creation of cells with different identities, destined to give rise to inner cell mass and embryo proper, trophectoderm, and extraembryonic endoderm. Recent studies reveal that developmental pluripotency remains dependent on early embryonic chromatin states, establishing one potential connection between early events and later progeny health. Artificial reprogramming methods, achieved by somatic cell nuclear transfer, induced pluripotency, and application of epigenetic drugs, display significant limitations. These limitations attest to the unique dialog between ooplasm and gamete/embryonic genomes, the continuously shifting demands of the embryonic system, the opposing needs that must be satisfied at key points in the process, and the potential points at which exogenous factors may intervene to compromise development. The factors responsible for the different phases of this unique and ongoing reprogramming dialog are still being discovered. A deeper understanding of the nature of these factors and the underlying chromatin states upon which they act should be beneficial in basic and applied arenas.

O-163 Mitochondrial stress in preimplantation development and opportunities for treatment

R. Robker

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Abstract text

Oocytes contain all of the cellular building blocks required to support embryogenesis until the blastocyst stage of development. However the quality, quantity and composition of these cellular building blocks is determined by physiological signals influenced by the maternal environment; which in turn have dramatic effects on the progression of embryogenesis. In particular oocytes are lipid-rich cells and oocyte lipid content is influenced by maternal nutrition. Similarly, oocyte mitochondria are highly sensitive to environmental signals which can change their capacity for energy production and biogenesis and thereby influence embryo development and mitochondrial inheritance. Using a number of approaches in mice we have sought to determine how these key aspects of oocyte quality, namely lipid content and mitochondrial activity, regulate embryo developmental kinetics during pre-implantation development to the blastocyst stage. This information is needed because accumulating evidence demonstrates that discrete changes to oocyte quality influence the timely formation of the inner cell mass as well as distinct aspects of offspring phenotypes into adulthood.

To examine the effects of relevant physiological and in vitro conditions of oocytes on subsequent developmental competence, oocytes were isolated from female mice that were either obese or aged or that were manipulated in vitro in order to deplete cholesterol lipid or induce oxidative stress. Embryo morphokinetics were then assessed by continuous time-lapse monitoring, and cell lineage allocation to the inner cell mass (Oct4+) or trophectoderm (Cdx2+) was identified by immunohistochemistry. The results show that exposing oocytes to different environmental conditions in vivo or in vitro causes distinct changes to cell cycle kinetics following fertilization and deficiencies in either inner cell mass or trophectoderm cell number by the blastocyst stage. In addition, embryos fertilized in vitro exhibit altered development compared to those fertilized in vivo, even from the 2 cell stage. These results further demonstrate that even subtle insults to oocytes during maturation and fertilization change zygote cell cycle kinetics, and identify differential effects on embryos in response to distinct physiological and in vitro environments. Most importantly, we are able to restore the developmental competence of poor quality oocytes, damaged by obesity, aging or in vitro stress, using specific pharmaceuticals.

INVITED SESSION

SESSION 46: PATIENT SESSION: SURROGACY - THE PATIENTS' PERSPECTIVE

Tuesday 3 July 2018 Room 117 14:00–15:00

O-164 Surrogacy in Portugal - from struggle to accomplishment

A. Galhardo

Instituto Superior Miguel Torga- CINEICC-Faculty of Psychology and Educational Sciences of the University of Coimbra- Associação Portuguesa de Fertilidade, Psychology, Coimbra, Portugal

Abstract text

Relevant medical advances in the field of reproductive medicine now allow people previously unable to become genetic parents due to medical factors (e.g., women without a uterus, uterine abnormalities or medical conditions that contraindicate pregnancy) or social motives (e.g., gay men or couples) to accomplish this usually valued objective. Gestational surrogacy policy in Europe varies by country and it remains a controversial topic due to ethical and legal concerns. This third-party reproductive technique is a complex subject involving the commissioning parents, the gestational surrogate and the child to be. Recently in Portugal, July 2017, the legal context changed and gestational surrogacy is currently possible. This was an important achievement from the patients' perspective and one of the last steps of a long journey of struggle. In 2012 a parliament working group was formed with the aim of revising the medically assisted reproductive legal framework. Two years later a group of Portuguese Fertility Association (APFertilidade) members wrote to all political parties in the parliament, to the Health Commission and the National Council for Medically Assisted Reproduction, asking for the

inclusion of surrogacy in their agenda. On 22nd April 2016 the first law project addressing gestational surrogacy was rejected and APFertilidade requested the main right wing party to vote for. A week later a left wing party proposed the text to be voted in a plenary session in the same date the revised version of the law would be presented (May 13th). At the same time, APFertilidade launched a video containing real testimonies of women without a uterus who needed the law change to accomplish their dream of becoming mothers and it was sent to all deputies. This was an interesting piece of campaign encompassing several messages such as exclusion and stigma as well as the idea of not being heard and being pushed to illegal and cross-board scenarios. On 13th May the new law was approved by the parliament and access to assisted reproductive technologies (ART) was extended to all women independently from their marital status or sexual orientation. Furthermore, gestational surrogacy was also approved but in June the President considered there were queries, mainly concerning the interest of the child and the surrogate, and these should be clarified before promulgation. In June 2017 the law received the approval of the Ministries Council. Finally, in July 2017, the President promulgated the law regulation. The approved framework states that women facing medical conditions such as not having a uterus or uterus malformations or other medical conditions that clearly contraindicate pregnancy are the ones who can benefit from this treatment approach. Within this regulation it is also worth noting that besides medical criteria, it is mandatory to include psychological support for the parties involved and this suggests that psychological and social implications were considered by the legislator. By the end of 2017 the National Council for Medically Assisted Reproduction approved the first application. Although this was a demanding and challenging process, a study conducted in 2015 (before the law change) with 518 participants to whom it was asked to state their opinion about surrogacy, 69% agreed with legalization, 19% did not agree and 12% were non-respondents. Moreover 38% stated that they would support a friend in need of a gestational surrogate and 32% would support a female friend who decided to act as a gestational carrier. Nevertheless, when the question was whether they would pursue such a treatment option in case they needed, 50% answered "no" and 37% would consider surrogacy if all other possibilities were to be excluded.

O-165 Surrogacy and stratified kinship

A. Pande

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Abstract text

Commercial surrogacy is a multi billion-dollar industry across the world, with India being one of the world leaders. Although often couched in dystopic terms, the topic of surrogacy is not restricted to medical or scientific circles and has been generating feminist, ethical, legal and social debates for over three decades now. Despite this rich and growing literature, the common perception of surrogacy is one of intense anxiety – with dystopic images of baby farms, breeding factories and women as cows and guinea pigs flooding the media. When intended parents travel from countries in the global north to access services in the global south, there is an additional anxiety that such travel reifies global inequalities by making the bodies of people in resource-poor countries easily rentable by others. In my previous works I have argued that while these images are justifiable, they do not get to the complexity of the industry, the way it is unfolding in India. Commercial surrogacy in India is a rapidly growing informal labour market, one that needs to be understood empirically and not as an ethical dilemma (Pande 2014, 2015, 2016). Once we start unpacking these empirical realities, we realize that much of the structural inequalities are inherent to commercial surrogacy. In fact this industry reflects the global trend of "stratified motherhood" – "the hierarchical organization of reproductive fecundity and birth experiences that supports and rewards the maternity of some women while despising or outlawing the mother-work of others" (Rapp 1991). In this paper I use my recent ethnographic findings from my field to expand on this notion of stratification by highlighting a fundamentally unequal aspect of this practice – what I label "stratified kinship" – whereby there is legal and social acknowledgement and celebration of only genetic parenting while making birth mothering invisible.

O-166 What is it like to be a wombless woman in the age of ART's? Patients' perspectives on gestational surrogacy in Finland

K. Kivipuro

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Abstract text

Each year in Finland, a number of fertile age women encounter an involuntary hysterectomy or find out they have never had a uterus. In my presentation, I examine how Finnish wombless women of fertile age cope with their condition and tackle their life between the imperative of procreation and the restrictions of reproduction. Based on my ongoing sociological study, I discuss what it means to be a wombless woman in Finland, where gestational surrogacy is forbidden, adoption is heavily regulated, and uterus transplants are still only a promise for the future.

Lack of uterus is always dramatic, because the indication for involuntary hysterectomy can be, for instance, a gynecological cancer, a life threatening complication during delivery, or it involves a teenage girl with congenital absence of a uterus (Mayer-Rokitansky-Küster-Hauser syndrome). Involuntary womblessness is a relatively rare incident, but it has a major effect on these women's lives and the possibilities to have a child. The situation forces to redefine the frames of reproduction and makes the woman to think strategically how founding a family would be possible. Furthermore, the lack of uterus does not only have a profound effect on the woman but also on her partner and immediate family.

Since 2007, the Assisted Fertility Treatment Act has prohibited gestational surrogacy in Finland. Based on research data, which includes 25 semi-structured in-depth interviews conducted with wombless women during 2017 and collaboration with two patient organizations for involuntary childlessness, I show that the prohibition of gestational surrogacy induces deep sorrow and resentment for majority of those affected by the condition. In practice, the legislative limitations are unable to prevent people from seeking and obtaining reproductive services elsewhere. In Finland, proximity to Russia prompts the intended parents with their Finnish surrogate to obtain the IVF from Russia, while giving birth to the baby in Finland. Intended parents are placed in highly contradictory situations, as they need to consider if they are willing to circumvent the legislation, invest a great amount of mental and financial resources, and leave their home country to seek the fertility services. Without regulatory support, evaluation of ethicality and riskiness of reproductive travel is left to individuals.

INVITED SESSION

SESSION 47: HOW TO INCREASE PEOPLE'S FERTILITY AWARENESS?

Tuesday 3 July 2018 Room 116 14:00–15:00

O-167 Development and impact of fertility campaigns

L. Schmidt

University of Copenhagen, Department of Public Health- Section of Social Medicine, Copenhagen K, Denmark

Introduction: Considerable gaps have been identified in men and women's knowledge of fertility. Fertility education and increasing the population's fertility awareness has been suggested as intervention strategies to contribute to prevention of infertility and to also reduce other negative consequences of delayed childbearing.

In response to this call, a small number of studies have measured the impact of fertility knowledge interventions. These studies show that these interventions are successful in increasing fertility knowledge in the short term. However, these interventions have often been developed for research and are not widely available to the public as in a widespread fertility campaign.

Results: A review of PubMed and searching keywords via Google showed that several fertility campaigns have been developed and launched across the globe. The campaigns are generally driven by one or both of the following goals:

(1) To increase men and women's fertility knowledge in order to promote informed and satisfying fertility/childbearing decision-making
(2) To increase knowledge and awareness of fertility in order to increase falling birth rates.

Content generally includes information about the fertility lifespan and risks to fertility.

The media plays a significant role in how the campaigns are understood and interpreted by the public and can facilitate or hinder the campaign goals. In some cases, the media provides a wider dissemination of the fertility campaign on a larger platform (e.g., ASRM).

In other cases, the media's portrayal of the campaign can result in the intended messaging being lost or misconstrued. For example, the media in Denmark linked the increased birth rate to fertility campaigns suggesting increasing the birth rate was the primary intention. In many cases, more coverage can be focused on the backlash rather than the content. The most common criticisms of fertility campaigns include blaming women for waiting too long and/or not being aware of their fertility, encouraging women and men to have children before they are ready, reducing women to their biological potential and presenting a one-sided story that does not acknowledge the structural and societal barriers and restrictions to younger parenthood.

A common problem across fertility campaigns is how to determine their impact. In the case of online campaigns, page views, social media hits and downloads can be tracked and provide some sense of the reach (example of YourFertility.org.au). The fertility campaigns have largely been unevaluated empirically so we do not know how or if they are impacting fertility knowledge and behaviour.

Conclusions: It is of importance that fertility campaigns are developed together with the many different stakeholders regarding fertility and family building; e.g., national health authorities, different groups of health professionals (medical doctors, midwives, nurses), NGO's on family building and sexual education as well as the target groups in the population. Further, evaluation is a necessary next step in fertility campaigns to gain a better sense of their impact and reach.

O-168 Preconceptional personalised mHealth lifestyle coaching: \rFirst results of a randomized controlled trial in couples undergoing IVF/ICSI treatment

R.P.M. Steegers-Theunissen

Erasmus MC, Dept of Obstetrics and Gynaecology Room Ee 22.71a, Rotterdam, The Netherlands

Abstract text

Mounting evidence demonstrates that healthy maternal lifestyles, including nutrition, significantly improve pregnancy chances as well as maternal pregnancy- and neonatal outcomes, with implications for health and disease risks across the lifecourse. Although the impact of paternal lifestyles has been largely neglected, more data are now available showing also their influence on semen quality and embryonic growth trajectories, with potential health consequences for the offspring. Again, this data very much illustrate the importance of the critical periconceptional window, defined as 3 months before up to 3 months after conception, during which most adverse reproductive outcomes originate. Therefore, in the first part of this presentation new evidence on the impact of periconceptional maternal and paternal lifestyles on the gametes and (pre) implantation embryo quality and growth trajectories will be demonstrated using questionnaire data and (non) invasive biomarkers using for example 3-dimensional ultrasound, time-lapse and offline virtual reality techniques.

As researchers we also have the responsibility to implement evidence-based data in patient care. Indeed more couples with a child wish and planning pregnancy, especially subfertile couples and health care professionals, are aware of the impact of lifestyle on reproduction. However, adopting healthy behaviors remains a big challenge for everyone. Therefore, following the implementation of a preconceptional clinic on lifestyle coaching (2007-2012) at the Erasmus MC, University Medical Center in Rotterdam, The Netherlands, we have developed the successful mHealth coaching programme 'Smarter Pregnancy'. This mHealth programme with CE certification is available in the Dutch (www.slimmerzwangeronderzoek.nl and www.slimmerzwanger.nl) and English language (www.smarterpregnancy.co.uk/research) and aims to empower couples of adopting healthy lifestyles before and during pregnancy. Between 2012 and 2014 a large survey was performed using 'Smarter pregnancy' as intervention which showed significantly positive results on compliance, feasibility, usability and first effectiveness. From 2014 onwards also a multicenter randomized controlled trial is conducted in 6 IVF clinics in the Netherlands using this mHealth programme. In the second part of this presentation the first results will be presented of this trial that demonstrate an even higher compliance than was revealed from the survey. Moreover, the significant effectiveness of this mHealth programme is conformed with regard to adopting short- and long-term healthy lifestyles in couples undergoing IVF treatment. Subgroup analysis also shows the beneficial effects in overweight and obese women. Since mHealth has the potential to reach and empower a large population, our next ambition is the development in co-creation with multiple stakeholders of a digital life course platform for implementation of lifestyle care in routine patient care from the preconceptional period onwards. This is a societal challenge which can also be a great opportunity for Big data collection to further improve (cost)effectiveness of health care.

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SELECTED ORAL COMMUNICATIONS

SESSION 48: EFFECTS ON TESTICULAR DEVELOPMENT AND GERM CELL DIFFERENTIATION

Tuesday 3 July 2018 Forum (Auditorium) 15:15–16:30

O-169 Exposure to acetaminophen and ibuprofen affects fetal germ cell development in both sexes in rodent and human

P. Hurtado-Gonzalez[1], J. MacDonald[1], K. Kilcoyne[1], S. Van den Driesche[1], A. Jorgensen[2], R. Anderson[1], R. Sharpe[1], R. Mitchell[1]

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Study question: We determined whether therapeutic doses of analgesics affect germ cell development in the human (and rodent) fetal testis and ovary using in-vitro, in-vivo and xenograft approaches.

Summary answer: Germ cell number was significantly reduced (28-49%) in human fetal testes and ovaries exposed to acetaminophen or ibuprofen, whilst rodent studies revealed altered epigenetic regulation.

What is known already: The majority of women use an analgesic (acetaminophen or ibuprofen) at least once during pregnancy. Whilst some epidemiological studies have reported associations between analgesic use during pregnancy and cryptorchidism in sons, the findings are inconsistent within and between the different study populations. A recent study which utilised a xenograft model of human fetal testis tissue showed that prolonged acetaminophen exposure at human-relevant dosing reduced plasma testosterone levels, whilst rodent studies have indicated potential for germ cell effects following acetaminophen exposure. However, whether germ cell effects occur in analgesic-

exposed human fetal gonads and the mechanisms involved have not been determined.

Study design, size, duration: First trimester human fetal testes and ovaries (n = 8) were cultured exposed to acetaminophen and ibuprofen for 7 days. Second trimester human fetal testes (n = 10) were xenografted into immunocompromised mice and exposed to vehicle, acetaminophen (60 mg/kg/day; 1 or 7 days) or ibuprofen (30 mg/kg/day; 7 days). To determine mechanism of action, a human GC tumor-derived cell line (NT2) was used (acetaminophen or ibuprofen +/- PGE2 agonists) as well as an in-vitro and in-vivo (n = 13-30) rat model.

Participants/materials, setting, methods: Fetal testis sections were double/triple immunostained for SOX9 (Sertoli cells), TFAP2C (gonocytes) and MAGEA4 (pre-spermatogonia). mRNA Expression of epigenetic regulators in human (TET1, EZH2, DNMT3a, DNMT3b) and rat (Tet1, Ezh2, Dnmt3a and Dnmt3b), in addition to germ cell pluripotency (POU5F1, TFAP2C and NANOG) genes were determined. Results were analyzed by two-factor ANOVA with replication. Statistical significance was indicated where $p < 0.05$

Main results and the role of chance: Gonocyte ($TFAP2C^+$) number was decreased in 1st trimester human fetal testes exposed in-vitro to acetaminophen (28% reduction; p = 0.008) or ibuprofen (22% reduction; p = 0.001) and in ovaries exposed to acetaminophen (43% reduction; p = 0.016) or ibuprofen (49% reduction; p = 0.005), compared with controls. Acetaminophen exposure decreased gonocyte number in xenografted 2nd trimester human fetal testes by 17% (p = 0.044) and 30% (p = 0.014) after treatment of host mice for 1 or 7 days respectively. NT2 cell number was decreased after exposure to acetaminophen (19% reduction, p = 0.0002), ibuprofen (18 %reduction, p = 0.006) or to prostaglandin E2 (PGE_2) antagonists (27% reduction; p = 0.0001), and PGE_2 agonists prevented acetaminophen-induced reduction in NT2 cell number (p = 0.029). Gene expression of regulators of DNA and histone methylation and of GC pluripotency genes, differed from controls following analgesic and PGE2 antagonist exposures. Gene expression changes also occurred in rat fetal testis/ovary cultures and following in vivo acetaminophen exposure of pregnant rats. One such example was expression of the epigenetic regulator TET1, which was significantly increased after exposure to acetaminophen in several model systems (human NT2 cells, rat fetal testis/ovary cultures, and fetal testes and ovaries after in vivo exposure of pregnant rats), demonstrating robust translatability across experimental models and species.

Limitations, reasons for caution: Although the xenograft/culture models can mimic many aspects of normal human fetal gonad development, they cannot recreate in vivo human conditions. Despite these limitations, the consistency of analgesic effects and concordance between humans and rats (including in-vivo), suggest that mechanisms for fetal GC effects are conserved between rodents and humans.

Wider implications of the findings: These findings provide novel evidence for potential adverse effects of analgesics during pregnancy. They suggest that exposure of human fetal gonads to therapeutically-relevant concentrations of acetaminophen and ibuprofen may impact fetal germ cell number and, potentially, epigenetic modifications. This could affect future fertility of the individual and/or future generations.

Trial registration number: n/a.

O-170 Acute myeloid leukemia (AML) impaired mouse male fertility by affecting testicular growth factors, premiotic/miotic and posmiotic spermatogenic cell counts

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Study question: How can AML affect mouse male fertility?

Summary answer: AML impaired the seminiferous tubules (STs) histology, reduced the number of spermatogenic cells, increased apoptosis in the STs and reduced production of testicular growth factors.

What is known already: It is known that some types of cancer can affect semen quality even before anti-cancer treatment. Some studies reported significant reductions in semen parameters (motility, concentration and morphology) in Leukemia patients before chemotherapy. In addition, a significant reduction in semen quality of thawed cryopreserved semen from leukemia patients compared to control patients was reported. Also, a reduction in some hormones was reported in cancer patients compared to control. However, the mechanisms behind these impairments parameters are not yet fully understood.

Study design, size, duration: Six-week-old males C57/BLACK mice were used. 1. Control group – injected with saline. 2. AML group – injected (intraperitoneal; i.p) with 1×10^5 AML cells (murine C-1498 AML cell line). Mice were sacrificed (4-6 mice in each time point) after 1,2,3,4 weeks of AML injection.

Participants/materials, setting, methods: Testes were removed and fixed in Bouin's solution for histological evaluation, or kept at -70^0 C for RNA extraction. The presence of premeiotic (SALL4,PLZF), meiotic (CREM-1) and postmeiotic (ACROSIN) cells were examined by immunofluorescence staining. Apoptosis was examined by tunel assay. The expression of growth factors in the testes was examined by qPCR analysis. Sperm cells were extracted from the epididymis. The fertility capacity of the mice was examined by mating with normal C57/BLACK females.

Main results and the role of chance: Mice were still alive 4 weeks after AML injection. A significant decrease in the number of cell layers and a significant increase in the size of the lumen of the STs were demonstrated in AML mice compared to control. The number of premeiotic (SALL4, PLZF) cells/ST and the percentage of STs with meiotic (CREM-1) or postmeiotic (ACROSIN) cells were significantly lower in AML mice compared to control. The percentage of STs with apoptotic cells significantly increased in AML mice compared to control. The expression of growth factors in testicular tissue [macrophage-colony stimulating factor (MCSF), glial cell-derived neurotrophic factor (GDNF), leukemia inhibitory factor (LIF)] was significantly lower in AML mice compared to control group. Sperm concentration, motility and morphology were significantly lower in AML mice compared to control. The fertility capacity of the AML mice was 50% of the control after 3 weeks of AML injection compared to control.

Limitations, reasons for caution: Animal model of leukemia may behave different from human model.

Wider implications of the findings: This study deepens our understanding of mechanism behind reduced fertility and infertility in AML male patients. Understanding these mechanisms may assist in the development of future therapeutic strategies for male fertility preservation.

Trial registration number: NA.

O-171 CRISPR/cas9 mediated HSD17B3 gene mutation on mouse Leydig cells: Effectivity of CRISPR/cas9 on testosterone synthesis pathway

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Study question: Can CRISPR/cas9 method be used for gene editing to interfere testosterone synthesis in mouse Leydig cells?

Summary answer: HSD17B3 gene knock-out via CRISPR/cas9 gene editing technology can be accomplished in mouse Leydig cells successfully to interfere and decrease secretion of testosterone.

What is known already: CRISPR/cas9 gene editing technology comprises programmable endonucleases that facilitate making models with precise genetic changes to study the progress and treatment of diseases, some of which are related to faulty testosterone production. Testosterone synthesis involves a highly complicated cascade of sequential reactions that precise regulations are not usually possible, since non-specific interventions affect the other steroid-related products in the cascade, as well. CRISPR/Cas 9 has made it possible to accomplish cell targeted interventions, which are widely used in a variety of cells in the body in order to obtain genome based therapies.

Study design, size, duration: This study involved the knock-out of HSD17B3 gene of Leydig cells isolated from adult CD1 mouse (n = 36) testis via the use of CRISPR/cas9, followed by evaluation of changes in secretion of testosterone within control group and experimental group throughout 15 days under the effect of 100 ng/ml human chorionic gonadotropin. Quantitative analysis was performed by qRTPCR, WB and ELISA in triplicates compared by student's t-test. The semiquantitative analysis was accomplished by superresolution microscopy.

Participants/materials, setting, methods: Leydig cells isolated from adult CD1 mice were cultured 2 days with human chorionic gonadotropin to induce maturity and secretion of testosterone before CRISPR/cas9 gene editing procedure. HSD17B3 gene was knocked-out with a specific guide-RNA specially designed for that mouse gene. Triplicate experiments for Western Blot, qRT-PCR, and super-resolution confocal microscopy were performed to show the reduction in this gene expression, with altered cell morphology, as ELISA was used to detect changes in testosterone levels.

Main results and the role of chance: This project was undertaken to design testosterone deficiency model in vitro and evaluate the effect along with the efficiency of CRISPR/cas9 method for Leydig cells.

The first set of experiments was set to confirm the isolation of Leydig cells and the effect of CRISPR/Cas9 gene edition on isolated cells. Leydig cells of mouse testis were isolated with high purity (>95%) in adequate amounts to carry out culture and gene edition experiments. The efficiency of CRISPR/cas9 for the edition of HSD17B3 gene was >85% in with no suspected off-target performance. The second set of experiments were designed to search for the efficacy of gene edition. Expression of HSD17B3 was significantly decreased in the gene edited group compared to control group as depicted by qRT-PCR, that was designed with triplicate controls, and Western Blotting ($p < 0.05$). Testosterone production of gene-edited Leydig cells in significantly lower ($p < 0.05$), as recorded by ELISA of the culture medium samples. Super-resolution microscopy revealed that morphological appearance of gene-edited cells which showed a less round cell structure with fewer steroid granules. 3BHSD stainings were found to be critically decreased in gene edited group compared to the normal group.

Limitations, reasons for caution: As this study is based on gene edition of primary mouse Leydig cells, **various outcomes can be expected for human** Leydig cells. This study design is very useful to create effective and simpler in vitro models; however, knocking out of HSD17B3 gene can be **lethal for in vivo studies.**

Wider implications of the findings: The limited number of *in vitro* models for testosterone deficiency limits research on this topic. Additionally; the literature on the effectiveness of CRISPR/cas9, which is very up-to-date knock-out and gene editing method, is insufficient regarding Leydig cells. This study releases an effective, feasible method to interfere testosterone production in vitro.

Trial registration number: not applicable

O-172 Busulfan distinctly affected Sertoli cell activity in immature mice

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Study question: What are the effects of busulfan on Sertoli cells from immature-treated mice?

Summary answer: Busulfan did not affect the number of Sertoli cells, but it significantly and distinctly affected their function to produce different growth factors.

What is known already: Busulfan (BU) is an aggressive chemotherapy, which may lead to male infertility. Sertoli cells (SCs) communicate directly with germ cells (GC) and thus regulate their development in the seminiferous tubules (STs) to sperm. Sertoli cells produce different factors that affect proliferation and differentiation of spermatogonial cells such as GDNF, LIF, SCF, transferrin, ABP and others. Busulfan was shown to increase some testicular growth factors such as GDNF and hormones such as AMH, but also to destroy vimentin filaments and ICAM-1 adhesion molecules that affect development of spermatogonial cells.

Study design, size, duration: Seven-day-old mice (immature) were injected intraperitoneal with BU (45 mg/kg) and sacrificed after 10 days [a time demonstrated by our group to severely affect spermatogenesis compared to control (DMSO)]. Twenty-twenty five mice were used in each experiment. Experiments were repeated at least 3 times.

Participants/materials, setting, methods: Cells from STs were enzymatically isolated and double immunofluorescence stained for SCs and growth factors was performed. Also, RNA expression of SCs marker and growth factors were evaluated by qPCR analysis.

Main results and the role of chance: A significant increase ($p < 0.001$) in the percentage of vimentin-positive cells (a SCs marker) and RNA expression, with no change in the overall number of SCs were demonstrated in BU-treated mice compared to the control group. Also, a significant increase ($p < 0.001$) in the percentage of SCs that produce CSF1 with no change in other growth factors (GDNF, SCF, LIF) was demonstrated compared to the controls. In addition, a significant increase in the expression of FSHR, androgen-receptor, inhibin B and transferrin, with a significant decrease in androgen binding protein was demonstrated in the BU-treated mice compared to controls.

Limitations, reasons for caution: Limitations, reasons for caution: Animal model treated with chemotherapy may behave different from human model.

Wider implications of the findings: We showed for the first time that BU treatment of immature mice doesn't affect SCs number, but increases their percentage in the testes. BU treatment distinctly affected regulatory molecules produce by SCs and play a key role in spermatogenesis. These results may partially explain testicular response to BU.

Trial registration number: NA

O-173 The nuclear envelope: an unknown actor of human spermiogenesis

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Study question: What roles do the nuclear envelope proteins play during normal human spermiogenesis?

Summary answer: In humans, B-type lamins, LEM-domain and BAF proteins are involved in the specific spermatid nucleus remodelling, SUN5 is required for solid sperm head-tail junction.

What is known already: The nuclear envelope (NE), composed by the nuclear membrane, the nuclear pore complexes and the nuclear lamina, is interposed between nuclear and cytoplasmic compartments.

In mouse, emerging evidence supports the involvement of many proteins of the nuclear membrane, the nuclear lamina (NL), and some of their partners, in the specific remodelling of the mammalian spermatid nucleus.

Recent studies in human, during normal spermiogenesis or in patients with abnormal sperm phenotypes, show a strong involvement of the NE, the NL

proteins and their associated proteins in the control of specific spermatid nucleus remodelling and formation of the sperm head-tail junction.

Study design, size, duration: A prospective qualitative and functional analysis of NE proteins and some partners, in human testis and ejaculated spermatozoa, supported by functional experiments in HeLa cells.

Participants/materials, setting, methods: Testicular samples and ejaculated spermatozoa from the Germetheque biobank, Hela cells, RNA extraction, RT-PCR, cell transfection, western blotting, immunofluorescence.

Main results and the role of chance: B-type lamins are present at the nuclear periphery, except in the region covered by the acrosome, and that as the spermatid matures the B-type lamins recede towards the posterior pole. We determine that, human lamin B3, but not lamin B2, induces strong nuclear deformation, when ectopically expressed in HeLa cells. LEMD1, LEMD2, ANKLE2, LAP2β, BAF and BAF-L, are present and Emerin, LBR and LEMD3 are absent. In spermatids, no LEM-domain protein localised to the nuclear periphery, but five were nucleoplasmic, receding towards the posterior nuclear pole as spermatids matured. The NL appears immature in globozoospermic spermatozoa. SUN5 is required for the formation of the sperm head-tail junction. Our work therefore establishes that the lamina-chromatin interface in human spermatids is radically distinct from that defined in somatic cells. Our results in human highlight the importance of the NE proteins in mammalian spermiogenesis and male fertility.

Limitations, reasons for caution: The number of samples and patients tested for rare phenotypes should be increased.

Wider implications of the findings: In general, our results illustrate the importance of spermatid NE components and partners in the coordination of the cellular events, between the nuclear and cytoplasmic compartments, that lead to the formation of functional spermatozoa. In human, spermiogenesis represents a new model of nuclear plasticity.

Trial registration number: NA.

SELECTED ORAL COMMUNICATIONS

SESSION 49: PREMATURE OVARIAN INSUFFICIENCY AND POOR OVARIAN RESPONSE

Tuesday 3 July 2018 Room 211 + 212 15:15–16:30

O-174 High resolution array comparative genomic hybridization contribution to uncover genetic etiologies of idiopathic premature insufficiency: a cohort study

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Study question: Can high-resolution array comparative genomic hybridization (CGH) contribute to uncover genetic etiologies of idiopathic premature ovarian insufficiency (POI)?

Summary answer: High resolution CGH array analysis can identify new candidate genes in the pathophysiology of POI as well as previously reported ones.

What is known already: Premature ovarian insufficiency (POI) is a heterogeneous disorder affecting 1% of women and remains idiopathic in the large majority of cases. It is defined by the presence of primary or secondary amenorrhea and hypergonadotropic hypogonadism, occurring before the age of 40. Familial history of POI is reported in up to 31% suggesting a significant genetic contribution, which remains largely unknown. Different genomic tools have been used to elucidate the genetic etiology of POI including array-CGH. However, different types of array platforms with various levels of resolution were used leading to heterogeneous results generally not replicated between studies.

Study design, size, duration: High resolution array CGH analysis was performed on DNA samples from 110 patients with idiopathic POI recruited prospectively from June 2016 to June 2017 in order to identify Copy number variants (CNVs). Data were compared to Database of Genomic Variants (DGV)

Participants/materials, setting, methods: Women were included if they experienced POI, as defined by the ESHRE guidelines. All patients had a normal karyotype. Patients with aneuploidies, fragile X premutation, adrenal/ovarian antibodies and iatrogenic POI were excluded from the study. Oligonucleotide array-CGH analysis was performed using the CytoSure™ Constitutional v3 Arrays (4×180k) from OGT and analyzed with CytoSure Interpret Software. The mean spatial resolution was approximately of 200 kb for the whole genome and higher for 502 highly-targeted developmental delay genes.

Main results and the role of chance: Thrirty-six patients (32.7%) presented CNVs of unknown significance (class III CNV) and one patient presented a pathogenic CNV (class V). As 9 of these patients presented 2 different class III CNVs, a total of 45 class III CNVs (23 microdeletions and 22 microduplications) were identified. These 45 CNVs were located in 32 genomic segments, 9 of them being involved in at least 2 patients (2p16.3, 4p15.32, 7q11.22, 10q21.1, 11q14.1, 17q11.2, 20p12.1, Xp22.11, Xp22.33). Three among those have been previously associated with POI (2p16.3, 17q11.2, Xp22.11). Different genes were included or interrupted by these CNVs, 16 of them being potentially relevant in the development of POI. Two of these genes were previously suggested as candidate genes for POI (CPEB1, DIAPH2), while the remaining ones could be considered as new candidate genes in POI as they were reported to be either implicated in the development of the stock of primordial follicles and the prevention of its depletion in non-human species, or in molecular pathways known to be associated with POI, or located in critical region of X chromosome or next to genes important for folliculogenesis.

Limitations, reasons for caution: This is a descriptive analysis: neither functional studies nor comparison to CNVs of patient's family members were performed. We have not considered benign CNVs for this study, which should be considered with caution as information from DGV is related to CNVs detected in healthy individuals without information concerning their fertility.

Wider implications of the findings: High resolution array CGH can uncover potential genetic etiologies of POI. This analysis should be integrated in POI assessment especially in idiopathic cases and in complement to a normal karyotype as long as whole genome sequencing is not used routinely.

Trial registration number: P2016/196/CCB B406201628264

O-175 ACTH-stimulation in women with low functional ovarian reserve (LFOR) confirms adrenal origin of hypo-androgenism

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Study question: Is the reported hypo-androgenism in women with LFOR adrenal or ovarian in origin?

Summary answer: Insufficiency in androgen-production by the adrenal zona reticularis is a characteristic feature of LFOR, profoundly contributing to hypo-androgenism.

What is known already: LFOR, whether due to age or premature ovarian aging (POA), also called occult primary insufficiency (oPOI), has been previously associated with hypo-androgenism (Gleicher et al., Hum Reprod 2013;28 (4):1084-1091).

Study design, size, duration: Prospective cohort study involving 62 women.

Participants/materials, setting, methods: 43 LFOR and 19 normal ovarian reserve (NOR) patients underwent 250 µg Cosyntropin (ACTH) stimulation in AM after overnight fast. Based on levels <95% CI, 12/43 LFOR patients were low-DHEAS. LFOR was defined as FSH > 95%CI for age and/or AMH < 95%CI for age for CHR's patient population (Barad et al., Reprod Biomed Online 2011;22(3):284-291; Barad et al., Obstet Gynecol 2007;109(6):1404-1410). Serum cortisol, 17-hydroxy-progesterone, DHEA, DHEAS, androstenedione and total testosterone (T) were evaluated at baseline and 30 mins.

Main results and the role of chance: Age did not vary between groups. As expected, FSH ($P = 0.0003$) and AMH ($P < 0.0001$) did. At baseline, with and

without LFOR, whether DHEAS was normal or low, all androgens, DHEA ($P < 0.0001$), DHEA ($P = 0.0002$), androstenedione ($P = 0.0005$), and T ($P = 0.0033$) differed significantly. At 30 minutes after ACTH infusion, however, only Δ DHEAS, the only exclusively adrenal androgen, was still significantly lower in LFOR than NOR patients ($P = 0.0265$), suggesting relatively insufficiency of adrenals.

Limitations, reasons for caution: To better understand adrenal control of ovaries via androgen production, further elucidation of endocrine signaling between adrenals and ovaries is required, including detection of yet unknown likely feedback loops.

Wider implications of the findings: It is becoming increasingly clear that adrenals, through diminished androgen production, may inhibit follicular function, producing a form of secondary ovarian insufficiency (SOI), like POI characterized by low estradiol and elevated FSH but, in contrast to POI, potentially treatable through androgen supplementation.

Trial registration number: Not applicable.

O-176 Are serum LH levels at the initiation of ovarian stimulation associated with oocyte retrieval yield after GnRH agonist triggering?

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Study question: To evaluate the relationship between basal serum LH levels and oocyte retrieval yield in GnRH antagonist cycles triggered with a GnRH agonist.

Summary answer: LH levels at the start of ovarian stimulation can identify patients with suboptimal oocyte retrieval yield following GnRH agonist triggering.

What is known already: GnRH agonist trigger may result in an inadequate oocyte yield in a small subset of patients. This failure can range from empty follicle syndrome to a retrieval of much fewer oocytes than expected. Suboptimal response has been defined as the presence of circulating LH values < 15 IU/l 12 h after triggering. It has been shown that patients with immeasurable LH levels on trigger day have an up to 25% risk of suboptimal response. However, it would be far more clinically relevant if one could identify these patients at the start of ovarian stimulation, namely by assessing basal LH levels.

Study design, size, duration: A retrospective cohort study of all patients from 2011 until 2017 who performed GnRH agonist triggering for final oocyte maturation (n = 3334) in GnRH antagonist cycles. Patients with known diagnosis of hypothalamic amenorrhoea were excluded from this treatment. Our primary outcome was oocyte yield, defined as the ratio between the total number of collected oocytes and the number of follicles with a mean diameter > 10 mm prior to GnRH agonist trigger.

Participants/materials, setting, methods: Final maturation triggering was performed using 0.2 mg of triptorelin. In order to evaluate whether LH levels at the start of ovarian stimulation predicted oocyte yield, we performed multivariable regression analysis adjusting for the following confounding factors: female age, body mass index, oral contraceptive pretreatment, basal and trigger day estradiol levels, starting FSH and LH levels and total gonadotropin dose. Suboptimal response to GnRH agonist trigger was defined as $<10^{th}$ percentile of oocyte yield.

Main results and the role of chance: The average age was 31.9 years and the mean oocyte yield was 89%. The suboptimal response to GnRH agonist trigger was 45% ($<10^{th}$ percentile) which was exhibited by 340 patients (10.2%).

Suboptimal responders had lower mean starting FSH, LH and estradiol levels compared to optimal responders 6.1 vs. 6.4 IU/l ($p = 0.04$); 4.74 vs. 5.9 IU/l ($p < 0.001$); and 33.3 vs. 36.4 ng/L ($p < 0.001$) respectively. They also had lower estradiol levels at trigger: 2277.5 vs. 2796.2 ng/L ($p < 0.001$), respectively. Pretreatment with oral contraceptive pill significantly affected the oocyte yield. In the suboptimal response group, 8.6% patients were pretreated with the pill compared with 4.4% ($p < 0.001$) in the optimal responders. Inadequate response was associated with longer stimulation 11.5 vs. 10.6 days ($p < 0.001$) and higher gonadotropin consumption 2086.4 vs. 1838.1 IU ($p < 0.001$).

Following confounder adjustment, multivariable regression analysis showed that LH levels at the initiation of ovarian stimulation remained a significant predictor of oocyte yield.

Patients with immeasurable LH levels at the start of stimulation (<0.11IU/l) had a risk of 45.2% of suboptimal response, while the risk decreased with increasing basal LH levels: < 0.5 IU/L, < 2 IU/L and <5 IU/L were associated with a 39.1%, 25.2% and 13.2% risk respectively.

Limitations, reasons for caution: The main limitation of the study is its retrospective design.

Wider implications of the findings: This is the largest study in GnRH agonist trigger cycles only, since most of the previous research on the predictive value of basal LH levels was performed in dual trigger cycles. LH levels at the start of ovarian stimulation are associated with suboptimal oocyte retrieval yield following GnRH agonist trigger.

Trial registration number: None.

O-177 Luteal phase stimulation compared with conventional follicular phase stimulation in poor ovarian responders: Results of a randomized control trial

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Study question: Does luteal phase stimulation (LPS) result in comparable efficiency to conventional follicular phase stimulation (FPS) in young bologna poor responders (POR)?

Summary answer: LPS results in similar outcomes than FPS in terms of the number of oocytes retrieved and gonadotropin consumption in a population of young Bologna POR.

What is known already: POR remains one of the main therapeutic challenges in women undergoing ovarian stimulation. In the last years, LPS emerged as a feasible way to obtain good quality oocytes. In POR, the use of LPS has been proposed within a strategy of double stimulation starting the gonadotropin administration immediately after the egg retrieval of a conventional stimulation. However, to date, the efficiency of LPS hasn't been tested in a population of POR. Therefore, the study was designed to explore benefits and pitfalls of LPS in POR.

Study design, size, duration: This is a pilot, prospective, single center, randomized controlled trial in a parallel two-arm design. The study included 60 patients < 40 years fulfilling the "Bologna" criteria who underwent ovarian stimulation to accumulate vitrified eggs for immediate IVF treatment. The randomization sequence was performed using a computer-generated randomization list.

Participants/materials, setting, methods: Patients were randomized to be stimulated with rFSH(300IU) + rLH(150IU) (Pergoveris® 2 ampoules per day). FPS arm started stimulation on the 3rd day of the cycle while patients in the group of LPS initiated the gonadotropin administration 4 days after an LH positive test. Both groups started antagonist administration in a flexible protocol. The primary outcome was the number of vitrified oocytes. Secondary outcomes included the duration of stimulation, gonadotropin consume and cancellation rate.

Main results and the role of chance: Using an intention to treat analysis the number of vitrified oocytes per patient did not differ significantly between LPS group 2,1 and FPS group 2,6 ($p = 0,31$). Other parameters of the ovarian stimulation were also comparable: number of cumulus-oocyte complex (COC) 2,7 in LPS and 3,1 in FPS ($p = 0,44$); duration of the stimulation 7,5 days in LPS and 8,0 in FPS ($p = 0,69$); gonadotropin consumption 16,7 ampoules in LPS and 16,3 in FPS ($p = 0,0,76$); maturation rate 77,5% in LPS and 84,0% in FPS ($p = 0,47$) and cancellation rate 20% in LPS and 3,3% in FPS ($p = 0,08$). However, a post-hoc analysis was necessary taking into account that ovarian reserve parameters were not comparable between groups. AMH in the 3rd day of the cycle was lower in the LPS group (4,65 pmol LPS vs 7,30 FPS $p = 0,021$). Other parameters as age, weight, BMI, AFC and number of previous stimulation attempts were not different. Creating a new variable to evaluate the ovarian response in function of the ovarian reserve (COC/AMH) resulted in a higher sensitivity in the LPS group 0,96 eggs per AMH pmol than in the FPS group 0,58 ($p = 0,037$)

Limitations, reasons for caution: This is a pilot study and the sample size was not calculated. The two arms of the study were different in terms of ovarian reserve.

Wider implications of the findings: Data show LPS comparable with FPS in terms of efficiency in POR patients. Therefore, this way of stimulation can be used in this group of patients when planning to accumulate eggs or embryos. Some data suggest a higher ovarian sensitivity so more trials will be necessary to explore this possibility.

Trial registration number: EudraCT: 2015-003856-31
ClinicalTrials.gov Identifier: NCT02625532

O-178 Double stimulation in a single menstrual cycle in patients with reduced ovarian reserve: hormonal characteristics, cumulus cell gene expression, embryological and clinical outcome

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Study question: Does double stimulation (DuoStim) improve the embryological outcome and increase pregnancy rate in patients with reduced ovarian reserve?

Summary answer: DuoStim results in retrieving more good quality oocytes in a single menstrual cycle which leads to a higher clinical pregnancy rate.

What is known already: Multiple follicular waves during the menstrual cycle make it feasible to start ovarian stimulation in luteal phase (LF). LF stimulation and DuoStim have been suggested with the aim of fertility preservation in cancer patients. Previous studies showed that this approach could be a good opportunity for poor-responder patients because it results in more oocytes and embryos. To predict oocyte competence, cumulus cell (CC) gene expression could be used. There are several genes which have a positive correlation with fertilization and embryo development. However, there is limited data about CC gene expression after LF stimulation and IVF treatment success after DuoStim.

Study design, size, duration: This prospective randomized (Microsoft Office Excel 2007) study included 148 patients with reduced ovarian reserve. Group 1 (n = 72) received stimulation on day 2 follicular phase. Group 2 (n = 76) received the DuoStim (stimulation started on day 2, then, on 4 day after the first oocyte retrieval (OR), stimulation repeated during LF. Plasma samples were obtained on stimulation starter day, 6 days after, on triggering and OR day. 40 patients were chosen to study CC gene expression.

Participants/materials, setting, methods: Inclusion criteria: age < 43 years; AMH <1,2 ng/ml; AFC < 6; basal FSH > 11 IU/ml. Exclusion criteria: uterine fibroids, deep endometriosis, cancer. Concentrations of LH, estradiol and progesterone in serum were evaluated with immunochemiluminometric assay. The CC gene expression was evaluated with Real-time PCR.

Main results and the role of chance: Age (36,0 ± 4,5vs.36,7 ± 3,8 in group 1 and 2, respectively) and antimullerian hormone concentrations (0,89 ± 0,3vs.0,94 ± 0,3) were similar in both groups. Comparisons of embryological outcomes showed significant difference between the number of retrieved oocytes (4,4 ± 2,1vs.8,8 ± 4,2, $p < 0,001$), MII (3,9 ± 2,0vs.7,4 ± 3,6, $p < 0,001$) and blastocysts (1,8 ± 1,5vs.2,7 ± 2,7, $p = 0,039$) with values being greater in group 2. The genes HAS2, COX2, GREM1, ALCAM, CD166, VCAN, SDC4, TP53I3, SPSB2, CALM2 expression in cumulus cells after LF group 2 (n = 17) were similar compared to group 1 (n = 23). After embryo transfer in cryo cycle, clinical pregnancy rate in group 2 was higher compared to group 1 (41.7% (30/72) vs. 51,3% (39/76), in group 1 and 2, respectively $p > 0.034$), but no significant differences were observed in the rate of early reproductive loss (16.7% (5/30) vs. 7.7% (3/39), $p = 0.06$). Progesterone (0,9 ± 1,67vs.16,7 ± 13,27) and estradiol (150,94 ± 83,41vs. 384,27 ± 334,44) levels on stimulation starter day were higher in LF group 2 compared to group 1, however, their concentrations on triggering day were similar in both groups (progesterone:3,49 ± 3,62vs.5,65 ± 4,58; estradiol:4630,17 ± 2469,52vs.3697,73 ± 2231,34 in group 1 and LF group 2, respectively).

Limitations, reasons for caution: Our study was carried out in a relatively small subset of patients whose CC gene expression was studied. Therefore, obtained results can not be extrapolated on other groups of patients and need to be confirmed in larger trials.

Wider implications of the findings: This study opens new possibilities for ovarian stimulation in the management of patients with impaired ovarian response.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 50: GENETIC AND EPIGENETIC IMPACT OF ASSISTED REPRODUCTIVE TECHNOLOGIES

Tuesday 3 July 2018 Room 113 + 114 + 115 15:15–16:30

O-179 Increased heteroplasmic load of coding mitochondrial DNA variants in tissues from ART children

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Study question: Is there an increase in heteroplasmic mitochondrial DNA variants in children born after ART?

Summary answer: We have found a statistically significant increase in mean variant load in individuals born after ART as compared to their naturally conceived peers.

What is known already: Children born after ART have an increased risk of a lower birthweight and a slightly abnormal cardio-metabolic profile later in life. Despite much research into epigenetic factors, the molecular cause of these differences remains unknown. Mitochondrial dysfunction is associated with lower birthweight, cardio-metabolic disease and it has been repeatedly suggested to play a role in infertility. Therefore, we hypothesized that an increase in variants in the mitochondrial genome (mtDNA) of ART individuals might explain the observed differences.

Study design, size, duration: We deep-sequenced the full mtDNA of 163 ART and 98 spontaneously conceived individuals, extracted from blood (58 ART, 65 control), saliva (77 ART, 6 control) and placental tissue (28 ART, 27 control). The samples were collected at the Center for Medical Genetics of the UZ Brussel and the Maastricht University Medical Center.

Participants/materials, setting, methods: The full mitochondrial genome was amplified using long-range PCR, sequenced on an Illumina platform and aligned to the reference genome (NC_012920.1) using BWA-MEM. mtDNA server was used to call single nucleotide variants, while insertions and deletions were detected using MuTect, with a lower threshold of 1.5%. Variants in the non-coding regions were excluded from the analysis. We calculated the cumulative heteroplasmic variant load as the sum of the loads of all variants in one individual.

Main results and the role of chance: We found that the three DNA sources followed similar differential distributions in cumulative mtDNA variant load in the ART and control groups. In blood, ART individuals carried a mean load of 9.1%±19.3, while in controls this was 5.4%±12.8. In the placental tissue, the ART carried a mean load of 12.6%±31.4, control samples of 5.7%±12.7. The saliva samples from ART carried a mean load of 8.5%±18.4. When all the

data from the different tissues were pooled per mode of conception, we observed a statistically significant higher mean load in the ART group (ART 9.4%±21.3, control 5.3%±12.4, p = 0.042 with one-tailed t-test). This difference is mainly due to the presence of more individuals in the ART group with higher loads. For instance,12.5% of ART individuals have cumulative loads >30%, while this is 5.1% for the controls (2.4 fold change, p = 0.04, one-tailed Fisher exact). We found no obvious correlation to maternal age and birthweight, although our current sample size is still too small to reliably test this.

Limitations, reasons for caution: The study is currently only able to show an increase in variant load, but not to correlate these to factors such as birthweight, maternal age or ART procedure. For this, we require a larger sample size; the study is still ongoing and we are currently sequencing control samples.

Wider implications of the findings: It is unclear what effect this type of mtDNA variants can have on an individuals' health, but studies in mouse have shown a significant impact of low load heteroplasmies on infertility, birthweight and premature aging. Further research is needed to clarify this and to identify the source of these mutations.

Trial registration number: NA.

O-180 Hypomethylation of imprinted genes in term placenta samples conceived by IVF/ICSI is associated with concomitant changes in post-translational histones modifications

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Study question: Is variability in DNA methylation at imprinted genes (IGs) differentially methylated regions (DMRs) following Assisted Reproductive Technologies (ART) associated with changes in post-translational histones modifications?

Summary answer: After IVF/ICSI, the histones marks at IGs' DMRs displayed enrichment of "active" and simultaneous loss of "repressive" modifications, consistently with the previously observed DNA hypomethylation.

What is known already: ART has been associated with an increased risk of placenta-related adverse pregnancy and perinatal outcomes, major malformations and imprinting disorders. Altogether, these data raise the concern of the potential epigenetic vulnerability associated with ART.

We previously demonstrated that DNA methylation in the placenta was significantly lower after IVF/ICSI than following natural conception at two IGs' DMRs: *H19/IGF2* and *KCNQ1OT1* (Choux *et al.*, *Human Reproduction* 2017). The aim of this original study was to profile active and repressive histones marks in placenta biopsies to reveal a better understanding of the epigenetic modifications in the context of ART.

Study design, size, duration: Patients were prospectively included from January 1st 2013 to April 30th 2015 in the Department of Obstetrics, Gynaecology and Reproductive Biology at Dijon University Hospital, France. Controls were singleton pregnancies of women that had conceived spontaneously within 1 year after stopping contraception. IVF/ICSI patients were singleton pregnancies achieved following fresh embryo transfer after two days of *in vitro* culture.

Participants/materials, setting, methods: In our previous study, we compared the placental DNA methylation by pyrosequencing in 51 IVF/ICSI *vs.* 48 control placentas for three IGs DMRs: *H19/IGF2*, *KCNQ1OT1* and *SNURF*. In the present study, we analysed histones marks abundance by Chromatin ImmunoPrecipitation (ChIP) in 16 patients who presented with below the 5th percentile of DNA methylation for at least one of these DMRs and compared with 16 controls matched for parity, new-born's sex, and gestational age at delivery.

Main results and the role of chance: DNA methylation of *H19/IGF2*, *KCNQ1OT1* and *SNURF* DMRs was significantly lower in the IVF/ICSI group (45.1 [43.2-48.9]; 32.8 [31.7-35.7] and 38.3 [35.5-40.5], respectively) than in the natural conception group (53.5 [49.6-59.3], P = 0.004; 39.4 [34.8-41.9], P = 0.001 and 41.2 [38.4-42.1], P = 0.036, respectively). Quantitative PCR targeting *H19/IGF2* and *KCNQ1OT1* DMRs in ChIP material precipitated with H3K4me2, a permissive modification, revealed significantly higher quantity of H3K4me2 in the IVF/ICSI group than in the natural conception group (P = 0.016 and 0.003, respectively). There was no significant difference for H3K4me2 for *SNURF*, or for the others permissive marks (H3K4me3, H3K9ac). The quantity of the repressive marks H3K9me3 and H3K9me2 at *H19/IGF2* and *SNURF* DMRs was significantly lower in the IVF/ICSI group than in the natural conception group (P = 0.011 and 0.027 for *H19/IGF2*, respectively; and P = 0.010 and 0.035 for *SNURF*, respectively) but there was no significant difference for *KCNQ1OT1*.

Limitations, reasons for caution: These data were assessed on a limited number of IGs DMRs. Therefore, it would be interesting to extend analyses to others DMRs.

Wider implications of the findings: These novel findings highlight that DNA hypomethylation at IGs' DMRs after ART is linked with increased active/decreased repressive histones marks, altogether promoting an "active" conformation of the chromatin. This concomitant change in epigenetic state at IGs at birth might be an important developmental event as a consequence of ART manipulations.

Trial registration number: not applicable.

O-181 Ovarian stimulation for IVF and risk of primary breast cancer in BRCA1/2 mutation carriers

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Study question: Does exposure to ovarian stimulation for IVF increase the risk of primary breast cancer for *BRCA1/2* mutation carriers?

Summary answer: No increased risk for breast cancer was found after ovarian stimulation for IVF in *BRCA1/2* mutation carriers.

What is known already: Female carriers of a pathogenic mutation in the *BRCA1* or *BRCA2* gene have a high risk of breast cancer. Their breast cancer risk rises from age 25 onwards and is particularly increased at reproductive age. In the general population no association between exposure to ovarian stimulation for IVF and breast cancer risk was found. However, a potential effect is rarely examined in *BRCA1/2* mutation carriers. This matter is of particular interest since *BRCA1/2* mutation carriers not only undergo IVF for infertility or fertility preservation, but also and in an increasing extent for preimplantation genetic diagnosis (PGD).

Study design, size, duration: 1,550 *BRCA1* and 964 *BRCA2* carriers were derived from (1) the nationwide HEBON study, a retrospective cohort with prospective follow-up among *BRCA1/2* mutation families, and (2) the national PGD registry. Observation time started at birth and ended at first invasive breast cancer diagnosis (event of interest), other cancer diagnosis or bilateral prophylactic mastectomy, whichever occurred first. In case these events did not occur, observation time ended at the age of questionnaire or last PGD contact.

Participants/materials, setting, methods: Eligible women were 18 years or older and had a pathogenic mutation in the *BRCA1* or *BRCA2* gene. Questionnaires were used to collect data on IVF exposure. Information on risk-reducing surgeries and cancer diagnoses was derived from clinical records and linkages with the Netherlands Cancer Registry, respectively. A time-dependent IVF exposure variable was used in Cox regression analyses, stratified for birth cohort and adjusted for infertility.

Main results and the role of chance: IVF exposure was not associated with risk of breast cancer for *BRCA1/2* mutation carriers (hazard ratio (HR) 0.79, 95%CI 0.46-1.36). Similar results were found for the subgroups of infertile women (n = 232; HR 0.73, 95%CI 0.39-1.37) and *BRCA1* mutation carriers

(HR 1.12; 95%CI 0.60-2.09). In addition, age at first IVF treatment and recency of IVF treatment were not associated with breast cancer risk.

Limitations, reasons for caution: Despite the availability of a nationwide cohort, power is still limited due to the low portion of women exposed to IVF. The subgroup *BRCA2* mutation carriers was too small for separate analysis. Hazard ratios were slightly biased to zero due to the oversampling of breast cancer cases.

Wider implications of the findings: Based on the current data, there is no reason to exclude *BRCA1/2* mutation carriers from IVF for fertility treatment, fertility preservation or PGD. Obviously, ruling out pre-existing lesions before IVF remains important because of the high *a priori* breast cancer risk in these women.

Trial registration number: Not applicable.

O-182 Higher gonadotropin dosage and longer ovarian hyperstimulation do not influence aneuploidy rates

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Study question: Does the gonadotropin dosage, duration of ovarian hyperstimulation, peak estradiol levels, or number of oocytes retrieved affect aneuploidy rates?

Summary answer: Aneuploidy rates are not affected by higher gonadotropin dosage, longer stimulation, higher estradiol levels, or larger number of oocytes harvested, regardless of a woman's age.

What is known already: Aneuploidy rates increase steadily with age, reaching >80% in women >42 years old. Preimplantation genetic screening (PGS) has been used along with blastocyst morphology to enhance embryo selection. The goal of controlled ovarian hyperstimulation (COH) is to overcome this high aneuploidy rate through the recruitment of several follicles, which increases the chances of obtaining a euploid embryo that results in a healthy conceptus. However, a recent study reported a significant difference in the aneuploidy rates (range: 39.5%–82.5%) between young oocyte donors who underwent stimulation at different fertility centers. These findings suggest that various methods of stimulation might affect aneuploidy rates.

Study design, size, duration: A retrospective cohort study including PGS data was conducted to compare aneuploidy rates. A total of 1,463 IVF/PGS cycles between January 2013 and December 2016 were included. PGS was performed using array comparative genomic hybridization. χ^2 test was used for categorical variables.

Participants/materials, setting, methods: A total of 7,980 embryos were analyzed for ploidy status. Cycles were divided into five age groups (<35, 35–37, 37–39, 40–42, and >42 years old), and aneuploidy rates were compared between different total gonadotropin dosages (<5,000 and ≥5,000 IU), duration of stimulation (<10, 10–13, and ≥14 days), number of oocytes harvested (<10, 10–19, and ≥20 oocytes), and peak estradiol (E2) levels (<1,500, 1,500–3,000, and >3,000 pg/mL).

Main results and the role of chance: Within the same age group, aneuploidy rates were not significantly different between cycles with different total gonadotropin dosages, duration of stimulation, number of oocytes retrieved, or peak estradiol levels. In the youngest group (<35 years, n = 2,366 embryos), aneuploidy rates were comparable between cycles of various total gonadotropin dosages (44.1% for <5,000 IU and 45.8% for ≥5,000 IU; $P = 0.7$), duration of stimulation (44.4% for <10 days, 44.2% for 10–13 days, and 40.4% for ≥14 days; $P = 0.8$), number of oocytes harvested (38.5% for <10 oocytes, 44% for 10–19 oocytes, and 45.8% for ≥20 oocytes; $P = 0.1$), and peak estradiol levels (45.7% for E2<1,500 pg/mL, 45.4% for E2 1,500–3,000 pg/mL, and 43.8% for E2>3,000 pg/mL; $P = 0.7$). Similarly, in the oldest group (>42 years, n = 741 embryos), aneuploidy rates ranged from 90.5% for gonadotropins <5,000 IU to 90.6% for gonadotropins ≥5,000 IU ($P = 1$), from 87.8% for <10 days of stimulation to 94.0% for ≥14 days of stimulation ($P = 0.3$), from 93.1% for <10 oocytes to 91.3% for ≥20 oocytes ($P = 0.1$), and from 88.6% for E2<1,500 pg/mL to 91.3% for E2>3,000 pg/mL ($P = 0.6$).

Limitations, reasons for caution: Although this large study (1,463 IVF/PGS cycles and 7,980 embryos) demonstrates the safety of COH in terms of aneuploidy, it would also be interesting to determine whether gonadotropin dosage affects the implantation potential of euploid embryos. Furthermore, a multicenter study may help to prove the generalizability of our single-center data.

Wider implications of the findings: The findings of this study reassure providers and patients that the total gonadotropin dosage, duration of ovarian hyperstimulation, estradiol levels, and number of oocytes retrieved do not influence aneuploidy rates, regardless of a woman's age.

Trial registration number: N/A.

O-183 Correlation between Embryo Ploidy Status and ovarian response after controlled ovarian Stimulation in ART cycles

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Study question: Is there a relationship between ovarian response and number of euploid embryos and aneuploidy rate in preimplantation genetic testing for aneuploidy (PGT-A)?

Summary answer: Ovarian response (OR) after conventional IVF stimulation is positively correlated with number of euploid embryos. Euploidy rate in younger patients decreases when OR is exacerbated.

What is known already: A positive relationship between ovarian response and the embryo aneuploidy rate has been described when mild controlled ovarian stimulation (COS) is performed in infertile patients, but this trend is not observed in patients who undergo conventional COS treatments with higher doses. It has been recently suggested by a published study that a threshold level for gonadotropin doses may exist and that no more competent oocytes can be obtained if it is exceeded. It has been shown in a retrospective study that high ovarian response to conventional ovarian stimulation does not increase embryo aneuploidy rates in aCGH-PGS cycles.

Study design, size, duration: 1560 blastocysts generated after ICSI between January 2016 to January 2017, derived from 210 patients (mean age 37.4 (SD 3.5) years), were included in study. This single centre retrospective cohort study was conducted at the Centre for Reproductive and Genetic health (CRGH), UK. Cycles were classified according to OR: poor (<4 oocytes collected), suboptimal (4-9), normal (10-14) and hyperresponse (>15). Embryo biopsy was performed at the blastocyst stage and PGT-A performed with Next Generation Sequencing.

Participants/materials, setting, methods: Female age was used to group the sample according to the Human Fertilisation and Embryology Authority (HFEA) categories into: <35 years (49/210), 35-37 years (43/210), 38-39 years (38/210), 40-42 years (40/210) and >42 (40/210). Number of euploid embryos, euploidy rate and relevant cycle parameters (i.e oocytes collected, embryos biopsied) was annotated for each of the categories. Statistical analysis was done using SPSS version 23.

Main results and the role of chance: Mean age for all patients in cohort 37.4+/-3.5 years. Analysis of euploid rate (number of euploid embryos/blastocysts biopsied) negatively corelates with an increase in ovarian response. This association is evident especially for young patients (<35 years) where euploidy rate for normal OR is 48% and drops to 32% in hyperresponse. Euploidy rate shows the same trend with a higher OR, but is not statistically significant in all age groups. Spearman's rank-order correlation was performed to determine the relationship between oocytes retrieved and euploidy rate in the same cycle (all ages). Analysis concluded a strong, negative correlation between both variables ($rs = 0.-062$, $p = 0.035$). The Poseidon group defined a "suboptimal response," (4-9 oocytes), which is associated, at any given age, with a significantly lower live birth rate compared with normal responders i.e., those with 10–15 oocytes. The euploidy rate in the "Poseidon" group was 28.1%. This rate is 0% in patients above 38 years of age when 4-9 oocytes are retrieved. Euploidy rate per oocyte collected decreases significantly for all age categories when number of oocytes retrieved is + 12. Maximum euploidy is obtained

when 8-10 oocytes are retrieved for patients (<35), 10-12 oocytes for ages 35-39 and 9-11 oocytes for patients above 40.

Limitations, reasons for caution: Larger studies (well-designed trials) with an increased number of biopsied embryos are needed to confirm our findings with analysis. The trials designed should also standardise the PGT-A technique being used as different laboratories have different reported sensitivity assays.

Wider implications of the findings: The first study that analysed the correlation between ovarian response and euploidy rate where PGT-A was performed using NGS technique on blastocysts. The degree of ovarian stimulation should be tailored to individual patient's ovarian reserve to optimise number of eggs retrieved thus resulting in a better yield of euploid embryos.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 51: IMMUNOLOGIC AND OTHER TREATMENT OPTIONS IN RM/RIF

Tuesday 3 July 2018 Room 117 15:15–16:30

O-184 Effect of Administration of Intravenous intralipid on implantation rate in women with implantation failure after IVF/ICSI: A randomized controlled trial

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Study question: To measure and compare the clinical pregnancy rate in women who receive intravenous intralipid versus placebo with previous implantation failure after fresh non donor IVF/ICSI.

Summary answer: Administration of intravenous intralipid in women with previous implantation failure after fresh non donor oocyte IVF/ICSI leads to a significant increase in clinical pregnancy rate.

What is known already: Proposed mechanisms for recurrent implantation failure is immunological abnormalities which reduce maternal endometrial receptivity, with focus on uterine natural killer (NK) cells and cytotoxic cytokines. Intravenous intralipid, a sterile fat emulsion containing soy oil, egg yolk, glycerin, and water, has traditionally been used in patients requiring total parenteral nutrition and has found to reduce the incidence of sepsis by regulating immune dysfunction. It is hypothesized that the fatty acids in the intralipid serve as ligands to activate peroxisome proliferators activated receptors (PPARs) expressed by the NK cells which decreases NK cytotoxic activity, which can enhance implantation and maintain pregnancy.

Study design, size, duration: Randomized controlled trial of 102 subjects with previous failed IVF undergoing IVF/ICSI from June 2016 to January 2018. Subjects were randomized after oocyte pickup into two arms.

Women with anatomical defects by diagnostic hysteroscopy, genital tuberculosis by endometrial aspiration for acid fast bacilli, acquired thrombophilias, and immunological co-morbidities were excluded.

Participants/materials, setting, methods: Women in the intervention arm(n = 52) received 2 doses of 20% intravenous intralipid (Fresenius Kabi), 4 mL diluted in 100 mL normal saline by slow infusion. The first dose was given immediately after oocyte recovery, second dose given one hour prior to transfer. The control group (= 50) received normal saline. All the women received routine luteal phase support. Outcomes measured were implantation rate and clinical pregnancy rate. No adverse effects of intralipid were observed.

Main results and the role of chance: The mean age of the participants was (31.6+/-4) in the intervention group and (32.1+/-3.5) years in control group (P = 0.47). The mean BMI was (24.1+/-3.1) in intervention and (24.6+/-3.3) kg/m2 (P = 0.394) in control group. The average number of attempts of IVF was (2.33+/-0.5) in both the groups, with minimum and maximum of 1 and 3 previous failed IVF.

The implantation rate (Beta HCG >/- 100 IU 14 days after embryo transfer) was 38.5% and 14% in intervention and control groups (P = 0.005). Clinical pregnancy rate as determined by sonographic fetal cardiac activity was 34.6% and 14% in intervention and control groups (P = 0.016). Ongoing pregnancy rate as defined as pregnancy continuing beyond 16 weeks gestation was 34.6% and 14% in intervention and control groups (P = 0.016). No adverse effects of intralipid were observed.

Limitations, reasons for caution: None

Wider implications of the findings: This study shows a statistically significant increase in implantation rate and clinical pregnancy rate in women who receive intravenous intralipid with prior implantation failure after IVF/ICSI, with no adverse effects.

Trial registration number: CTRI/2017/01/017206

O-185 Recombinant Human Granulocyte - Colony Stimulating Factor (rhG-CSF) in women with unexplained Recurrent Pregnancy Losses (RESPONSE Study): randomised, double-blind, multicentre, placebo controlled trial

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Study question: Does administration of recombinant human granulocyte - colony stimulating factor in the first trimester of pregnancy improve outcomes in women with a history of unexplained recurrent pregnancy losses?

Summary answer: There is no evidence that administration of rhG-CSF in the first trimester of pregnancy improves outcomes, among women with history of unexplained recurrent pregnancy losses.

What is known already: rhG-CSF is shown to improve pregnancy outcomes by immunomodulation. The evidence prior to this study is only one RCT which was a smaller single centre study and another 4 observational study which suggested statistically significant increase in pregnancy rates and live birth rates.

Study design, size, duration: Women between the age of 18 to 37 years with a BMI of 19-35 (at the time of consent) and actively trying to conceive naturally after being diagnosed with a history of unexplained recurrent pregnancy losses were eligible. 340 women were screened into the study of which 150 were randsomised between June 2014 and June 2016. This was a double bind, placebo controlled randomised trial.

Participants/materials, setting, methods: The participants were recruited from 21 hospitals with established recurrent pregnancy loss clinics from across the United Kingdom. Eligible participants were randomised to receive rhG-CSF 130 mcg or placebo in a 1:1 ratio. Stratified permuted block randomisation was used with number of prior miscarriages (3, >3), and age (<35, 35-37 years)as the stratification factors. An online computerised system was used for randomisation. The primary outcome was ongoing clinical pregnancy at 20 weeks of gestation.

Main results and the role of chance: A total of 340 participants were screened for eligibility, of whom 150 women were randomised. 76 were randomised to rhG-CSF and 74 were randomised to placebo. All women were followed-up to primary outcome, and beyond to live birth. The clinical pregnancy rate at 20 weeks, as well as the live birth rate, was 59.2% (45/76) in the rhG-CSF group, and 64.9% (48/74) in the placebo group, giving a relative risk of 0.9 (95% CI: 0.7 to 1.2; p = 0.48). There was no evidence of a significant difference between the groups for any of the secondary outcomes.

Limitations, reasons for caution: The limitation of this study was that participants were not screened prior to inclusion to demonstrate immune dysfunction as the reason for their pregnancy losses. This is because there is no accepted test(s) for immune dysfunction in reproductive immunology. This trial was therefore exclusively for women with unexplained recurrent miscarriages.

Wider implications of the findings: G-CSF is widely used in reproductive medicine to treat recurrent miscarriages. Observational studies suggested

statistically significant improvements in clinical pregnancy rates after administration of G-CSF. However, we now have high quality evidence suggesting that G-CSF is not an effective treatment for patients with unexplained recurrent miscarriages.

Trial registration number: Eudract Number –2014-000084-40.

O-186 A reduction in uterine Natural Killer (uNK) cell density in response to luteal-phase endometrial scratch (ES) is associated with improved pregnancy outcome

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Study question: Does a change in uNK cell density following endometrial tissue injury relate to subsequent pregnancy outcome in women with recurrent reproductive failure?

Summary answer: A reduction in uNK cell density in response to endometrial scratch is associated with higher rates of ongoing pregnancies (≥12 weeks) and live births (P = 0.007).

What is known already: Work from our laboratory demonstrated an important role for uNK cells in maintaining tissue haemostasis in cycling endometrium. Specifically, IL-15 activated uNK cells target and clear acute senescent decidual cells during the window of implantation. Some but not all clinical trials have suggested that endometrial injury from scratch improves pregnancy outcome. This conflicting evidence could be explained by an inter-patient variation in response to injury. We hypothesised that changes in uNK cell density normalised to the day of biopsy relative to the LH surge, could be a marker of endometrial plasticity and by extension, subsequent pregnancy outcome.

Study design, size, duration: This is a 3 year prospective cohort study of 60 women with recurrent reproductive failure (2014-2017). Participants had either a history of recurrent miscarriage or implantation failure following IVF/ICSI. All participants had two LH-timed, mid-luteal endometrial biopsies at 1 to 6 months apart and a pregnancy or embryo transfer within one year of the second biopsy (2015-2017).

Participants/materials, setting, methods: Women were recruited from a dedicated Implantation Research Clinic for couples with recurrent reproductive failure. Participants had two pre-conceptual, mid-luteal endometrial biopsies. Each biopsy was subjected to immunohistochemistry staining for CD56 (uNK cell marker). uNK cell density was calculated using image analysis and normalised for day of biopsy relative to LH-surge, using a published centile chart based on 1900 samples. Inter-cycle variation was defined as the difference between uNK cell centiles of the two biopsies.

Main results and the role of chance: There was a significant variation in the inter-cycle difference in normalised uNK cell densities between participants with some patients showing minimal or no inter-cycle variability whereas the response was marked in others. In order to assess the clinical importance of this variance, we analysed uNK cell variance in two clinical groups according to subsequent pregnancy outcome following the second biopsy. Group 1 included women who achieved a subsequent ongoing pregnancy beyond 12 weeks gestation or live birth (n = 29) and Group 2 consisted of women who experienced a further first trimester miscarriage or IVF/ICSI implantation failure (n = 31). Group 1 had a significant reduction in uNK cell density [P = 0.007, median difference: -13% (CI -4 to -26.7)]. In comparison, Group 2 showed no significant inter-cycle variation [P = 0.58, median difference: -1.5% (CI -16.6 to 10.8)].

Limitations, reasons for caution: Linking endometrial factors to pregnancy outcome is hindered by the fact a proportion of miscarriages and implantation failures will be due to chromosomally abnormal embryos. The findings in this study are not yet robust and confidence intervals are large. To confirm or refute these findings, a larger cohort is required.

Wider implications of the findings: Our findings support the emerging concept of endometrial plasticity, i.e. the ability of the endometrium to adapt from cycle to cycle in response to stress signals. In addition, the patient-specific response to tissue injury likely determines the clinical benefit, or lack thereof, of the scratch procedure in clinical practice.

Trial registration number: N/A.

O-187 Combinatorial use of Endometrial Receptivity Array (ERA) and PGT-A can improve the clinical outcome in cases with previous ART failures

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Study question: This study investigates whether ERA and PGT-A approaches can be properly integrated in order to improve the clinical outcome in cases with previous ART failures.

Summary answer: Endometrial receptivity assessment by ERA and euploidy assessment by PGT-A can be effectively coupled in order to maximize the clinical outcome in recurrent ART failures.

What is known already: It has been shown that ERA test, which is based on the transcriptomic signature of 236 genes of a given endometrial sample, can successfully and objectively assess the endometrial receptivity in patients undergoing ART. By using this tool, recent reports indicate that in around 30-35% of the implantation failures can be due to pre- or post-receptive state of the endometrium and subsequent pET can rescue this defect. It is also well-accepted that majority of implantation failures can be of embryonic origin and by transferring euploid embryos high pregnancy rates can be obtained after PGT-A by comprehensive chromosomal screening (CCS).

Study design, size, duration: This retrospective clinical study includes clinical data of 632 patients with previous ART failures, whose endometrial receptivity were assessed by ERA between November 2013 – August 2017. During the study period, based on the ERA results, embryo availability as well as the clinical treatment protocol, patients were subsequently scheduled for either frozen embryo transfers (With embryos of unknown or known euploidy status), a freeze-all cycle or a PGT-A cycle.

Participants/materials, setting, methods: All endometrial biopsies were performed by a pipelle under general anesthesia after a hormone replacement therapy cycle (HRT) adjusted for expected window of implantation (WOI; at P + 5). Same HRT approach was also used in the subsequent frozen embryo replacements in patients with receptive results. In patients with displaced receptivity, embryo warming and transfer was personalized according to producer's recommendations. All PGT-A cycles involved Day5/6 trophectoderm biopsy, embryo cryopreservation and CCS analysis by aCGH or NGS.

Main results and the role of chance: Of these 632 patients, mean female age and number of previous ART trials were 35.8 ± 4.7 and 2.2 ± 2.3 respectively. Out of 684 endometrial biopsy samples obtained, 657 (96.1%) were successfully diagnosed as receptive (n = 432; 65.8%), pre-receptive (n = 160; 24.3%) and post-receptive (n = 65; 9.9%). In 27 (4.1%) of the samples, results were not informative. Based on the test results and clinical conditions, a total of 516 frozen embryo transfer cycles were performed either by embryos with unknown euploidy status (not screened by PGT-A; n = 171; Group I) or by euploid embryos obtained from a previous PGT-A (n = 345; Group II) cycle. Sixtyfour cycles (37.4%) in Group I and 108 cycles (31.3%) in Group II were performed as pET (p = 0.35). Biochemical pregnancy, clinical pregnancy, implantation as well as missed abortion rates were 47.4%, 43.2%, 31.8% and 20.3% for Group I and 75.9%, 70.4%, 65.8% and 16.4% for Group II respectively and these rates were found to be significantly higher in the latter (p < 0.01). At the time of data collection, the percentage of ongoing pregnancy/live birth cases in Group II was also significantly higher (58.2%) than the one in Group I (34.5%; p < 0.001).

Limitations, reasons for caution: Implantation is a complex process, involving synchronization of a variety of known as well as yet unknown embryonic & maternal factors. This study aimed at analyzing the possible impact of embryonic euploidy and endometrial receptivity assessment in patients with previous ART failures.

Wider implications of the findings: Results indicate that around 30% of patients with history of ART failures in this study show displaced endometrial receptivity. Personalization of FET cycles by the integration of ERA test in

conjunction with PGT-A can significantly improve the clinical outcome in these cases.

Trial registration number: None.

O-188 Immune therapies for women with history of unsuccessful implantation undergoing IVF/ICSI treatment - A Cochrane collaboration systematic review

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[°]*Contributed Equally,*

Study question: Do immune therapies improve live birth rate in women with a history of recurrent implantation failures (RIF), who are undergoing another cycle of assisted reproduction?

Summary answer: There is some evidence that intralipid may improve live birth rate with PBMC,GCSF treatment conferring potential benefit with regards to clinical pregnancy rate. However, the safety profile of these agents needs further investigation.

What is known already: RIF remains a clinically and emotionally challenging diagnosis for most patients undergoing IVF. Currently, there is lack of evidence to support specific screening tests and therapeutic measures. This has led to an offering of "empirical" treatment with immuno-suppressive agents to patients who experience RIF. However, this approach has been based on very little clinical evidence and treatment is offered to patients, without fully assessing the risks and benefits.

Study design, size, duration: The Cochrane Gynaecology and Fertility Group Specialised Register, the Cochrane Central Register of Studies Online (CRSO), MEDLINE, Embase, CINAHL and PsyINFO were searched from inception to September 2017. Randomised controlled trials (RCTs), where immunotherapy was given to women with a history of unexplained RIF who were undergoing assisted reproduction, were included for data analysis. The primary outcomes were live birth and miscarriage rates below 24 weeks of gestation. Secondary outcomes included clinical pregnancy rate and adverse events such as preterm delivery, multiple pregnancy and drug side effects. All outcomes were reported per woman randomised.

Participants/materials, setting, methods: The systematic review included 19 studies with a total of 1748 patients. G-CSF was used in 8 studies, heparin in 4, intralipid in 2 and leukocyte inhibitory factor (LIF) in 2 studies.

Risk ratios (RRs) with 95% confidence intervals (CIs) were calculated for each study and the data were synthesized using a fixed-effect model. The quality of the evidence was assessed using the GRADE method.

Main results and the role of chance: The evidence showed that intralipid probably improves the live birth rate compared to no treatment (RR 2.13, 95% CI 1.35 to 3.36; participants = 303; studies = 2; I2 = 6%, moderate certainty evidence)). Similarly heparin may slightly improve the live birth rate compared to aspirin, however the evidence for this was graded as low certainty evidence (RR 2.56, 95% CI 1.17 to 5.58; participants = 126; studies = 1; I2 = 0, low certainty evidence).

The evidence for miscarriage rate for intralipid, PBMC and LIF was insufficient or not reported for heparin and GCSF.

There was moderate evidence that PBMC, GCSF and Heparin treatment may improve the clinical pregnancy rate.

The data on adverse effects, either during the IVF procedure or pregnancy were either sparse or missing. The majority of studies had no means of blinding and were at high risk of performance and detection bias.

Limitations, reasons for caution: The use of intralipid is still under investigation and should be used with caution; PBMC is still at the experimental stage. Therefore, these results should only be applied to the research setting; further work is needed before it is offered in the clinical context.

Wider implications of the findings: Once the pathophysiology of RIF is understood, and its definition is agreed upon, targeted immune therapies may be proved effective in increasing pregnancy and live birth rates.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 52: REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

Tuesday 3 July 2018 Room 116 15:15–16:30

O-189 The DOD (Distance Oocytes Donation) strategy: A safe, efficient and "patient friendly" way to overcome lack of egg's donors in different countries

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Study question: DOD (Distance Oocyte Donation) as fresh IVF-eggs donor's cycles with individual blastocyst vitrification and subsequent shipping to the recipient's country for thawed embryo transfers: a good alternative to reproductive tourism?

Summary answer: As a "patient friendly" strategy with good success rates, DOD cycles are a valid alternative to reproductive tourism in countries with lack of egg's donors.

What is known already: In some countries, legal statements as well as ethical, cultural and social aspects generate a huge discrepancy between the need for egg donation treatments and the availability of egg donors. Therefore, many recipient patients will travel to a different country to seek for egg donation treatments: it is not astonishing that in Europe more than 1/3 of all egg donor's cycles is performed in Spain (European registers 2013 by ESHRE). The cost of reproductive tourism is very high for patients: physical and psychological distress, difficulties in the medical follow-up during and after treatment, travel expenses, loss of working days, etc.

Study design, size, duration: This is a retrospective study of 215 DOD cycles preformed during a two years time period (may 2015 - may 2017) between two IVF clinics, based in two different European countries, as Spain (Barcelona) and Italy (Turin).

Participants/materials, setting, methods: For 195 couples, a frozen sample of husband's sperm was shipped from the Italian clinic to the Spanish clinic. In 20 cases, we used donor's sperm samples proceeding from a Spanish sperm bank (Barcelona, Spain). At the Spanish clinic, a total of 215 fresh IVF-eggs donor's cycles were completed and blastocyst stage embryos individually vitrified. Frozen embryos were shipped back to the Italian clinic in liquid nitrogen and 215 recipients underwent thawing transfer cycles.

Main results and the role of chance: When comparing DOD cycles with standard fresh egg-donor's cycles completed in the same time-period at the Spanish clinic in Barcelona, no statistically significant differences were observed in the mean number of good morphology blastocysts/cycle obtained (3.4 vs 3.4), mean age of recipients (41.7 years vs 42.1 years) and donors (26 years vs 26 years). A mean number of 1.58 (1-4) thawed transfers/DOD cycle was

performed. A single blastocyst transfer was performed in 90.9% of the DOD thawed transfers, being 1.09 the mean number of transferred embryos in each transfer. Clinical pregnancy rate was 64.6% (139/215) miscarriage rate was 12.6% (27/215), live birth rate was 52% (112/215), and twins rate was 0.9% (2/215).

Limitations, reasons for caution: Efficiency and safety of the DOD strategy depends on a strong and consistent blastocyst freezing/thawing method. Moreover strong partnership and shared IVF protocols between clinics, are needed to ensure a good outcome. Strict shipping rules are applied when frozen gametes and embryos are shipped between different countries.

Wider implications of the findings: The DOD strategy seems to be a safe and efficient alternative to the reproductive tourism in those countries where - for different reasons - there is a lack of eggs donors. DOD cycles are "patient friendly" treatments, allowing patients to receive reproductive care in their own country.

Trial registration number: Not applicable.

O-190 You did not turn up… I did not realise I was invited…: Understanding male attitudes towards fertility awareness and poor engagement

B. Grace[1], J. Shawe[2], S. Johnson[3], J. Stephenson[4]

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Study question: What are the underlying reasons of poor male involvement in fertility awareness and reproductive health?

Summary answer: Improving male involvement in fertility awareness reproductive health will require more male focused initiatives in order to engage men better.

What is known already: Involvement of men in fertility and reproductive health is important for healthy pregnancies and positive outcomes for mother, father and child. However in research studies in these areas, there is a dearth of information on men's perspective. Whilst many studies have postulated the numerous reasons for lack of inclusion of men in reproductive health research, few studies have actually included men. Poor engagement by men is often cited as reason for this. In our study, we interviewed different groups, including men, to understand the underlying reasons for the lack of engagement.

Study design, size, duration: The study was a qualitative component of a wider mixed methods study. We carried out 35 qualitative in-depth interviews on 3 population groups: 13 women, 13 men and 9 healthcare professionals. Interviews were conducted between October 2016 and February 2017. Interviewees were purposively sampled to include men and women from the reproductive age range (18-45 years) and of varying ethnic and educational backgrounds.

Participants/materials, setting, methods: Participants were sampled from a UK cross-sectional survey on Fertility Awareness with 1080 participants who agreed to a follow-up interview. Survey participants were recruited nationwide via online newspaper and social media adverts. Healthcare professionals (HCPs) included doctors and nurses. Healthcare professionals were recruited from professional bodies such as RCN, RCGP, RCP, doctors.org.uk. Data was transcribed and analysed via framework analysis. Favourable ethical opinion was given by UCL.

Main results and the role of chance: The difficultly experienced recruiting the required samplesize of men highlighted a disconnect between men and reproductive health issues. We assessed this issue from the men's, women's and HCPs' perspectives. The reasons different groups gave for lack of male involvement were varied and reflected a need to evaluate different approaches for improving male involvement.

We found recurring themes towards men's reluctance to engage in fertility discussions. Women expressed that this was due to stereotypical male and female roles. They discussed the impact of societal norms and the perception that fertility is the 'woman's territory'. HCPs supported this view but also highlighted that poor male involvement was across healthcare needs; not just unique to fertility.

Contrary to expectations, we found that men wanted to be involved in family building discussions and wanted to improve their knowledge. However, men felt they did not have a voice on the topic because discussions have traditionally focused on women. The notion that men were not expected to be interested and engaged thus becomes a self-fulfilling prophecy.

To encourage male involvement, services, education programmes and researchstudies should widen scope to include men. Additionally, typical methods for attracting and incentivising women in studies will not necessarily work for men.

Limitations, reasons for caution: Although we gathered rich data, interviewees were self-selected and results principally reflect views of those who we willing to participate. Due to the online recruitment method, there is a bias towards more educated respondents with online internet access.

Wider implications of the findings: To encourage male involvement, the current female-oriented services and education programmes on fertility and reproductive health should be reviewed and revised to involve men. Additionally, educational programs on sexual and reproductive health should be engaging and structured to include boys and adolescents from a young age.

Trial registration number: Not applicable.

O-191 Risk of prostate cancer in ICSI treated men

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Study question: At what risk for prostate cancer are clinically sub-fertile men who have undergone ICSI as compared to men conceiving naturally?

Summary answer: Men who had undergone ICSI had a statistically significant increased risk of prostate cancer, in particular, early-onset prostate cancer, compared to men conceiving naturally.

What is known already: Register-studies have reported lower risk of incident prostate cancer for childless men than biological fathers. Other studies have indicated that men with impaired fertility are at higher risk for prostate cancer than fertile men. The majority of the men undergoing ICSI treatment are subfertile and since they are in contact with the health care system, these men are well suited as target for preventive measures.

Study design, size, duration: This register-based study sourced data from the Swedish Medical Birth Register, the Swedish Cancer Registry, and the Swedish Quality Register for Assisted Reproduction. All fathers and their first child born 1994-2014 were identified. ICSI fathers were compared to those who had become fathers by natural conception (controls) and IVF fathers regarding incident prostate cancer during a follow up of total 51990101 person-years until 2016. Sensitivity analysis stratified upon age at diagnosis of prostate cancer.

Participants/materials, setting, methods: Among all fathers (n = 1 181 490), 20 618 and 14 882 had undergone IVF and ICSI, respectively; and 3 211 were diagnosed with prostate cancer. Associations between mode of conception (ICSI/IVF/natural) and subsequent prostate cancer were investigated using Cox regression models with their date of birth as start of follow-up and adjusted for education level. Early and late-onset prostate cancer was defined according to age at diagnosis: ≤50 and >50 years.

Main results and the role of chance: Fathers who had undergone ICSI had a higher risk of prostate cancer (at any age) as compared to controls (HR = 1.47, CI 95% 1.15-1.89; p = 0.002). Conversely, IVF-men did not have an increase in prostate cancer risk when compared to controls (HR = 1.14, CI 95% 0.91-1.43; p = 0.25).

When stratified into age groups at cancer, the fathers who had conceived through ICSI had higher risk for early-onset prostate cancer (HR = 2.94, 95% CI = 1.84–4.71; p < 0.001) i.e. diagnosed before 50 years of age. However, ICSI-men did not have an increased risk for late-onset prostate cancer compared to controls. No increased risk of early onset PCa was detected for IVF-fathers (HR = 1.06, 95% CI = 0.57–1.98; p = 0.86).

In sensitivity analysis excluding fathers who were diagnosed with cancer prior to their offspring conception date, ICSI-fathers still had a statistically significant increased risk of PCa (HR = 1.32, 95% CI = 1.01–1.72; p = 0.045) and of early onset PCa (HR = 2.54, 95% CI = 1.52–4.24; p < 0.001).

Limitations, reasons for caution: Even with a cohort of this size, the prostate cancer cases within the ICSI group were quite limited (n = 63). The study design did not allow inclusion of the heterogeneous group men who had never fathered children.

Wider implications of the findings: The results show immense risk for early-onset prostate cancer, generally considered more aggressive, in men referred for ICSI. These men may already have a latent tumor at the time of ICSI, why the possible benefits of targeted screening could be considered.

Trial registration number: N/A.

O-192 The Canadian Assisted Reproductive Technologies Register (CARTR) Plus database: A validation study

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Study question: Are data in the Canadian Assisted Reproductive Technologies Register (CARTR) Plus database valid and reliable?

Summary answer: Markers of validity were strong for the majority of variables evaluated. Those with moderate agreement were FSH levels, oocyte origin and elective single embryo transfer.

What is known already: Health databases and registries are excellent sources of data. However, as these databases are typically not established for the primary purpose of performing research, they should be validated prior to utilization for research both to inform the study design and to determine the extent to which key study variables are accurately documented in the database. CARTR Plus is Canada's national register for collecting information on in vitro fertilization (IVF) and corresponding pregnancy outcomes, and it has yet to be validated.

Study design, size, duration: This validation study of the CARTR Plus database examined IVF cycles performed in 2015 using patient chart reabstraction. Six clinics across Canada were recruited to participate using a purposive sampling strategy. Fixed random sampling was employed to select 146 patient cycles at each clinic, representing unique patients.

Participants/materials, setting, methods: Twenty-five data elements were reabstracted from patient charts, which was declared the reference standard. Data were reabstracted by two independent auditors with relevant clinical knowledge after confirming inter-rater reliability. Data from the chart were then compared to those in CARTR Plus. We calculated kappa coefficients, sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals (CI) for categorical variables, and mean differences, paired t-tests and intraclass coefficients for continuous variables.

Main results and the role of chance: Six clinics agreed to participate in this study representing 5 Canadian provinces. The mean age of patients was 35.5 years, which was similar between the two data sources, resulting in a near perfect level of agreement (ICC = 0.99; 95% CI: 0.99, 0.99). The agreement for FSH was moderate, ICC = 0.68 (95% CI: 0.64, 0.72). There was nearly perfect agreement for cycle type, kappa = 0.99 (95% CI: 0.98, 1.00). Over 90% of the cycles in the reabstracted charts used autologous oocytes; however, data on oocyte source was missing for 13% of cycles in CARTR Plus, resulting in a moderate degree of agreement, kappa = 0.45 (95% CI: 0.37, 0.52). Embryo transfer and number of embryos transferred had nearly perfect agreement with kappa coefficients greater than 0.90, whereas that for elective single or double embryo transfer was much lower (kappa = 0.55; 95% CI: 0.49, 0.61). Validity markers for pregnancy type, number of fetal sacs, and number of fetal hearts on ultrasound were nearly perfect, with kappa coefficients greater than 0.90.

Limitations, reasons for caution: CARTR Plus contains over 200 variables, of which only 25 were selected for this study. This study, therefore, represents the first step in the validation process for the national database. This foundational validation work should be extended to other database variables in future studies.

Wider implications of the findings: This study provides the first assessment of the quality of the CARTR Plus database and we found very high data quality for the majority of the variables that were analyzed. The rigorous methodology used can serve as a guide for future validation projects.

Trial registration number: Not applicable.

O-193 Assisted reproductive technology (ART) treatment and risk of ovarian cancer

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Study question: Is ART treatment associated with risk of ovarian cancer?

Summary answer: An increased risk of ovarian cancer after ART treatment was mainly apparent during 12 years after treatment initiation and is likely influenced by detection bias.

What is known already: Ovarian stimulation in ART treatment has been suggested to increase the risk of ovarian cancer. Previous findings are inconsistent and often based on few diagnosed cases, and it has proven difficult to assess potential confounders such as endometriosis in large-scale studies. Nulliparity has been shown to be associated with ovarian cancer, although the causal relation is not clear. Ovarian cancer is often detected at an advanced stage due to a lack of symptoms during the early stages of disease. Latency time from initiated cancer until diagnosis has been estimated to be 30-40 years.

Study design, size, duration: The Danish National ART-Couple II (DANAC II) cohort includes all women treated with ART at Danish fertility clinics in 1994-2015. Each woman in ART treatment was age-matched with ten women from the background population without a history of ART treatment. The women were followed until first cancer diagnosis, death, migration or end of study December 31st 2015. The cohort consisted of 58,472 women treated with ART and 549,210 women without a history of ART treatment.

Participants/materials, setting, methods: Multivariable analyses were conducted using cox proportional hazards regression. Having a primary cancer diagnosis other than ovarian cancer was incorporated as a competing risk. Adjustment for confounders included baseline nulliparity, educational level, partnership status, endometriosis and PCOS and time-dependent adjustment for age and treatment year. Analyses were stratified on baseline nulliparity and on cause of infertility. Further, the risk of being diagnosed with ovarian cancer was observed over time in order to detect potential patterns.

Main results and the role of chance: During follow-up 393 (0.06%) women were diagnosed with ovarian cancer, 64 (0.11%) among ART-treated women and 329 (0.06%) among non-ART women. Women undergoing ART treatment had a higher risk of ovarian cancer than non-ART women (HR 1.20, 95% CI 1.10-1.31). ART treatment was associated with an increased risk of ovarian cancer among parous women (HR 1.34, 95% CI 1.11-1.62) and among nulliparous women (HR 2.38, 95% CI 2.17-2.60) versus (HR 2.03, 95% CI 1.89-2.19). ART treatment due to female factor infertility was associated with an increased risk of ovarian cancer (HR 1.36, 95% CI 1.25-1.48), while ART treatment due to male factor/unexplained infertility was associated with a lower risk (HR

0.87, 95% CI 0.76-1.00). ART treatment was not associated with a long-term increased risk of ovarian cancer which would be expected if caused by ovarian stimulating hormones. The excess risk of ovarian cancer among ART-treated women was highest during the first two years after ART treatment initiation (HR 1.24, 95% CI 1.06-1.45). The excess risk gradually declined and 12 years after ART treatment initiation the risk was similar to the background population (HR 1.05, 95% CI 0.87-1.27). This pattern suggests an influence of detection bias while undergoing ART treatment.

Limitations, reasons for caution: Although we did not find any indications of harmful effects of ovarian stimulating hormones, it is uncertain to what extent detection bias explains the higher risk among ART-treated women. ART treatment without female factor infertility was not associated with an increased risk of ovarian cancer.

Wider implications of the findings: Disentangling between the effect of underlying causes of infertility and the ovarian stimulation on the risk of ovarian cancer is a challenge. Further, detection bias should be thoroughly considered prior to drawing conclusions.

Trial registration number: Not relevant.

SELECTED ORAL COMMUNICATIONS

SESSION 53: MACHINE LEARNING AND ARTIFICIAL INTELLIGENCE

Tuesday 3 July 2018 Room 211 + 212 17:00–18:00

O-194 A pre-treatment counselling tool for predicting the cumulative probability of live birth

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Study question: Can we predict the chance of live birth after a single cycle of ovarian stimulation and transfer of all resulting embryos using only pre-treatment variables?

Summary answer: Using ovarian reserve and age, our model achieved a positive predictive value of 80% and AUC of 0.776 (95%CI 0.71 – 0.83).

What is known already: Published IVF prediction models calculate the probability of live birth per embryo transfer. More recently the cumulative probability of live birth (CPLB) has been predicted based on number of eggs collected after ovarian stimulation (Drakopoulos et al. 2016). While this information is useful, couples often want to know what their CPLB would be before they make the decision to engage with IVF. A model based on pre-treatment variables to calculate CPLB after one stimulated cycle of IVF and transfer of all frozen embryos would be helpful. To our knowledge there is no such model in current published work.

Study design, size, duration: This was a retrospective cohort study of patients who underwent their first ovarian stimulation between January 2014 and December 2015 in a tertiary referral hospital in central London. We reviewed all electronic and paper based records for each couple. 551 cycles were reviewed of which 335 complete cycles of IVF were included in the study.

Participants/materials, setting, methods: To estimate the CPLB we only included couples who had used all embryos from their initial stimulation or achieved a live birth. We excluded: (i) cycles that involved oocyte or sperm donation or in vitro maturation; (ii) unstimulated cycles; (iii) cycles that resulted in neither fresh nor frozen–thawed embryo transfer; and (iv) patients who did not have a live birth and had frozen embryos left or had discarded or transferred out their frozen embryos.

Main results and the role of chance: The cumulative live birth rate was 80% (268/335, [95% CI 76.0-84.0]). Women with a live birth had significantly lower median age (34 [IQR 6] vs. 36 [IQR 5], p = 0.005) and median FSH 6.6 [IQR 2] vs. 7.7 [IQR 2], p = 0.001). They had significantly higher median AMH (22.0 pmol/L [IQR 19] vs. 9.98 [IQR 6], p = 0.001) and AFC (18 [IQR 12] vs. 11 [IQR 8], p = 0.001). There were no significant differences in BMI, cause of infertility, previous pregnancy and duration of infertility.

We constructed a logistic regression model using live birth as the dependent variable and then used a backward elimination method to create the best fitting regression model to predict the probability of a cumulative live birth without removing age as this is a known predictor of live birth. Our model has a positive predictive power of 80% and the area under the curve (AUC) was 0.74 (95% CI 0.71 – 0.83).

The model was internally validated using 72 couples who had their first IVF cycle between January 2016 and December 2016. This model was able to correctly predict 96% of couples who had a live birth.

Limitations, reasons for caution: This was a small study and requires external geographical validation. The study population were only those eligible for NHS funded IVF treatment which had strict ovarian reserve criteria. The low specificity of this model could be a result of exclusion of women with very low ovarian reserve.

Wider implications of the findings: This model is a useful tool for couples considering whether to undergo ovarian stimulation allowing them to appreciate the full potential benefit of ovarian stimulation and embryo freezing. Couples with low probability can have realistic expectation on how many cycles of ovarian stimulation may be needed to achieve live birth.

Trial registration number: N/A.

O-195 Development and validation of an artificial intelligence based algorithm for the selection of an optimal stimulation protocols in IVF patients

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Study question: Are artificial intelligence (AI) algorithms able to reliably predict ovarian response in patients undergoing their first IVF treatment according to different proposed ovarian stimulation protocols?

Summary answer: AI can predict with high reliability both the number of cumulus oocyte complexes (COCs) and of MII obtained in patients undergoing their first IVF cycle.

What is known already: Patients accessing IVF treatment for the first time are prescribed a stimulation protocol based on their reproductive characteristics and clinical history. Nevertheless, such initial treatment might not optimize their ovarian response, and the objective of averting non optimal outcomes (i.e. low response or hyperstimulation) might not be met. The analysis of large databases by machine learning algorithms should allow predicting with great accuracy the results of ovarian stimulation with different stimulation protocols, thus assisting the clinician in selecting the most appropriate treatment course since the first IVF cycle.

Study design, size, duration: Cohort database of 6952 first IVF cycles. Predictor variable included: patient age, BMI, blood type, smoking habit, reason for treatment, ethnicity, antral follicular count, FSH, estrogen and LH levels, age at menarche. 80% of the cycles in the database were used to train the algorithm, and the remaining 20% were used to validate the best model. As control in the training process we used a 5 times repeated 10-fold cross validation technique.

Participants/materials, setting, methods: Algorithm tested included Random Forest (RF), and two types of black box algorithms such as Support Vector Machine (SVM, linear kernel), and Artificial Neural Network (ANN). All of them capable of regression prediction, as the response variables that the algorithms were trained to predict were: number of COCs and of MII retrieved after stimulation.

Main results and the role of chance: Women in the database analyzed were on average 37.6 ± 4.7 years old (range 18-45), and had a BMI was 24.2 ± 2.8, mean number of COCs and MII obtained was 10 ± 7.1, and 7.2 ± 5.3, respectively. The best results were obtained using the RF algorithm (mtry = 51) and the response variable number of OCCs (correlation = 0.829, R squared = 0.676, mean absolute error = 2.405). Nevertheless, the difference with the RF model (mrty = 34) using as response the number of MII is

minimum (correlation = 0.816, R squared = 0.632, mean absolute error = 2.079), and it is better to be able to predict MII as the presence of these determine the success possibilities of the cycle. In other word, the AI algorithm could predict with 81% accuracy the number of MII obtained by a patient only considering pre-treatment characteristics and comparing them to a large database of known cycles' characteristics, stimulation protocols and stimulation outcomes.

Limitations, reasons for caution: Although the dimension of the database and the presence of both a training and a validation sample population should ensure the robustness of the model developed, AI based applications should be tested prospectively before release.

Wider implications of the findings: AI and machine learning algorithms can offer the opportunity to model the outcome of the first IVF cycle under different stimulation protocols, and therefore help clinicians in selecting the most appropriate treatment for first time patients.

Trial registration number: Not applicable.

O-196 Optimization of prediction models for live birth and miscarriage rate in intrauterine insemination between homologous and frozen donor semen

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Study question: Can we identify the factors predicting a positive and negative clinical outcome in the homologous and donor intrauterine insemination?

Summary answer: It is possible to establish a prognostic model of live birth and miscarriage rate before the intrauterine insemination (IUI) depending on female and male factors.

What is known already: Although IUI is a commonly used treatment, it is difficult to predict the chances of gestation. The overall success rate of IUI remains controversial and depends on several factors. Determinants of IUI success can be classified into three categories: female infertility factors (age, weight, hormones on the third day of the cycle, follicle development and aetiology), male infertility factors (semen analysis and preparation technique), and technique-dependent factors (type of insemination, number of cycles, type of stimulation received, and total dose administered). There is a controversy between researchers if pregnancy rates are influenced or not by these factors among others.

Study design, size, duration: A retrospective cohort study. A total of 7582 subfertile couples undergoing IUI between April 1997 and December 2017 were analysed. 5491 couples underwent IUI with homologous semen (IUI-H) and 2091 couples underwent IUI with frozen donor semen (IUI-D).

Participants/materials, setting, methods: All the infertile couples that underwent IUI cycles in the last ten years. Predictive factors evaluated were female infertility diagnosis, female age, body mass index (BMI), number of preovulatory follicles, number of the IUI cycle, endometrial thickness and sperm quality (total motile sperm count, TMSC). Binary and ordinal multinomial logistic regression analysis by the stepwise procedure were used to selecting the potential variables related to the live birth rate and miscarriage rate.

Main results and the role of chance: The overall pregnancy rate (PR) in IUI-H was 10.4% and 20.5% in IUI-D. Ordinal PR was 0.5% and 1.6%, respectively. The overall live birth (LB) rate was 8.3% in IUI-H and 17.5% in IUI-D, and the overall miscarriage rate (MR) was 2.1% in IUI-H and 3.0% in IUI-D. Binary and ordinal multinomial logistic regression analysis identified number of follicles ($p = 0.03$), number of IUI cycle ($p = 0.02$), female infertility diagnosis ($p = 0.00$) and TMSC ($p = 0.00$) as significant predictive factors of LB in IUI-H cycles. Number of follicles ($p = 0.01$), female age ($p = 0.00$) and NTSM ($p = 0.00$) were significant predictive factors in IUI-D cycles. IUI-H achieves the best results with two or three follicles, carrying out a maximum of 5 cycles, a high TMSC number and in cases of female infertility from ovulation disorders or a normal ovarian reserve. Moreover, patients under 30 years would significantly increase the LB rate in the IUI-D. A single preovulatory follicle ($p = 0.04$) and patients with genital malformation ($p = 0.00$) would be considered as prognostic miscarriage factors in IUI-H cycles. The risk of abortion would also be greater as the age increases in IUI-D cycles ($p = 0.01$).

Limitations, reasons for caution: The limitations of our study include lack of a prospective design, instead relying on a retrospective analysis.

Wider implications of the findings: There are not studies analysing the predictive factors of live birth between homologous and donor IUI, under the same conditions. This study will allow defining which couples will obtain a greater benefit with the IUI technique for more effective counselling and assisted reproduction treatment planning.

Trial registration number: This is not a clinical trial.

O-197 Comparison of machine learning models for the prediction of live birth following IVF treatment: an analysis of 463,669 cycles from a national database

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Study question: What is the comparative ability of different machine learning models in predicting live birth following IVF based on the analysis of a large national database?

Summary answer: Deep neural networks were associated with the highest accuracy and specificity for live birth prediction compared to other machine learning models.

What is known already: The increasing wealth of IVF-treatment data has presented the opportunity, but also the challenge, to develop models for accurate and personalized outcome prediction facilitating optimal treatment design and patient counseling. Machine learning allows the construction of algorithms that can 'learn' from data and make predictions. It is a powerful way to analyze large and complex datasets, which may not be effectively interpreted by the use of conventional statistics. Machine learning has been applied in several fields of healthcare and may prove to be a useful tool in developing accurate personalized predictions in fertility treatment.

Study design, size, duration: This population-based cohort study used anonymous data obtained from the register of the Human Fertilization and Embryology Authority (HFEA), the ART statutory regulator in the UK. A total 463,669 fresh autologous IVF/ICSI, non-PGD cycles, with a full set of data, performed between 1991 and 2012 were analysed to predict live birth per cycle started.

Participants/materials, setting, methods: A predictive model of live birth constructed using latest-technology deep neural networks (DNN) was compared with random forest (RF), decision trees (DT) and Naive Bayes (NB) machine learning models. Cycles were randomly divided into a training and testing set at a 80:20 ratio. The training set was used to develop the prediction model and the testing set to validate model performance. Comparisons were performed using McNemar's test.

Main results and the role of chance: After exclusions, a total 463,669 cycles were included, of which 99,537 [(21.5% (95% CI: 21.4-21.6%)] resulted in a live birth. 370,935 and 92,734 cycles were assigned to the training and testing sets, respectively. Variables up to and including the day of embryo transfer were included in the analysis.

The DNN model was associated with significantly higher accuracy, specificity, positive predictive value (PPV), positive and negative likelihood ratios compared to other machine learning models. Conversely, sensitivity and negative predictive value (NPV) were lower in DNN compared to other models. All differences compared to DNN were statistically significant ($p < 0.0001$).

Details of predictive parameters for all machine learning models are shown in Table 1. ratios and 95/ratios and 95% confidence intervals.

Table I

	Deep neural networks	Random forest	Decision trees	Naive Bayes
Accuracy %	76.83 76.59-77.08	74.94 74.66-75.22	72.90 72.61-73.18	35.91 35.61-36.22
Specificity %	94.86 94.72-95.01	91.16 90.95-91.36	87.07 86.82-87.31	18.30 18.02-18.58
Sensitivity %	11.09 10.71-11.49	16.16 15.65-16.67	21.53 20.96-22.11	99.76 99.68-99.82
Positive Likelihood Ratio	2.16 2.07-2.26	1.83 1.76-1.90	1.67 1.61-1.72	1.22 1.22-1.23
Negative Likelihood Ratio	0.94 0.93-0.94	0.92 0.91-0.93	0.90 0.89-0.91	0.01 0.01-0.02
PPV %	37.20 36.16-38.25	33.52 32.65-34.40	31.48 30.79-32.19	25.20 25.13-25.27
NPV %	79.55 79.48-79.63	79.76 79.65-79.86	80.09 79.96-80.21	99.63 99.51-99.72

Limitations, reasons for caution: The anonymised HFEA database holds no information on the number of cycles performed per patient. Therefore, necessary adjustments and calculation of cumulative live birth were not possible. Furthermore, availability of features related to patient demographics, ovarian reserve, baseline and stimulation characteristics, would likely improve the models' predictive ability.

Wider implications of the findings: The DNN model offered the highest specificity and accuracy, efficiently predicting cycles not leading to live birth. Prediction of unsuccessful treatment may be of value for counseling and patient assessment by clinics and funding bodies. Ongoing research using large datasets will attempt further improvements of the model's overall predictive performance.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 54: BIOMARKERS FOR MALE FERTILITY

Tuesday 3 July 2018 Room 111 + 112 17:00–18:00

O-198 Afamin, a novel human vitamin E-binding glycoprotein, as a marker of male infertility

R. Nunez-Calonge[1], S. Cortes[2], R. Kireev[3], L.M. Gutierrez[4], J.A. Guijarro[5], P. Caballero[6]

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[6]*Clinica Tambre, Reproduction Unit, Madrid, Spain*

Study question: Are afamin levels and single nucleotide polymorphisms in the 5'-untranslated region of the afamin gene associated with male infertility?

Summary answer: Our results show increased afamin levels in the patient group when compared to controls. Also, suggest that afamin genetic variations might be associated with infertility.

What is known already: The plasma glycoprotein afamin has been previously identified and described as an alternative carrier protein for vitamin E in extravascular fluids such as plasma, ovarian follicular and seminal fluids. The role of afamin in male fertility was discovered by the afamin knockout mouse model. These results from a gene-deleted mouse model indicate a central role of afamin in fertility possibly due to its vitamin E-binding properties. However, so far, we have not observed any study which was able to establish a relationship in-between the levels of the protein, polymorphisms and expression of afamin genes with male and female infertility.

Study design, size, duration: This observational prospective study evaluates afamin levels in serum and seminal fluids from infertile men and compares them with healthy controls Plus, it also studies the association in-between single nucleotide polymorphisms (SNPs) in the 5'-untranslated region (5'-UTR) of the afamin gene with infertility. The study was performed from samples collected during the period from May to September 2017.

Participants/materials, setting, methods: Semen samples from 20 oligoasthenoteratozoospermic patients attending the Andrology Laboratory were included in the study. The control was formed by 39 men with normal semen parameters. Concentration of afamin was quantified by sandwich-type ELISA. Peripheral blood samples were analysed to examine the presence of specific sequences from the afamin gene (AFM) by PCR amplification followed by direct sequencing. T-Student test was applied to verify normality distribution of the variables.

Main results and the role of chance: Subjects with low sperm motility or/and sperm concentration had higher median sperm afamin (18,9 ± 2,9 ng/mg of proteins) and serum afamin concentrations (24,1 ± 4,0 ng/mg of proteins) than those without sperm alterations (10,6 ± 1,4 ng/mg of proteins) ($p < 0,02$); (15,6 ± 1,4 ng/mg of proteins) ($p < 0,002$).

A total of five different polymorphisms have been found, these include one deletion and the remaining four considered as single nucleotide polymorphisms (SNP).

A new transversion (A/T) (position 4:73481093) was identified in an OATz patient and was associated with high levels of afamin in plasma and seminal fluids. The prevalence of this variant in our study in the case homozygous for TT is 0.985 (98.5%), whereas in the case heterozygous for TA is 0.015 (1.5%).

5'Upstream single nucleotide polymorphisms rs115041046, rs371710441, rs35680917, rs72856618 of AFM were polymorphic in both patient and control groups. However, our data showed that the AG genotype (rs115041046) was higher among patients compared with controls, and that the TA genotype (rs371710441) was predominant in the group of male patients.

Limitations, reasons for caution: The main limitation of our study is the small number of replicates. The results observed in this study should be further confirmed with a larger sample number.

Wider implications of the findings: Afamin concentration is higher in the patient group, potentially due to a compensatory mechanism caused by a possible decrease in vitamin E levels. Accordingly, these patients could benefit from an antioxidant therapy. Moreover, these findings could significantly enhance our understanding of the molecular-genetic causes of infertility.

Trial registration number: FT 115

O-199 combining RNA-seq and proteomic profiling in cryopreserved human sperm exposed to sublethal stress: new biomarkers for stress-tolerance in sperm

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Study question: What is stress tolerance pathways and changes in the level of proteome and transcriptome in cryopreserved human sperm exposed to sublethal stress?

Summary answer: Sub lethal stress induced by nitric oxide(NO) increased sperm cryoservival by induce expression and modifications of proteins but, did not effect on transcriptome level.

What is known already: Stress preconditioning of sperm before cryopreservation is a novel approach for increase sperm cryosurvival. Beneficial effects of sublethal stress may attributed to the protein response and biosynthesis of stress-related proteins such as heat shock proteins (HSP) and cellular antioxidant enzymes. These proteins could reduce activation of the apoptotic cascade and maintain the quality of sperm after freeze. This response may be regulated at translational or posttranslational levels. Understanding the cellular and molecular modifications involved in the offensive controllable strategy by whole genome sequencing and proteomics could yield valuable information on the mechanism of cryotolerance in sperm.

Study design, size, duration: This was a control versus treatment study. We selected 60 semen samples from normozoospermic men, 28-35 years old. Basic semen analysis performed according to the WHO guidelines, samples which had not the oxidative stress (ROS-TAC score ≤ 50) were processed by density gradient centrifugation. Each sample was divided into the following three subsamples: fresh, freezing without treatment and group exposed to 0.01 µM NO for 1 h at 37°C and 5% CO2 before cryopreservation.

Participants/materials, setting, methods: Sperm suspensions were cryopreserved according to the rapid freezing method and stored for one week. The post-thaw sperm was evaluated for motility parameters (CASA), percentage of apoptosis rate (flow cytometry) and fertilizing ability (heterologous piezo-ICSI). Protein and transcript profiles were investigated between groups by TMT technology coupled to LC-MS/MS and RNA-seq respectively. Bioinformatics analyses were performed using DAVID. Candidate proteins and mRNA were further validated by western blot and Quantitative RT-PCR analysis respectively.

Main results and the role of chance: Data were analyzed using SPSS software and one-way ANOVA. Chi-Square analysis was used for fertilization data, Level of $p < 0.05$ was considered statistically significant. NO-0.01 group significantly increased total motility and progressive motility compared to the cryopreserved group. The lowest percentage of apoptotic rate observed in the NO-0.01 group in compared to the freezing group. In the fertilization trial, the rate of 2- cell formation was higher in fresh and NO-0.01 groups when compared with the control freezing group. In proteomic analysis,out of 2,912 proteins identified, 428 proteins were differentially expressed in the fresh, freeze and NO-0.01 groups. Gene ontology analysis of differentially proteins showed that the gluconeogenesis pathways, and fertility related proteins such as PLCz significantly down-regulated under cryopreservation condition. whereas in the NO-0.01 group, HSP70 and redox proteins such as thioredoxin were up-regulated in compared to cryopreserved group. Using RNA sequencing, Alignment mapped to the human reference genome (hg19).We determined that 368 transcripts were differentially expressed ($p < 0.05$) between fresh and freeze groups. The main down-regulated genes were PRM1 and PIK3R2, whereas SPATA family was up-regulated in cryopreserved sperm. We did not find a significant differentially in transcript level between NO-0.01 and freeze groups.

Limitations, reasons for caution: Due to lack of an adequate amount of protein and RNA for proteomics and RNA-seq analysis,extracted proteins and RNAs were pooled in each experimental group. Ethical committee not allowed using human oocytes for research, therefore, for evaluate oocyte activation capacity,we used mouse oocytes.

Wider implications of the findings: Cryopreservation effects on the proteome and transcriptome of sperm which may ultimately impair the sperm function. Stress precondition human sperm before cryopreservation could induce expression and modifications of proteins that can increase resistance to cryostress. understand mechanisms of stress-induced stress tolerance might improve sperm conservation protocols in assisted reproductive techniques.

Trial registration number: not applicable.

O-200 Attenuation of peroxiredoxin activity induce apoptosis in seminal plasma of men with idiopathic male infertility: striking the chord for evaluation of a novel biomarker

S. Kanta Goswami[1], R. Chattopadhyay[2], S. Ghosh[2], P. Chakraborty[3], M. Chakraborty[2], B. Chakravarty[2]

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[3]Institute of Reproductive Medicine, Infertility, Calcutta, India

Study question: Is expression of the antioxidant gene panel is altered in seminal plasma of patients with idiopathic male infertility (IMI) compared to normozoospermic men?

Summary answer: Our results show that expression of peroxiredoxin and glutathione peroxidase was lower in IMI suggesting them as critical oxidative-stress regulators for fertilization and embryonic development.

What is known already: Most spermatozoa experience DNA repair, additional reactive oxygen species (ROS) being the main cause for DNA injury. High concentrations of ROS can alter biosynthesis kinetics of stress-related proteins(Heat Shock Protein, intracellular antioxidants etc.). Peroxiredoxin (PRDX), glutathione peroxidase 6 (GPX6), thioredoxin reductase 1 (TXNRD1) are chief antioxidant genes present in semen and are associated with various biological processes such as protection against DNA damage, detoxification, oxidative stress and cell apoptosis. Our previous studies have shown that combination of antioxidants may reduce pregnancy loss by correcting DNA abnormality induced by OS. However, the molecular mechanism responsible is yet to be explored.

Study design, size, duration: Prospective study; 147 men with IMI served as study cohort. Normozoospermic men (WHO criteria 2010) were treated as control ($n = 156$). The study was carried out from October 2014 to September 2017and was approved by Institutional Ethics Committee of Institute of Reproductive Medicine, Kolkata. All participants signed written informed consent form.

Participants/materials, setting, methods: Sperm parameters (velocity, motility), apoptosis status, DNA fragmentation, intracellular ROS was evaluated by Makler's chamber, flow cytometry, HaloTech DNA assay, and H2DCF-DA (2',7'-dichlorofluorescein diacetate) fluorescent probe respectively after freezing-thawing of ~200 spermatozoa. mRNA expression of PRDX, GPX6, TXNRD1and controls was measured by qRT-PCR using TaqMan probes. Relative changes in gene expression were calculated using the 2-ΔΔCT method. Statistical significance was set at $p < 0.05$ as evaluated by Pearson's correlation test and student's T-test.

Main results and the role of chance: In comparison with normozoospermic spermatozoa, there was a significant decrease ($P < 0.03$) in motility and velocity parameters and increase in Caspase+/propidium iodide- (PI-) cells ($P < 0.04$) in cryopreserved spermatozoa. As expected, small halo producing dispersed DNA loops in IMI cohort showed good correlation ($r = 1.39$; $p < 0.001$) with high DNA fragmentation index (DFI) and were significantly higher ($P < 0.01$) than control. Intracellular ROS levels were significantly higher in samples with increased caspase+ cells compared to normozoospermic men ($p \leq 0.02$) (57 ± 5.5 vs. 16 ± 7.8). We found that increased expression of PRDX2, PRDX5 and PRDX6 was associated with a significant reduction ($r=-0.69$, $p < 0.02$; $r=-0.61$, $p < 0.02$; $r = 0.55$, $p < 0.01$) in sperm motility parameters, and viability whereas ROS levels, DNA fragmentation, and loss of mitochondrial membrane potential as measured by rhodamine 123 were increased. qRT-PCR analysis revealed that the mean relative levels of mRNA coding for PRDX2, PRDX5, and PRDX6 were significantly decreased in seminal plasma of men with IMI (PRDX2 $p < 0.02$; PRDX5 $p < 0.05$; PRDX6 $p < 0.01$). TXNRD1 showed no differential regulation. Attenuation in GPX6 expression,

although lower than PRDX family, was comparable when correlated with ROS levels ($r = 0.87$, $p < 0.04$).

Limitations, reasons for caution: The results observed in this study should be confirmed on a larger sample number.

Wider implications of the findings: The cellular antioxidant defense is compromised by the attenuation of PRDXs and GPX6 leading to an increase in oxidative damage thus affecting the fertility potency of patients with IMI. Thus, current findings provide a strong platform for new therapeutic strategies through targeting PRDXs.

Trial registration number: Not applicable.

O-201 Seminal plasma cell-free mitochondrial DNA copy number is associated with human semen quality

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Study question: Whether is there association between seminal cell-free mitochondrial DNA (mtDNA) copy number and human semen parameters.

Summary answer: Seminal cell-free mtDNA copy number is associated with semen parameters, which may serve as a novel diagnostic marker of semen quality.

What is known already: Mitochondria are the major source of ATP through the electron transport chain and play important roles in sperm function and spermatogenesis. Recently, it has been suggested that abnormal sperm mitochondrial DNA copy number has been associated with defective sperm function. Despite that the cell-free mtDNA in body fluids has ignited great interest in exploiting its diagnostic value, there have no report about seminal plasma cell-free mtDNA and its probable clinical implications.

Study design, size, duration: Semen samples were collected from 205 men who were undergoing fertility assessment because of idiopathic inability to conceive. The population included men with normal and abnormal semen parameters.

Participants/materials, setting, methods: Seminal cell-free mtDNA copy number was measured using Real-time PCR. Seminal reactive oxygen species (ROS) level was analysed by using the in vitro OxiselectTM assay kit. Sperm quality was assessed using World Health Organization criteria, while sperm motility parameters were determined by computer-aided sperm analysis.

Main results and the role of chance: We applied PCR to detect seminal cell-free mtDNA from infertile men. All seminal plasma samples had mitochondrial MTF3212/R3319 gene expression. Average cell-free mtDNA copy numbers were 310.3, 199.4, 44.6 and 23.1 in the group of Normal, Oligozoospermia, Asthenozoospermia and Oligoasthenozoospermia. There were significant differences in mtDNA copy number among the groups of infertile men ($P < 0.05$). Comparing with the Normal group, seminal cell-free mtDNA copy numbers were decreased significantly in the Asthenozoospermia group ($P < 0.05$) and Oligoasthenozoospermia group ($P < 0.05$). In contrast with the Normal group, ROS levels were increased significantly in the Asthenozoospermia group ($P < 0.05$) and Oligoasthenozoospermia group ($P < 0.05$). Cell-free mtDNA copy number positively correlated with sperm concentration ($r = 0.345$; $P < 0.05$), motility ($r = 0.673$; $P < 0.05$), morphology ($r = 0.291$; $P < 0.05$), curvilinear velocity ($r = 0.263$; $P < 0.05$), straight-line velocity ($r = 0.247$; $P < 0.05$), and average path velocity ($r = 0.271$; $P < 0.05$). ROS level negatively correlated with sperm concentration ($r = -0.455$; $P < 0.05$), motility ($r = -0.676$; $P < 0.05$), curvilinear velocity ($r = -0.273$; $P < 0.05$), straight-line velocity ($r = -0.261$; $P < 0.05$), and average path velocity ($r = -0.264$; $P < 0.05$). There was negative correlation between seminal cell-free mtDNA copy number and ROS level ($r = -0.753$; $P < 0.05$).

Limitations, reasons for caution: The results need to be confirmed in more samples.

Wider implications of the findings: The strong positive correlation between cell-free mtDNA and conventional semen parameters suggest seminal cell-free mtDNA copy number could be used as a novel biomarker in the study and diagnosis of male infertility.

Trial registration number: N/A.

SELECTED ORAL COMMUNICATIONS

SESSION 55: FEMALE AND MALE FERTILITY PRESERVATION

Tuesday 3 July 2018 Room 113 + 114 + 115 17:00–18:00

O-202 Freezing ovarian tissue and simultaneously obtaining an average of 8 mature oocytes without ovarian stimulation - is it a reality?

D. Nikiforov[1], C. Junping[1], S.E. Pors[1], J. Gruhn[2], S.G. Kristensen[1], C.Y. Andersen[1]

[1]*University Hospital of Copenhagen- Rigshospitalet, Laboratory of Reproductive Biology, Copenhagen, Denmark*
[2]*University of Copenhagen, Centre for Chromosome Stability- Department of Cellular and Molecular Medicine- Faculty of Health Sciences, Copenhagen, Denmark*

Study question: Can immature oocytes found in the medium after cortical tissue preparation for fertility preservation, constitute a reliable source of mature eggs?

Summary answer: GV oocytes released during cortical tissue preparation, mature to MII stage with an average 23% success rate, resulting in average 8 MII oocytes per patient.

What is known already: Cryopreservation of ovarian tissue by slow freezing has become a reliable method of fertility preservation for many groups of the patients. During the procedure of cortical tissue preparation, many small antral follicles within the tissue are cut open resulting in release of immature oocytes into the medium. These oocytes have the potential to develop into mature oocytes that may be used for fertility purposes. The aim of present study was to improve the IVM protocol for oocytes deriving from small follicles which was previously rarely used in the context of fertility utilization.

Study design, size, duration: Oocyte-cumulus complexes (COCs) were collected into HEPES-buffered medium supplemented with 10 mg/mL of human serum albumin and cultured following an IVM protocol established in our laboratory (315 oocytes). After 48 hours oocytes were denudated and their maturation stage was determined.

Participants/materials, setting, methods: A total of 9 patients aged 21-36 years (mean age 29) who had one ovary excised for fertility preservation were included. After collection GV oocytes were divided into one of three groups: COCs with large amount of cumulus cells, small amount of cumulus cells and naked oocytes. After maturation measurements of oocyte diameter, diameter of egg's cytoplasm and zona pellucida thickness were taken and related to the maturation status of the oocyte.

Main results and the role of chance: On average, 35 immature oocytes per patient from one ovary were collected with the range of 13-60 oocytes. 23% ($N = 74$) of all oocytes matured to the MII stage within 48 hours. On average each patient had 8,2 mature oocytes. Maturation rate for the oocytes from large COCs was 37,5%, small COCs matured with the rate of 22,7%, while success rate for the naked ones was only 6,3% ($p < 0,001$). Our morphometric observations showed how diverse the pool of immature oocytes was. All found oocytes were within the range of 114-167 µm in diameter (including zona pellucida - ZP). Oocytes matured to MII had a significantly larger diameter than MI and GV ones ($p < 0,001$). No differences were found in relation between zona pellucida thickness and different stages. No significant relationship was observed between the number of collected oocytes and the age of patients.

Limitations, reasons for caution: More patients are needed to evaluate the developmental potential of oocytes obtained by the described method.

Wider implications of the findings: The unexpected developmental capacity of immature oocytes from small antral follicles is likely to augment chances for conception in this group of patients in addition to the cortical tissue. The

use of IVM oocytes collected from small antral follicles during normal IVF treatment may be of interest in the future.

Trial registration number: n/a.

O-203 Recovery of ovarian function after chemotherapy for Hodgkin lymphoma is limited by age, not pretreatment ovarian reserve

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Study question: How much do age and pretreatment ovarian reserve impact on ovarian function recovery after low toxicity chemotherapy with A(B)VD (doxorubicin, (bleomycin), vinblastine, dacarbazine) for Hodgkin lymphoma?

Summary answer: Anti-mullerian hormone (AMH) showed complete recovery after A(B)VD in women aged <35 but not in those ≥35, whereas recovery was similar across pretreatment AMH levels.

What is known already: Chemotherapy can be very damaging to the ovarian reserve, reflected in post-treatment amenorrhoea of variable duration, and reduced AMH levels in those without overt POI. Chemotherapy regimen and dose, age and pretreatment ovarian reserve have all been implicated in determining recovery of ovarian function after treatment, but previous studies have often included women with a range of diagnoses and treatment regimens, precluding clear interpretation. We have investigated recovery from A(B)VD treatment, regarded as having low gonadotoxicity, in a relatively young adult population as a more standardised model to investigate the relative contributions of age and pretreatment ovarian reserve.

Study design, size, duration: Women were recruited from the RATHL trial of treatment for newly diagnosed advanced Hodgkin lymphoma. Blood samples from 57 women treated with ABVD or AVD chemotherapy (grouped as A(B)VD) were taken pretreatment, at the end of treatment and at 1, 2 and 3 years later and serum stored.

Participants/materials, setting, methods: Women were aged 27.4 ± 0.9 years (range 18-44). Samples were analysed in a single batch for AMH using the Roche Elecsys automated assay. AMH recovery was calculated as concentration at 2 years/pretreatment. Spearman correlation coefficients were calculated and multiple linear regression used to analyse age and pretreatment AMH against AMH recovery. Groups were compared by Mann-Whitney U test.

Main results and the role of chance: AMH levels fell markedly in all women during chemotherapy, 13.8 ± 1.8 to 3.5 ± 0.7 pmol/L at end of treatment then recovered to pre-treatment levels by 1 year (13.1 ± 1.9 pmol/L) with no decline over the following 2 years. FSH showed opposite changes. There was a very good correlation between AMH pretreatment and at 2 years (Spearman $r = 0.71$, $p = 0.0002$) but not with AMH recovery ($r=-0.02$, $p = 0.9$). There was no significant correlation between age and pretreatment AMH ($r=-0.2$, $p = 0.1$) but there were significant negative correlations between age and 2 year levels ($r=-0.53$, $p < 0.0001$) and with AMH recovery ($r=-0.48$, $p = 0.001$). This negative impact of age on recovery was supported by mean AMH recovery in women <35 yrs being complete at 1.27 ± 0.12, whereas it was markedly reduced at 0.37 ± 0.10 in those ≥35 ($p < 0.0001$). Analysis by pretreatment AMH below/above the median value (9.77 pmol/L) showed that recovery was similar in the 2 groups (low group 1.23 ± 0.21 vs high group 1.03 ± 0.09, ($p = 0.8$). Multiple linear regression analysis also confirmed a significant impact of age (beta -0.43, $p = 0.004$) but not pretreatment AMH (beta -0.15, $p = 0.3$) on AMH recovery.

Limitations, reasons for caution: This analysis may be relevant only to women treated with this chemotherapy regimen, for Hodgkin lymphoma. It is unclear whether this impact of age on ovarian recovery can be generalized to other diagnoses/treatments. The mechanisms through which ageing impacts on recovery from chemotherapy are unknown.

Wider implications of the findings: The limiting effect of age on recovery of growing follicle populations requires further study to more fully understand the effects of age and chemotherapy on the ovary. This is likely to involve effects on both on the stroma and vasculature, in addition to depletion of the ovarian reserve.

Trial registration number: NCT00678327

O-204 Recombinant AMH prevents primordial follicle loss and long term fertility alteration in cyclophosphamide treated mice

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Study question: What is the effect of concomitant recombinant Anti-Müllerian Hormone (rAMH) and cyclophosphamide (Cy) administration on ovarian reserve and fertility in a mouse model?

Summary answer: Co-treatment of rAMH with Cy preserves primordial follicle (PMF) loss induced by Cy and might improve fertility outcome after treatment.

What is known already: An endowment of oocytes is established during fetal development in women. The majority of oocytes are present within non-growing PMF. This PMF stockpile may decline as a result of age or in some pathological conditions. Mechanisms controlling the activation of PMF imply a balance between factors activating the initiation of follicular growth, mainly actors of the phosphatidyl-inositol-3-kinase (PI3K) signaling pathway, and inhibiting factors, such as AMH. Cy induces both the initiation of dormant follicle growth via an activation of PI3K pathway and the apoptosis of growing follicles leading to a major depletion of the follicular reserve.

Study design, size, duration: Forty-eight pubertal Swiss female mice received one intra-peritoneal injection of either vehicle, Cy (150 mg/kg), rAMH (100 µg) or Cy + rAMH and were sacrificed 24 hours or 8 days after treatment. To assess fertility outcome, a subgroup of pubertal mice was treated with one weekly intra-peritoneal injection of either vehicle, Cy (75 mg/kg), AMH (10 µg) or Cy + AMH through 4 weeks and then mated with proven fertile male along 14 weeks. Four weeks after male removing, ovulation tests were performed.

Participants/materials, setting, methods: Ovaries were removed one week after treatment, serially cut into 4 µm sections and stained with hematoxylin eosin. One out of five sections was analyzed and all follicles were counted. Western blot analysis of whole ovaries was performed to study PI3K pathway signaling. Fertility assessment included estrous cycles monitored by daily vaginal smears, mating experiment, and counting of cumulative pups. At last the number of ovulated oocytes after superovulation was evaluated.

Main results and the role of chance: The number of PMF into Cy-treated mice ovaries was significantly decreased as compared to controls (597.7 ± 35.9 vs 813.4 ± 68.7 follicles, $p = 0.010$) and was similar to normal range in Cy +rAMH mouse ovaries (875.5 ± 83.9 follicles). In addition, the number of early growing follicles was higher after a single injection of Cy as compared to control (802.4 ± 64.8 vs 507.7 ± 55.4 follicles $p = 0.02$). Western blot of ovary homogenates analysis showed that Cy increased significantly the phosphorylation of PI3K-pathway key proteins (P70S6K and FOXO3a). Co-administration of rAMH does not impact PI3K signaling pathway. Nevertheless, phosphorylation of the transcription factor FOXO3a was significantly decreased in ovaries of mice treated with rAMH alone as compared to control mice suggesting that AMH via FOXO3a blocks the PMF activation.

Cy-treated mice exhibited a significantly reduced number of estrous cycles when compared to controls (6.5 ± 0.4 vs 9.2 ± 0.3 cycles, $p = 0.006$). Co-treatment with AMH did not modify this alteration of cyclicity. The number of pups was slightly decreased in Cy-group as compared to controls or Cy+ rAMH mice. At last, the mean number of ovulated oocytes was decreased in the Cy-group as compared to controls and significantly increased after AMH co-administration.

Limitations, reasons for caution: This original study using a mouse model supports a potential protective role of rAMH against Cy-induced follicular loss. Further research and clinical studies are needed to confirm these results.

Wider implications of the findings: PMF activation has been recently reported as mechanisms for Cy-induced gonadotoxicity. This follicular activation might not be specific of Cy and has been described for other drugs.

Therefore rAMH could become a new option in the therapeutic arsenal of fertility preservation for young women having to undergo chemotherapy.

Trial registration number: not applicable.

O-205 Organotypic culture of fresh and cryopreserved-thawed human prepubertal and pubertal testis

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Academic Medical Center, Center for Reproductive Medicine, Amsterdam, The Netherlands

Study question: Can organotypic culture of fresh and cryopreserved-thawed human (pre)pubertal testicular tissue equally maintain spermatogenesis?

Summary answer: Though spermatogonia could be maintained *in vitro* for 5 weeks, the percentage of tubules containing MAGEA4+ cells decreased independently of tissue status and culture condition.

What is known already: Cryopreservation of testicular tissue is increasingly offered as a fertility preservation method for (pre)pubertal patients prior to gonadotoxic treatment, whereas strategies for fertility restoration still remain under development. One of the proposed experimental approaches is to differentiate germ cells *in vitro*. In fact, this has already been accomplished in mouse using an organ culture method to both fresh and cryopreserved-thawed testicular fragments. Despite these encouraging results, the spermatogenic yield is yet relatively low and could not be successfully applied to cryopreserved human testis tissue.

Study design, size, duration: Testicular tissue was collected from nine (pre)pubertal boys (6-14 years) admitted for fertility preservation procedure prior to gonadotoxic treatment. In all cases, ethical approval was obtained and informed consent was received from the boys' parents in order to use a portion of the testicular biopsy for research purposes.

Biopsies were either immediately processed or cryopreserved-thawed before organotypic cultured in two different media for a maximum period of 5 weeks, depending on the initial biopsy size.

Participants/materials, setting, methods: Fresh or cryopreserved-thawed testis fragments (1-2 mm^3 pieces) were cultured at a gas-liquid interphase (34°C, 5% CO_2) in weekly refreshed α-MEM + 10% KSR media containing 10^{-7} M melatonin and 10^{-6} M retinoic acid, with or without 3IU/L FSH/LH supplementation. A minimum of 3 cultured pieces were weekly fixed for further histological and immunohistochemical assessment of spermatogonial survival and proliferation (MAGEA4/PCNA). Percentage of tubules with MAGEA4 positive spermatogonia was determined in 25-100 tubular cross sections from each fragment.

Main results and the role of chance: Our data shows that, under the described conditions, organ culture of human (pre)pubertal tissue supported spermatogonial maintenance, but no further germ cell differentiation was detected over a period of 5 weeks. Quantitative analysis revealed a progressive decrease in the percentage of testicular tubules containing MAGEA4 positive cells during culture. Nevertheless, spermatogonial survival was comparable between fresh and cryopreserved-thawed cultured fragments and independent on culture media (without or supplemented with FSH/LH). Only in a single case (14 years patient), when elongating spermatids were already present in the testicular biopsy before culture, the percentage of tubules with MAGEA4 positive cells increased in fresh cultured tissue.

Depending on patients' age, spermatogonia remained proliferative active in the first weeks of culture and Sertoli cells regained proliferative ability *in vitro*.

Limitations, reasons for caution: These results should be interpreted considering that it is necessary to further optimize the organ culture method for human testicular tissue, ensuring better spermatogonial survival as well as differentiation potential. Additionally, investigations comprising the supporting somatic environment should be performed.

Wider implications of the findings: Our data suggests that fresh and cryopreserved-thawed testicular fragments are maintained similarly *in vitro*. Therefore, although the culture conditions need to be further optimized, these results support the concept that cryopreserved testis fragments from (pre) pubertal cancer survivors could be used for fertility restoration in the future.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 56: BIOLOGY OF OOCYTE AND CUMULUS COMPLEX

Tuesday 3 July 2018 Room 117 17:00–18:00

O-206 Mitochondrial DNA sequence variation in human oocytes by single-cell mtDNA sequencing

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Study question: Using single-cell mtDNA sequence to explicit variation of mitochondrial DNA in human oocytes during maturation.

Summary answer: All of the oocytes mtDNA were heteroplasmy. Most of mtDNA sequence variations were de novo distict to cumulus and located in MT-RNR1 and MT-RNR2 locus.

What is known already: Mitochondria's quantity and quality are essential to oocytes development, fertilization and early embryo development. mtDNA is unique from the nuclear genome, and mitochondrial inheritance is solely maternally inherited (non-mendelian patterning). And mtDNA shows a higher rate of mutation than nuclear DNA. It is assumed that most *de novo* mutations accumulate through the process of heteroplasmy. However, it is unknown whether mutations initiated, occur and accumulate early in the oocytes.

Study design, size, duration: Human oocytes were obtained from 8 patients at our Centers for Clinical Reproductive Medicine through written informed consent and institutional approval. 36 oocytes and its cumulus: Germinal vesicle (GV) (13), MI (4), and MII (19). After digestion them, oocytes or cumulus were used to construct mtDNA library and sequence. The whole mitochondrial genome was sequenced on the Illumina HiSeq platform and haplogroups and mtDNA variants were identified through mapping to reference mitochondrial genomes.

Participants/materials, setting, methods: After controlled ovarian hyperstimulation, oocytes were collected after 34–36 hours of hCG injection. Oocytes were treated with Tyrode's acidic solution to remove zona pellucida then were used to construct mtDNA library using VariantPro™ PCR reaction. The libraries were sequenced on the Illumina HiSeq 4000 platform. After removing low quality reads, clean data were analyzed and mapped to the reference human mitochondrial genomes using MtoolBox toolkit, BWA, GATK HaplotypeCaller, UnifiedGenotyper and so on tools.

Main results and the role of chance: It revealed the crucial mtDNA variations in different stages of oocytes. All samples showed different degree of mtDNA heterogeneity, and the proportion of heterogeneity and homogeneity (Het/Hom Ratio) was 0.03 to 0.46. The mitochondrial heteroplasmies were different among different stages of oocytes from the same patient, and mitochondrial heterogeneities were slightly different between the same stages of oocytes in the same individual.

A total of 196 mitochondrial variations or mutants were detected, and about 30% of them distributed in the non encoding region of (D-loop), e.g. MT-RNR1 and MT-RNR2 locus, which had high mutation rates. All of oocytes had different number of 25-50 mutants, and most of them were single nucleotide polymorphism (SNP). The types of SNP were different between individuals. It was found that most of the SNP (75.5%) was common to the oocytes and granulosa cells, and which was a genetic marker for the patients. But there are also oocytes and granulosa cells specific for the SNP, of which there were 22 SNP in oocytes included 9 missense mutations, and there were 26 SNP mutations in granulosa cells included 6 missense mutations and 2 synonymous, but most of the missense mutations with low frequency.

Limitations, reasons for caution: In the study, every stage of human oocytes was not achieved in natural cycles but induced using a GnRH agonist

and GnRH antagonist. And extremely-low-level variations were not anaylzed in this paper.

Wider implications of the findings: The results provide a valuable resource to comprehend for mtDNA duplication regulatory mechanisms underlying progressive development of human oocytes, and our study provides new insight into mitochondrial genetic disease occurrence and therapy.

Trial registration number: not applicable.

O-207 Transcriptional profile of tubulin post translational modification (PTM) enzymes throughout human oocyte meiotic progression in vivo and in vitro

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Study question: How do PTM enzyme transcripts in human germinal vesicles (GVs) change during the process of meiotic progression to the MII stage of maturation?

Summary answer: 17 PTME transcripts are detectable in GVs, MII oocytes and GVs cultured *in vitro* to the MII stage; only 3 PTMEs vary between maturation stages.

What is known already: Oocyte maturation requires the formation of the meiotic spindle, mainly composed of tubulin heterodimers (microtubules). Tubulin PTMs alter microtubule properties, making PTM enzymes potential regulators of spindle formation and chromosome segregation. Tubulin acetylation, glutamylation, formation of Δ2- tubulin, and tyrosination have been described in the meiotic spindle. In mice, increased acetylation impairs spindle migration to the subcortical region. Deacetylase overexpression can compensate for altered spindle formation and chromosome alignment in aged mouse oocytes. A role for glutamylation and tyrosination has not yet been defined in oocyte meiosis. Importantly, PTM and PTM enzymes have not been systematically characterized in human oocytes.

Study design, size, duration: This is a basic research study. GVs or MII stage oocytes were retrieved from hormonally stimulated oocyte donors aged 20-35 years old. Three experimental groups were defined: GVs (n = 6), *in vivo* matured MII oocytes (n = 8) and GVs cultured *in vitro* (without cumulus cells) until the MII stage (n = 6). Expression levels of 22 PTM enzymes responsible for all known tubulin PTMs were determined by single–cell real time quantitative PCR (qPCR) in the each oocyte.

Participants/materials, setting, methods: All women were stimulated by GnRH antagonist protocol, with GnRH agonist trigger. Trigger criterion was 2 or more follicles >18 mm, and OPU was carried out strictly 36 h later. Total RNA was isolated from each oocyte independently, cDNA generated by random hexamer priming and amplified for 24 cycles. qPCR for PTME transcript detection was performed in triplicate and expression levels were normalized to ACTB, RPLP1, DNMT1, GADPH, SDHA and UBC, using the $\Delta\Delta C_t$ method.

Main results and the role of chance: Out of 22 transcripts measured, 17 transcripts were detected. 14 transcripts out of 17 detected showed no variation between GVs and *in vivo* matured MII stage oocytes. NAA50 (acetylation), TTLL4 (monoglutamylation) and TTLL6 (polyglutamylation) showed the highest overall expression between both groups. In contrast, CCP2 (involved in deglutamylation) had low expression in both groups. In particular, expression levels of TTLL3 and TTLL10 (responsible for glycylation) were remarkably low, consistent with the presence of this modification only in exceptionally stable microtubules, such as in cilia and flagella. All other PTME had intermediate levels of expression. Notably, CCP2, TTLL4 and TTLL12 (unkown function) were significantly lower (Mann-Whitney test, $p < 0.05$) in MII oocytes as compared to GVs. PTME expression levels in MII stage oocytes obtained by culturing GVs in G2-plus medium did not show significant differences when compared to MII oocytes, except in the case of TTLL12 and TTLL5 (involved in monoglutamylation), which showed significantly higher expression levels than those observed in MII oocytes matured *in vivo* (Mann-Whitney test, $p < 0.05$). SIRT2 (involved in deacetylation) showed a similar tendency but did not reach statistical significance.

Limitations, reasons for caution: Variability may be underestimated by the relatively low number of samples per group. mRNA and protein expression levels of PTM enzymes may not be correlated, as transcripts may be stored in the oocytes in an inactive state, or be left-over from translation during oocyte growth.

Wider implications of the findings: This is the first characterization of tubulin PTM enzyme during human oocyte meiosis. Overall, expression levels of PTM enzymes did not change, with the exception of TTLL4, TTLL12 and CCP2, which decrease significantly during meiotic progression. We hypothesize that these PTM enzymes may play a role in meiotic spindle regulation.

Trial registration number: Not applicable.

O-208 Dual trigger vs. hCG for final oocyte maturation. A prospective randomized controlled, double blinded study: preliminary results

J. Haas[1], R. Bassil[2], R. Casper[2]

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Study question: Does co-administration of GnRH agonist and hCG in IVF cycles improve the number of oocytes and oocyte quality compared to hCG alone.

Summary answer: The dual trigger increases the number of mature oocytes, and number of top quality blastocysts compared to triggering with hCG alone in normal responder patients.

What is known already: Human chorionic gonadotropin (hCG) is usually used at the end of controlled ovarian hyperstimulation as a surrogate LH surge to induce final oocyte maturation. Recently, based on retrospective studies, the co-administration of GnRH agonist and hCG for final oocyte maturation (dual trigger) has been suggested to improve IVF outcome and pregnancy rates.

Study design, size, duration: A single center, prospective, randomized controlled, double-blinded clinical trial between July 2016 and January 2018.

Participants/materials, setting, methods: Normal responder patients were randomized either to receive hCG or dual trigger for final oocyte maturation. Data on patients age, BMI, AMH, number of follicles >10 mm and >15 mm on day of hCG administration, number of oocytes retrieved, MII, zygotes and blastocysts were assessed and compared between the dual trigger group and the hCG group.

Main results and the role of chance: One hundred and forty six patients were included in the study, 73 patients in each arm. The age (35.9 vs. 35.7 p = NS), BMI (23.7 kg/m^2 vs. 24 kg/m^2, p = NS), AMH (20.7 pmol/l vs. 19.5 pmol/l P = NS) and FSH (6.5 vs. 5.8 p = NS) were comparable between the two groups. The total amount of gonadotropins, the length of the stimulation and the number of follicles >10 mm and >15 mm in diameter on day of hCG administration were also similar in the two groups. The number of eggs retrieved (12.9 vs. 10.6, p = 0.02), the number of MII (10.1 vs. 8.6, p = 0.03) and the number of zygotes (7.9 vs. 6.3, p = 0.04) were significantly higher in the dual trigger group compared to the hCG group. The oocyte recovery rate (oocytes/follicles≥11 mm) was significantly higher in the dual trigger group compared to the hCG group (97% vs. 78% p = 0.001). The number of top quality blastocysts (2.8 vs. 1.8 P = 0.005) was significantly higher in the dual trigger group. The clinical pregnancy rate and the ongoing pregnancy rate were similar in both groups.

Limitations, reasons for caution: None.

Wider implications of the findings: The co-administration of hCG and GnRH agonist increases the number of oocytes, and the number of top quality embryos compared to triggering with hCG alone in normal responder patients. This increase may improve the outcome of the IVF cycle but a larger study powered by pregnancy outcome is needed.

Trial registration number: NCT02703584

O-209 Anti-Müllerian hormone promotes granulosa cell proliferation in the preantral-to-early antral follicles, but induces granulosa cell apoptosis during the subsequent follicular development, in the primate ovary

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Study question: Do granulosa cells in follicles at the different stages of folliculogenesis respond differently to anti-Müllerian hormone (AMH) in vivo in the primate ovary?

Summary answer: AMH may enhance preantral and early antral follicle growth by promoting granulosa cell proliferation, and prevent subsequent antral follicle maturation by inducing granulosa cell apoptosis.

What is known already: AMH action in the primate ovary is stage-dependent, directly promoting preantral follicle survival and growth while inhibiting antral follicle maturation in vitro. Blocking AMH actions in vivo interferes with dominant follicle selection process in nonhuman primates.

Study design, size, duration: Hemi-ovariectomized animals (n = 9) served as their own controls and were assigned randomly to 6 treatment sequences in a crossover design. Intraovarian treatments were administered during the follicular phase (cycle day -2 to mid-cycle estradiol, or E2, surge) of menstrual cycles 1, 3, 5 and 7, with 1 recuperation cycle between treatment cycles. Treatments included (a) control vehicle, (b) recombinant human AMH (rhAMH; 25 ng/hr), and (c) neutralizing anti-human AMH antibody (AMH-Ab; 1000 ng/hr).

Participants/materials, setting, methods: Female rhesus macaques (6-10 years) received intraovarian infusions via an osmotic pump. The menstrual cycle length was recorded. Blood samples were measured daily during treatment cycles for E2 and progesterone (P4) levels by immunochemiluminescence assays. Ultrasonography was performed at mid-cycle to assess antral follicle cohorts. One hour before the ovariectomy at the mid-cycle of the last treatment, animals received 20 mg bromodeoxyuridine (BrdU; iv). Ovaries were evaluated by hematoxylin/eosin staining, BrdU immunostaining, and TUNEL assay.

Main results and the role of chance: In the control cycles, only one large (5-9 mm) antral follicle was observed at the mid-cycle E2 surge. However, 3 rhAMH-treated ovaries displayed multiple medium-sized (2-4 mm) antral follicles, while the other 6 developed a single medium-to-large antral follicle with diameters less than those of controls (6.1 versus 7.0 mm; $P < 0.05$). Seven AMH-Ab-treated ovaries developed multiple medium-to-large (3-10 mm) antral follicles. The average serum E2 levels during the follicular phase decreased following rhAMH infusion, but increased following AMH-Ab infusion, relative to those of the control cycles (109.9/167.6 versus 136.1 pg/ml; $P < 0.05$). There were no differences in the menstrual cycle length, overall ovarian size at the mid-cycle E2 surge, peak E2 levels, or average P4 levels during the luteal phase between the control and rhAMH/AMH-Ab treatment cycles. While the preantral and early antral follicles of the control and rhAMH-treated ovaries appeared to be healthy, with the rhAMH-treated follicles containing abundant BrdU-positive granulosa cells, those in AMH-Ab-treated ovaries exhibited abnormal morphology with TUNEL-positive granulosa cells. In contrast, healthy medium-to-large antral follicles containing BrdU-positive granulosa cells were identified in the control and AMH-Ab-treated ovaries, whereas those in the rhAMH-treated ovaries contained abundant TUNEL-positive granulosa cells or became atretic.

Limitations, reasons for caution: The current study was performed in vivo with follicle cohorts from different developmental stages co-existing in the macaque ovary. Therefore, effects of the follicle-follicle interactions could not be ruled out. Hematoxylin/eosin, BrdU and TUNEL staining was descriptive, but could be quantified in future studies using cell and follicle counting methods.

Wider implications of the findings: The findings are consistent with previous in vitro data on the stage-specific actions of AMH in follicle cohorts. The study provides valuable information relevant to understanding and/or treating ovarian dysfunction. For example, elevated serum AMH in patients with polycystic ovary syndrome may contribute to the pathology of this endocrine disorder.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 57: APPROACHING THE CLINIC: STEM CELLS TREATMENTS FOR INFERTILITY

Tuesday 3 July 2018 Room 116 17:00–18:00

O-210 Autologous mitochondrial transfer as a complementary technique to ICSI to improve oocyte and embryo quality in IVF patients. A Randomized Pilot Study

E. Labarta Demur[1], S. Herraiz[2], M.J. De los Santos[3], A. Marzal[1], M.J. Escribá[3], A. Buigues[2], A. Pellicer[4]

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[4]IVI RMA Rome, Human Reproduction, Rome, Italy

Study question: Does Autologous mitochondrial transfer (AUGMENT® treatment) improve oocyte/embryo quality and pregnancy rates in patients with previously failed IVF treatments?

Summary answer: AUGMENT does not seem to improve oocyte/embryo quality in this poor prognosis patients. No differences regarding live birth rates per embryo cohort were observed.

What is known already: AUGMENT is a proprietary new technology launched in 2014 by OvaScience℠. Previous clinical use suggested that the addition of autologous mitochondria during IVF is safe, improves embryo quality, increases the success of IVF and avoids concerns associated to heteroplasmy. Many studies described the existence of egg precursor cells (EPCs) in the outer lining of the ovarian cortex, however others have failed to find them. OvaScience reports that mitochondria isolated from EPCs are of high quality and morphologically like oocyte mitochondria.

Study design, size, duration: Triple blind, randomized, single-center, controlled experimental pilot study.

Study period: October 2015- June 2017.

A biopsy of the ovarian cortex was performed to isolate the EPCs to obtain their mitochondria. Sibling MII oocytes obtained after stimulation were allocated though computerized randomization to two groups: AUGMENT protocol (experimental) and Conventional ICSI (control).

Sample size was calculated to detect a 20% difference (15-35%) in the OPR between both groups. An interim analysis was performed in 59 patients.

Participants/materials, setting, methods: Infertile patients undergoing an IVF-PGT-A cycle, aged ≤42, BMI < 30, AMH ≥4 pmol/mL, >5 million/mL motile sperm, ≥1 unsuccessful previous IVF cycle with ≥5 MII eggs collected and extremely low embryo quality.

Primary endpoint was ongoing pregnancy rate (>12w).

In the experimental group, ≈1-2 picoliters of mitochondrial suspension were injected along with the sperm during ICSI. Viable blastocysts from both groups were biopsied and cryopreserved. The genetic content of the embryos was evaluated through CGH arrays.

Main results and the role of chance: Two of 59 enrolled patients spontaneously conceived (one ended in miscarriage). A total of 57 ovarian cortex biopsies were performed. In one case, a suboptimal number of EPCs were isolated; a second biopsy was performed yielding sufficient EPCs for inclusion in the study. Twenty-six patients did not undergo embryo transfer for the following reasons: no blastocysts available for biopsy (n = 16); all aneuploid (n = 8); no fertilization (n = 1); no survival after thawing (n = 1). A total of 253 MII were inseminated in the AUGMENT and 250 in the Control group, with a fertilization rate of 64.0 % and 70.8%, respectively ($p = 0.11$).

Day 5 blastocyst formation rates were significantly reduced in the AUGMENT group (27.2%) compared to the Control group (43.5%), $p = 0.002$. The number of euploid blastocysts/biopsied blastocysts was 42.2% (19/45) in the AUGMENT and 50% (37/74) in the Control group ($p = 0.42$). Statistical differences in euploidy rate/MII oocytes between both groups (7.5% vs. 14.8%, $p = 0.01$) were observed.

No differences were observed regarding mtDNA content in euploid embryos, $p = 0.56$.

The live birth rate per transferred embryo was 41.2% (7/17) in the AUGMENT and 39.1% (9/23) in the Control group ($p = 0.90$).

Limitations, reasons for caution: The technique requires a laparoscopy. Isolation of EPCs and mitochondrial extraction was performed in a separated laboratory set up from OvaScience. Data regarding final mitochondrial quantity and quality was not available for this analysis.

Wider implications of the findings: In this difficult to treat patient population, the ratio of euploid embryos obtained per MII was significantly lower with

AUGMENT compared to the Control group. No differences regarding live birth rates per embryo cohort were seen. Therefore, the study is no longer been continued.

Trial registration number: The www.clinicaltrials.gov registration number is NCT02586298

O-211 3D Architecture of endometrial glands in relation to stem cell organisation

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Study question: Is there an epithelial stem cell(s) in the human endometrium that can regenerate all epithelial cell subtypes and what is the 3D architecture of endometrial glands?

Summary answer: Epithelial stem cells producing luminal, functional and basal epithelial subtypes exist in human endometrium; 3D glandular architecture demonstrates region-specific, horizontal branching and vertical singular arrangement.

What is known already: Menstrual shedding and repair of the endometrial functionalis is unique to humans and higher-order primates. The current dogma is that endometrial epithelium regenerates from endometrial epithelial stem cells residing in the base of the glands; this theory presumes endometrial glands to have a single tubular architecture with a blunt end. The precise 3D organisation of the human endometrial glandular epithelium is unknown. The existence of a human endometrial epithelial stem cell(s) that can produce the 3 epithelial regions (luminal, functionalis and basalis) is yet to be directly confirmed.

Study design, size, duration: Prospective observational study examining full thickness human endometrial samples from 50 normal pre (menstrual/proliferative/secretory) and postmenopausal women undergoing hysterectomy for benign gynaecological conditions without endometrial pathology.

Participants/materials, setting, methods: The gold standard method, in vivo cell lineage tracing, detecting unique mitochondrial DNA mutations (mtDNAm) in laser captured micro-dissected single epithelial cells, was used to confirm the existence of endometrial epithelial stem cells. 100 consecutive paraffin sections stained with H&E / cytochrome *c* oxidase (CCO) were used for 3D reconstruction. Manual drawing of gland boundaries in 2D images were connected along the third dimension. Epithelial subtypes were examined with 10 stains in consecutive endometrial sections.

Main results and the role of chance: CCO deficient patches exist in human endometrium, first appearing in a 27 year old. A unique CCO-mtDNAm, in a partially mutated gland within a CCO deficient area confirmed a clonal population evidently with its origin in a stem cell and suggesting there may be more than one stem cell responsible for regenerating that gland. Basalis glands interconnect (branch) and take a horizontal course along the myometrium; whilst non-branching single functionalis glands appear to germinate perpendicularly from these horizontal basal glands. mtDNAm occur in the basalis glands, and the vertical tracking of such mutations along the functionalis glands, confirmed that at least one population of endometrial epithelial stem cells resides in the basalis glands, responsible for endometrial epithelial regeneration. The sequential tissue sections stained for ten known epithelial cell markers also demonstrated the existence of all these cell types in a CCO negative clonal patch, confirming their common origin.

(I have 3D reconstruction videos that look great in a presentation)

Limitations, reasons for caution: Since the different epithelial cell subpopulations with phenotypical and functional differences are not fully characterised in the human endometrium, the ability of the potential stem cells to produce all different epithelial cells cannot be conclusively demonstrated.

Wider implications of the findings: More than one endometrial epithelial stem cell contributes to the monthly glandular regeneration. The complex 3-dimentional histo-architectural arrangement, highlights endometrial epithelium cannot be treated as a single entity. The existence of multiple endometrial epithelial stem cells with possible functional diversity warrants further interrogation, essential for understanding endometrial pathology.

Trial registration number: NA.

O-212 Human pluripotent stem cell-derived cells expressing functional follicle-stimulating hormone receptor (FSHR) - a tool for functional studies and disease modeling

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Study question: Can human pluripotent stem cells (hPSCs) be utilized in modeling FSHR function during early human development, and to study *FSHR* expression and function in FSH resistant patients?

Summary answer: hPSC-derived cells expressing human *FSHR* were derived. These cells responded normally to FSH while cells from patients with inactivating *FSHR* mutation failed normal responses.

What is known already: FSH regulates follicular growth and spermatogenesis. FSH bioactivity is exerted through binding to FSHR, where upon intracellular cAMP synthesis and other downstream signaling pathways are activated. Besides ovaries and testes, *FSHR* expression has been reported in extragonadal tissues including developing placenta and endothelial cells of female reproductive tract. We have previously shown that the inactivating *FSHR* mutation (A189V) leads to arrest of follicular development and infertility in females. Affected males have reduced sperm counts but are able to father children. Thus far, the expression or function of the mutated FSHR has not been studied in cells endogenously expressing the receptor.

Study design, size, duration: The study was conducted in a control human embryonic stem cell line (hESC H9, 46, XX) and two patient derived human induced pluripotent stem cell lines (hiPSC HEL127.6 and HEL128.5, 46, XX). The patient lines contained a A189V mutation in the *FSHR* gene, known to inactivate normal receptor function. Generation of patient lines was approved by Ethics Committee.

Participants/materials, setting, methods: Cells were differentiated with Activin A, CHIR-99021 and Y-27632 (1d), BMP7 and CHIR-99021 (1d), CHIR-99021 and dorsomorphin (2d), and B27 supplemented media (4d). Cells at different stages of differentiation were studied by qPCR. At d6 or d8, cells were stimulated with FSH for flow cytometry, localization, western blotting and qPCR studies and for cAMP assay. Single-cell sequencing was performed on control cells. Experiments were repeated at least three times for each cell line.

Main results and the role of chance: hPSCs differentiated by our protocol endogenously expressed functional FSHR. These cells responded dose-dependently to FSH stimulation by increasing cAMP production, downregulation of *FSHR* and inhibin βA (*INHBA)* mRNA expression and upregulation of inhibin α (*INHA*) expression. Patient derived iPSCs carrying A189V mutation also expressed *FSHR*, but the receptors did not respond to FSH stimulation by cAMP production or regulation of *FSHR* or inhibin gene expressions. Preliminary qPCR and single cell sequencing results indicate that the cells resemble extraembryonic cell types. Interestingly, hPSCs differentiated into trophoblast-like cells with another protocol also expressed *FSHR* at mRNA level but did not respond to FSH stimulation. Hence, we have established a method for differentiating human cells endogenously expressing *FSHR* that can be used for studying wild type and mutant receptors. However, the true identity of these cells is yet to be confirmed.

Limitations, reasons for caution: The cells express functional FSHR and can thus be used for studying the FSHR biology, but the biological role of these cells have to be further evaluated. So far, the localization of the receptor on the cell has not been elucidated due to lack of functional FSHR antibodies.

Wider implications of the findings: This study presents novel information about *FSHR* gene expression patterns during differentiation. The model generated here provides an efficient method to study the regulation and function of cells endogenously expressing FSHR obtained from both healthy subjects and subjects with abnormal FSHR function.

Trial registration number: Translational research, no trial.

O-213 A novel two-step transplantation procedure using adipose tissue-derived stem cells increases follicle survival by boosting angiogenesis in xenografted frozen-thawed human ovarian tissue

D.D. Manavella I.[1], L. Cacciottola[1], J. Donnez[2], C.A. Amorim[1], M.M. Dolmans[1]

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[2]*Society for Research into Infertility, Fertility Preservation, Brussels, Belgium*

Study question: Do adipose tissue-derived stem cells (ASCs) increase follicle survival by enhancing oxygenation and vascularization in xenografted ovarian tissue using a two-step transplantation approach?

Summary answer: Higher rates of oxygenation and vascularization of ovarian tissue, as well as increased follicle survival rates, were detected in the early post-grafting period.

What is known already: ASCs have multilineage differentiation potential, proangiogenic properties and enhance vascularization in a peritoneal grafting site. Some studies suggest that using ASCs may improve quality of grafted ovarian tissue by enhancing graft angiogenesis.

Study design, size, duration: In vivo experimental model. A total of 15 severe combined immunodeficient (SCID) mice were intraperitoneally grafted with frozen-thawed human ovarian tissue from 5 different patients for a short-term period (7 days). A peritoneal transplantation site had been previously prepared in a first step using either empty fibrin (Fi + OT group [n = 5]) or ASC-loaded fibrin (Fi/ASCs+OT group [n = 5]) 14 days prior to grafting. Five mice underwent the standard one-step transplantation procedure and served as controls (OT group).

Participants/materials, setting, methods: Prospective experimental study conducted at the Gynecology Research Unit, Université Catholique de Louvain. Lithium phthalocyanine (LiPc) crystals were inserted into all grafted human ovarian tissue before transplantation. On days 3 and 7: pO_2 measurements (EPR oximetry); on day 7: mice were euthanized and samples were collected for histological (H-E and Masson's trichrome staining), IHC (anti-mouse and human double CD34 and anti-human Ki-67) and TUNEL analyses.

Main results and the role of chance: A significant increase in pO_2 was observed in all groups between day 3 and 7 ($p < 0.001$). A significantly higher pO_2 level was observed in the Fi/ASCs+OT group compared to the OT group on day 7 ($p = 0.028$). Total CD34-positive vessel area on day 7 was greater in the Fi/ASCs+OT group than in any other group (vs non-grafted group: $p = 0.0014$; vs OT group: $p = 0.013$; vs Fi + OT group: $p = 0.018$). Follicle survival rates after grafting were also higher in the Fi/ASCs+OT group compared to all the rest (vs OT group: $p = 0.0059$; vs Fi + OT group: $p = 0.0138$). TUNEL-positive follicle percentages after grafting were significantly lower in the Fi/ASCs+OT group than in any other grafted tissue (vs OT group: $p = 0.045$; vs Fi + OT group: $p = 0.0268$). Percentages of Ki-67-positive primordial follicles were significantly higher in all grafted groups compared to non-grafted tissue controls ($p < 0.01$). No differences in were observed fibrotic areas.

Limitations, reasons for caution: As demonstrated by our results, the proposed two-step ovarian tissue transplantation procedure using ASCs enhances vascularization in the early post-grafting period, leading to increased follicle survival rates and decreased apoptosis. However, mechanisms involved in the proangiogenic behavior of ASCs remain to be elucidated.

Wider implications of the findings: Our results suggest that the proposed transplantation procedure with ASCs is a promising step towards potentially solving the problem of massive follicle loss after ovarian tissue grafting.

Trial registration number: N/A.

INVITED SESSION

SESSION 58: COCHRANE SESSION: INDUSTRY AND EVIDENCE IN REPRODUCTIVE MEDICINE: MANAGING THE CONFLICTS

Wednesday 4 July 2018 Room 211 + 212 08:30–09:30

O-214 Conflict of interest and bias: not your problem or is it?

L. Bero

The University of Sydney, D17- The Hub- 6th Floor- Charles Perkins Centre, Sydney NSW, Australia

Abstract text

Bias in research is a problem that concerns researchers, consumers, policy makers and other users of evidence. Researchers strive to reduce bias in their research and make it transparent. A variety of methods have been used to identify "bias"—the systematic error or deviation from the true results or inferences of a study—in clinical pharmaceutical research. Bias related to funding sources or investigator conflicts of interest can be introduced throughout the entire research process (questions asked, design, conduct or publication). Global transparency initiatives have enabled the detection of previously hidden financial ties between researchers and pharmaceutical companies. I will discuss a number of ongoing efforts aimed at identifying and reducing bias in clinical research and practice.

O-215 We need some sunshine - making gifts from industry Transparent

P. Vercellini

Università degli Studi and Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico- Milan- Italy, Department of Clinical Sciences and Community Health, Milano, Italy

Abstract text

The collaboration between academic investigators and industry is invaluable for the advancement of medical knowledge and improvement of health care. However, whereas the main objective of medicine is to develop increasingly clinically effective and cost-effective therapeutic options, that of pharmaceutical industries and medical device manufacturers is to generate profit. Physicians are accountable to their patients and, more in general, to society. Industry CEOs and senior executives are accountable to their board of directors and, more in general, to shareholders. In spite of different main aims, a "convergence of interests" may arise between medicine and industry when crossing the boundaries of science and accepting the weight of financial dynamics. Physicians may desire money for themselves, but also for their research programmes, scientific societies, conferences, and medical journals. In an era of scarce public funding, industry becomes an attractive and abundant source of money for the entire medical community. In this condition, the risk for medicine would be to favour the interest of industry to the detriment of that of patients, thus derailing from its mission, whereas industry can continue to pursue its main interest (i.e., profit) without mission distortion. According to entrepreneurial logic, any industry financial investment should generate revenues, in this case through modification of prescribing patterns. In this regard, scientific information is crucial to increase sales of drugs and devices. The way scientific information is generated, selected, disseminated, and conveyed to patients is precisely the process that industry may be tempted to influence. To accumulate funds for their research programmes, academicians could accept to conduct trials expressly designed to systematically favour the experimental drug. The reports of these trials may appear in prestigious journals that would gain from selling advertisement and reprints. The publication of these results could influence the drafting of guidelines issued by scientific societies, especially when members of the panel of experts received industry funds. Annual conferences, which are indispensable for the financial survival of many professional organisations, could be supported by drug and device manufacturers, provided sponsored symposia including selected key opinion leaders are strategically positioned within the scientific programme. Industry may focus not only on investigators tempted to facilitate their career and increase their visibility, but also on patient associations, generally considered by citizens as honest and reliable sources of information. Awareness campaigns can be organized by façade committees behind which industries operate. Several stakeholders would profit from this "convergence of interests", except patients, their families, and public health services that eventually would pay the bill. If influenced scientific information is the cornerstone of this ambiguous compromise, then medicine appears much guiltier than industry, because no distortion of evidence would ever be possible without the active role of academia. The validity of the shared decision-making process, i.e. the

currently accepted paradigm of care in chronic medical conditions, is based on a properly conveyed, complete and truthful information. In case the professional contract is breached to the benefit of industry, who is more to blame: the individual physician who accepted meals, gifts, and travels to conferences, or those academicians with vested interests who, playing several different roles, generated and disseminated distorted information used to influence the end-prescriber? With the objective of limiting the "convergence of interests", an open peer-review system could be readily implemented by major journals for those manuscripts dealing with novel medical interventions with a potential impact on prescribing patterns. If names of editors and reviewers, as well as their reports, were publicly accessible, probably the gatekeepers of scientific information would be less indulgent toward industry interests.

INVITED SESSION

SESSION 59: NON-GENETIC FACTORS INFLUENCING EMBRYO VIABILITY

Wednesday 4 July 2018 Room 111 + 112 08:30–09:30

O-216 Influence of maternal diet on preimplantation embryo development and long-term potential

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Abstract text

Epigenetic and physiological mechanisms permit embryos at the onset of development to adapt to environmental conditions such as maternal dietary quality, leading to altered phenotype of both extra-embryonic (placental) and embryonic (fetal) cell lineages. Thus, poor maternal diet around the time of conception in mouse models induces compensatory responses in extra-embryonic lineages that collectively enhance the capacity for nutrient delivery from the mother. Peri-conceptional poor diet also causes fetal tissues to coordinate growth with nutrient availability through control of ribosome biogenesis, mediated by DNA methylation level of the rDNA promoter. Such adaptations to peri-conceptional maternal diet appear mediated through nutrient sensing, particularly levels of select amino acids and insulin, at the blastocyst stage and modulation of mTOR signalling. Whilst this maternal-embryonic 'dialogue' supports embryo survival, if environmental conditions change, adaptations can become maladaptive and associate with long-term risk of cardiometabolic and neurological disease in adult offspring. Evidence of related environmental programming of early embryos has been forthcoming across mammalian species including the human, and can occur in response to diverse conditions, not only maternal diet but also in vitro embryo techniques as used in assisted conception. Funding: BBSRC, MRC, EU, NIH, Rosetrees Trust, Kerkut Trust.

O-217 Extrinsic factors disrupting embryo physiology in vivo and vitro

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Abstract text

Hypo fertility or delay/failure to conceive is an increasing multifactorial problem in western populations. Increasing parental age due to delaying the decision to have a baby is one problem, while a decrease in sperm quality and premature ovarian failure (POF) and diminished ovarian reserve (DOR) are today observed with increasing frequency. Obesity and diabetes are on the increase and now affect 30% of the population. The link between these chronic pathologies, oxidative stress (OS) and epigenetics and their impact on embryo quality is now clear. Endocrine disruptors (EDCs) are strong effectors in the contribution to hypofertility. Pesticides, xenoestrogens, endocrine disrupting chemicals involved in plastic technology such as polychlorinated bisphenols (PCB), bisphenols (BP), phthalates, alkyl phenols are now present in the environment, food and even in cosmetics. They induce OS that affects DNA (but not only) quality via the estrogen (ER) and androgen (AR) receptors leading to hormonal anomalies. Via the peroxisome proliferator-activated receptors (PPARs), they perturb metabolic signaling and induce endocrine pathologies (obesity). Recent research suggests that they negatively affect correct DNA methylation in offspring tissue and thus epigenesis and imprinting. Epigenetic mechanisms modify the expression of specific genes without changing the underlying DNA sequence: they are involved in important cellular processes: chromosome stability (and inactivation), transcription and cellular growth and differentiation; all major effectors for a correct embryonic development. Fetal male and female gametes are submitted first to a methylation/imprinting erasure and then a full reset of a complete profile. EDCS may affect this resetting as these chemicals are found in urine of children and adults; they induce ovarian resistance during pre-implantation development, there is a strong methylation maintenance driven by the DNA methyltransferase 1 highly expressed in oocytes and early embryos (Menezo et al. 2011). This maintenance may be affected in vitro as well as the majority of culture media do not contain methyl donors; these compounds are the only molecules able to counteract the negative effects of the EDCs. Recent publications have demonstrated that IVF babies have different DNA methylation profiles compared to babies obtained by natural conception (Katari et al. 2009, Song et al. 2015). All these negative features may be increased by the endogenous backgrounds affecting methylation process such as mutations affecting the one carbon cycle (1-CC), such as MTHFR (methylene tetrahydrofolate reductase) and MS (Methionine synthase) mutations.

In conclusion, many aspects of modern day life have increased the difficulty to conceive. Nutritional backups that support the 1-CC, and therefore methylation processes, and endogenous protection against oxidative stress are strongly recommended for men and women before trying to conceive since avoiding negative factors from environmental pollution now seems impossible.

INVITED SESSION

SESSION 60: NURSES/MIDWIVES INVITED SESSION: TOWARDS PARENTHOOD

Wednesday 4 July 2018 Room 113 + 114 + 115 08:30–09:30

O-218 Antenatal care in transition from fertility treatment to parenthood

C. Warmelink, M. Boekhout, E. Tros, L. Kool

Midwifery Academy Amsterdam-Groningen, Midwifery Academy Groningen, Groningen, The Netherlands

Study question: What are the antenatal maternity care needs of MAC-parents in the transition from fertility treatment to parenthood?

Summary answer: The study will be ready in July 2018

What is known already: Nearly five percent of newborns in the Netherlands are now born following fertility treatment, Medically Assisted Conception (MAC), a steadily increasing number. Pregnancies conceived through MAC are associated with higher risks of complications, such as small for gestational age, preterm birth, low birth weight, perinatal death and delivery complications. Next to the medical impact, the fertility treatment and the pregnancy after MAC can have psychological and social impact for the MAC-parents, such as higher levels of anxiety in early pregnancy and perception of the pregnancy as being more risky and demanding.

Little is known about the antenatal care needs of MAC-parents in transition from fertility treatment to parenthood. There is a lack of awareness and training for midwives and other maternity care providers on the impact of MAC on the pregnancy.

Study design, size, duration: Mixed Methods Review – a systematic review of qualitative, quantitative, and mixed method studies guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist and methodological quality appraised in accordance with the Mixed Method Appraisal Tool (MMTA). Data sources such as Medline/PubMed, Cochrane library, Cumulative Index to Nursing and Allied Health (Cinahl) and Psychology and behavioral science were searched to find eligible studies.

Participants/materials, setting, methods: Two student-midwives researchers conducted the searches and selected the studies together and abstracted the data independently in Winter and Spring 2018. Two researchers (psychologist and educational scientist) supervised the study.

A PRISMA flowchart present the screening process and results of the bibliographic search.

Main results and the role of chance: In progress.

Limitations reasons for caution: As with all research, the value of this systematic review depends on what was done, what was found, and the clarity of reporting. As with other publications, the reporting quality of systematic reviews varies, limiting readers' ability to assess the strengths and weaknesses of those reviews. Even though systematic review and meta-analysis are considered the best evidence for getting a definitive answer to a research question, there are certain inherent flaws associated with it, such as the location and selection of studies, heterogeneity, loss of information on important outcomes, inappropriate subgroup analyses, and duplication of publication.

Wider implications of the findings: The number of women treated with and babies born after fertility treatments is mounting. Also, there is clear empirical evidence of the postponement of the first child in Western societies, which has implications on the (dis)ability of women to conceive and parents to produce additional offspring. Subfertility and fertility treatment can be a potential medical, social and psychologist burden. This places additional and changed demands on the competence and knowledge of professionals working in maternity care.

The study output can lead to the implementation of innovative interventions, care models and methods and guidelines for midwives, gynaecologists and other maternity care takers.

Study funding/competing interests: This work is not funded. All authors declare s.

Registration number: N/A.

O-219 Focussing on infertile men's needs

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Infertile men and their needs

Abstract text

Childlessness concerns both partners in the couple; however until now only limited focus has been on men's needs regarding their fertility treatment. Men with low sperm count are frequently a complex group to treat at the fertility clinic. Women undergo the majority of the physical aspects of treatment, thus the focus of the information and physical care is generally directed toward the women. Even in couples with male factor infertility, men's role in treatment is generally limited to providing a semen sample. Research has shown that men wish to talk about the psychosocial topics and be included more.

Approximately one third of men in heterosexual couples in fertility treatment have low sperm count. For the individual man this often means that he will consider why his sperm count is low, try to find explanations and consequences of this. Male infertility is a potential severe low-control stressor, and research has shown that many men with low sperm quality would like to talk about it more widely. Men, unlike women, rarely express their problems and needs in the health service or are more reluctant to do so. Mikkelsen et al. found that 72% of 210 Danish men undergoing fertility care lacked information about the psychological consequences of male infertility.

Previous studies on male fertility patients' satisfaction with treatment differ in results. A study among Australian men has shown that the contentment with the fertility treatment was not influenced by the outcome of the treatment. A Danish study among 909 male fertility patients treated in four public clinics and initiating treatment in 2000-2001 showed higher satisfaction ratings of medical care and patient-centered care with decreasing social class and if the couple had achieved a pregnancy after treatment. A Danish qualitative interview study showed that infertile couples preferred that their treatment took place in a separate clinic, with few employees, short waiting list and in a setting where the plan of treatment was known by the doctor and the couple. The patients saw the fertility treatments as a psychological and emotional strain and they did not feel that these problems acknowledged by the staff. Further, that the patients who disclosed their fertility problems to others were the patients who expected most of the health professionals. These participants wanted detailed technical assessments along with psychosocial and sexual advice.

We conducted a qualitative study among infertile men who initiated ART with ICSI-treatment to explore their expectations and needs in order to optimize the treatment and handling of this patient group. The results showed that men experienced the process of going from diagnosis to treatment like 'being in a maze', which affected their personal life. The men were ready to become fathers and felt impatient because they needed medical assistance to achieve this life goal. The men felt like they were placed on the sideline but they expressed a desire to have information about the treatment plan from medical professionals and they wanted to discuss the psychological consequences of their low semen quality.

Fertility care and treatment needs to be considered as a couple issue and not only as a woman issue. Fertility staff needs to focus on involving both the man and the woman in the treatment, and more focus is needed on the men and their psychosocial needs.

INVITED SESSION

SESSION 61: ARTHIQS SESSION: THE EVOLVING ROLES OF COMPETENT AUTHORITIES, PROFESSIONAL ORGANISATIONS AND THE EUROPEAN COMMISSION IN ART

Wednesday 4 July 2018 Room 117 08:30–09:30

O-220 Introduction: The roots and findings of ARTHIQS

O-221 Competent authorities: From inspectorates to support structures

N. Jones

Human Fertilisation and Embryology Authority HFEA, London, United Kingdom

Abstract text

The session will introduce examples of how competent authorities can undertake their mission. Firstly, by carrying out efficient and effective 'core' work in setting standards and inspecting ART tissue establishments to maintain and improve standards, doing so in an environment of mutual respect where dialogue about practise is engendered; and secondly building on this environment so policy discussion takes place openly. As such, this may create an opportunity to ensure that innovation is introduced carefully, together with practices that would not ordinarily have been introduced – given the country's context, culture and history. For example, consultation and discourse on, the treatment of same sex couples; single woman; pre-implantation genetic diagnosis and other ethically controversial areas. The two approaches are not mutually exclusive to a Competent Authority.

O-222 The relationship between competent authorities and the European Commission

W.N. Strang

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Abstract text

During and since the preparatory phases of the 2004 EU Tissue and Cells Directive that created Competent Authorities in all European Union Member

States, the European Union has worked closely with these Member States and emerging Competent Authorities in order to align community legislation as closely as possible to the needs of the different situations found in the different Member States.

This cooperation occurs within the formal consultations of the advisory groups, but also via more informal stakeholder consultations and in particular, through Joint Actions, such as ARTHIQS, where the Commission and its executive Agency CHAFEA establish frameworks within which the Member State Competent Authorities can work together, but also with Commission Services and external organisations such as ESHRE, to share and develop expertise amongst themselves.

For the European Commission, ARTHIQS was an important Action allowing the increase of shared knowledge and know-how in the EU-28 Member States in the fields of reproductive medicine and haematopoietic stem cell transplantation. These fields are increasingly important for a large number of EU citizens in need of treatment for infertility problems on the one hand, and for blood cancers on the other.

Both these areas are characterised by rapid development. It is therefore of utmost importance that the national Competent Authorities which oversee these activities in the 28 EU Member States, keep pace with these developments and have a minimum and common understanding of how to ensure safety and quality in these therapies. ARTHIQS built on our European model of collaboration, bringing together the best expertise in these fields and making it available to authorities in all Member States of the European Union.

In domains as complex as reproductive medicine it is essential to create conditions of mutually beneficial synergy whilst defining and developing legislative and good practice frameworks. As such, the relationships between the Commission and the Competent Authorities and Professional Societies have evolved into a quasi-partnership over the last decade. The relationships between CAs, tissue establishments and professional societies are also developing and are, overall, positive and constructive. The missions of all these organisations are aligned with the need to protect donors and patients affected by assisted reproduction.

O-223 The role of medical societies in promoting good practice and the scope for reprovigilance

R.G. Farquharson

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Abstract text

The role of specialist medical societies was historically confined to research, education and training for the benefit of its membership. A more modern approach has enlarged that sphere of interest to include core behaviour standards and patient safeguarding in the belief that collaboration is the gateway to the future. The European Society of Human Reproduction and Embryology (ESHRE) has become more involved with relevant competent authorities (CA), patient organisations and European funded projects in helping to frame policy making and act as a focus for direction of travel.

With ART specifically in mind, the journey from gamete to child via pregnancy and the newborn requires safety assurance and dialogue with regulatory agencies to protect patients from harm and to optimise patient outcomes in achieving a family. Reprovigilance is a term coined by ESHRE that involves monitoring of treatment activity and intervention profiling that mirrors the ethos of our core values.

O-224 Closure: Future perspectives for competent authorities' Cooperation

C. Plancha[1,2,3]

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Abstract text

The ARTHIQS Joint Action brought clarity to the diversity of practice and legislation in the field of Assisted Reproductive Technologies (ART) within and between European Union (EU) Member States (MS). It highlighted the specific aspects of ART and how ART dedicated Competent Authorities (CA) might implement regulation in the field.

The ARTHIQS Joint Action provided several opportunities of interaction between different regulators regarding ART, and of these with the professionals, all recognized as beneficial for the advancement of quality and safety measures in this sector. The ART dedicated CA have much to learn from each other, given their different experience in several MS, and need also to work closely with the healthcare professionals, their representatives and the general public, given the sensitive and exceptional nature of ART.

How to maintain a close cooperation between CA following the ARTHIQS Joint Action, emerges as a major organizational challenge. However, this may also become an opportunity for ART dedicated CA to fertile exchange of experience and expertise, adoption of shared policies, and to facilitate harmonization of practices among the different MS, maintaining and enhancing national and European registers, namely those regarding the safety of patients, donors and recipients.

We defend that a network of ART CA, could conveniently follow the ARTHIQS Joint Action, in order to provide a forum to bring this experience together improving quality and safety for the patients, donors and children born of ART. This could involve proposing joint initiatives, working closely with the European Commission to analyse amendments to policy and legislation and resolving difficulties encountered in the complex evolving field of ART. The tissues and cells CA could provide the basis for the creation of such a network, which might be constituted as a specific working group, with the support of DG Sante, with transparent declarations of conflicts of interest and adhesion to the EU institutions guidelines. The ART CA network or working group should call upon the experience of and cooperate with professional and patient's organisations, in particular ESHRE. Professional societies play a fundamental role in the complex and evolving field of ART which continues to be driven by rapid scientific progress.

SELECTED ORAL COMMUNICATIONS

SESSION 62: FROM OOCYTE MATURATION IN VIVO TO BABY BIRTH

Wednesday 4 July 2018 Forum (Auditorium) 10:00–11:45

O-225 Immature oocyte incidence: Contributing factors and effects on intracytoplasmic sperm injection cycles

B.F. Zanetti[1], **D.P.A.F. Braga**[2], **R.C.S. Figueira**[3], **A. Iaconelli Jr.**[4], **E. Borges Jr.**[4]

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[3]*Fertility Medical Group, IVF Laboratory, Sao Paulo, Brazil*
[4]*Fertility Medical Group, Clinical Department, Sao Paulo, Brazil*

Study question: Which factors contribute to the incidence of immature oocytes and how this impacts the outcomes of intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: The immature oocyte incidence is affected by pituitary blockage regime,gonadotrophin type and dose, and negatively impacts on fertilization rate, embryo quality, implantation and pregnancy outcomes.

What is known already: Oocyte maturation is defined as the restart and completion of the first meiotic division with accompanying cytoplasmic maturation, which includes storage of enzymes, mRNAs, organelles, and substrates that are necessary for fertilization and early embryo development. Controlled ovarian stimulation (COS) for IVF apply supra-physiologic gonadotropin doses to induce coordinately multiple follicular growth and maturation, however up to 30% of the retrieved oocytes are still immature. The ovarian asynchrony can be indicative of less responsiveness of ovarian follicles to stimulation, and the incidence of immature oocytes may reflect the competence of the mature oocytes cohort for proper embryo development.

Study design, size, duration: This retrospective cohort study included data from 3,920 ICSI cycles performed from June/2010 to August/2016 of couples undergoing first or second ICSI cycle with fresh embryo transfer performed on day five. Immature oocytes rates were defined as the number of Germinal Vesicle and Metasphase I oocytes by the number of retrieved oocytes in each cycle. The FSH/oocyte rate was defined as total dose of FSH administered by the number of oocytes retrieved in each cycle.

Participants/materials, setting, methods: The study was performed in a private university-affiliated IVF center. The influence of administered FSH doses, FSH type, pituitary blockage protocol, and E2 peak on the day of hCG administration on the incidence of immature oocytes were evaluated by generalized linear models. The effects of immature oocytes rates on normal and abnormal fertilization, embryo quality on cleavage stage (days two and three), blastocyst formation, implantation, pregnancy, and miscarriage rates were analyzed by regression models.

Main results and the role of chance: The FSH/oocyte rate was positively correlated with MI/oocyte (β=-0.043, $p = 0.014$) and GV/oocyte (β=-0.107, $p < 0.001$) rates. A higher MI/oocyte rate was observed in patients who received GnRH agonist and recombinant FSH in relation to GnRH antagonist and recombinant FSH (13.34 ± 0.09 vs. $10.73 \pm 0.28\%$ $p = 0.007$). Patients who received recombinant FSH had about 30% increase in GV/oocyte rate in comparison to urinary FSH, both with GnRH agonist (11.29 ± 0.90 vs. 4.18 ± 4.52 $p = 0.048$), and antagonist (11.47 ± 0.28 vs. 8.84 ± 1.41 $p = 0.021$) protocols. The fertilization rate was negatively correlated to MI/oocyte and GV/oocyte rates (β=-0.096, $p < 0.001$; β=-0.059, $p < 0.001$), while abnormal fertilization rate (1PN or 3PN) was positively correlated with GV/oocyte rate (β = 0.155, $p = 0.049$). The incidence of immature oocytes affected negatively the high-quality embryos rate at day two (MI/oocyte β=-0.102, $p < 0.001$; GV/oocyte β=-0.066, $p < 0.001$), and day three (MI/oocyte β=-0.090, $p < 0.001$; GV/oocyte β=-0.087, $p < 0.001$), as well as the blastocyst rate (MI/oocyte β=-0.066, $p < 0.001$.; GV/oocyte β=-0.053, $p < 0.001$). The negative effect of the incidence of MI and GV was also noted in the implantation (MI/oocyte β=-0.074, $p < 0.001$; GV/oocyte β=-0.042, $p = 0.033$) and pregnancy (MI/oocyte B=-0.011 $p = 0.002$; GV/oocyte B=-0.066, $p = 0.013$) rates.

Limitations, reasons for caution: The retrospective nature of this analysis limits the relevance of the study.

Wider implications of the findings: During COS, pharmacological gonadotrophins doses creates a supra-physiological environment, which allows the maturation of oocytes, which under natural conditions, would regress. Our evidence suggests that oocytes derived from a cohort with high incidence of maturation fail may have an inefficient biological machinery, which would have detrimental effects on clinical outcomes.

Trial registration number: None.

O-226 The influence of denudation and injection on ICSI outcome - does timing matter?

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Study question: Does the time interval between oocyte retrieval and denudation or injection have an effect on fertilization and clinical pregnancy rates in ICSI cycles?

Summary answer: Our findings suggest that ICSI outcome is improved when oocytes are cultured for at least 3 hours before denudation and injected 5-6 hours after retrieval.

What is known already: Oocyte maturation is a complex and dynamic process involving structural and biochemical modifications in the cell necessary to support fertilization and early embryo development. While meiotic competence is easily identifiable by the presence of an extruded first polar body, cytoplasmic maturation cannot be assessed microscopically. Culturing oocytes with their surrounding cumulus cells prior to fertilization can enhance the completion of in vitro cytoplasmic maturation; however, prolonged culture may also induce cell degeneration. The optimal culture intervals prior to oocyte denudation and/or injection have not yet been established and may prove relevant for the improvement of ICSI outcome.

Study design, size, duration: Single-centre retrospective analysis of 1583 ICSI cycles performed between 2002 and 2017 in women <40 years old. All women performed controlled ovarian hyperstimulation using a long GnRH-agonist protocol and oocyte retrieval was undertaken 34-36 hours after final ovulation triggering. Time intervals between oocyte retrieval, denudation and injection of the oocytes were exclusively dependent on laboratory workload. The effects of these timings on fertilization and clinical pregnancy rates were compared.

Participants/materials, setting, methods: The effect of oocyte culture duration prior to injection of the oocytes was considered in two separate analysis: (i) time interval between oocyte retrieval and denudation (3.2 h±1.0) and (ii) time interval between oocyte retrieval and injection (5.4 h±0.8). Only cycles with at least one injected oocyte were included. The effect of the before mentioned time intervals on fertilization and clinical pregnancy rates were compared using multivariable regression adjusted for potential confounding variables.

Main results and the role of chance: 10359 metaphase II oocytes were injected and checked for the presence of pronuclei. The fertilization rate was found to be independent of the time interval to denudation and/or injection. Oocyte degeneration rate after ICSI was also comparable considering different time intervals to denudation and injection but the abnormal fertilization rate was significantly increased for longer incubation periods before injection, even after accounting for the time elapsed until denudation (<5 h, 6.1%; 5-6 h, 7.1%; ^{3}6, 9.0%, $p = 0.01$). Extending oocyte culture before denudation significantly improved clinical pregnancy rates, particularly if the time interval comprised of at least 3 hours (<2 h, 34.5%; 2-3 h, 33.7%; 3-4 h, 41.6%; ^{3}4 h; 46.8%, $p < 0.001$). Clinical pregnancy was also dependent on time to injection and optimal results were obtained when ICSI was performed 5-6 hours after retrieval (<5 h, 35.3%; 5-6 h, 42.3%; ^{3}6 h, 39.3%, $p = 0.043$).

Limitations, reasons for caution: This is a retrospective study, with its inherent limitations, and restricted to long agonist cycles. Furthermore, the effect of very short or very long periods of culture before denudation and/or injection was not evaluated and should not be extrapolated from these results.

Wider implications of the findings: Our findings support the importance of extending oocyte culture with their surrounding cumulus cells, but more importantly provide new evidence of a correlation between denudation and injection intervals. Establishing an optimal time window to perform denudation and ICSI and adjusting laboratory practice may prove beneficial and improve clinical pregnancy rates.

Trial registration number: Not applicable.

O-227 The impact of intracytoplasmic sperm injection (ICSI) on embryo development as assessed by Time-Lapse (TL) microscopy on 4126 embryos

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Study question: Does the utilization of ICSI versus conventional insemination (CONV) impact either early- or late- morphokinetic parameters in embryos cultured to blastocyst in a TL-monitored incubator?

Summary answer: Time periods from either pronuclei fading (tPN*f*) or 5-cell stage to early blastulation were significantly prolonged when *usable* ICSI embryos were compared to CONV ones.

What is known already: While some studies suggest that correcting for time of fertilization by normalizing all times to tPN*f* may eliminate differences in early cleavage times between embryos undergoing ICSI versus CONV, other data suggests a potential effect of fertilization method on early cleavage times. At the

same time, the potential impact of fertilization method on late-morphokinetic parameters remains unclear.

Study design, size, duration: Retrospective review of data from 4126 embryos (derived from 364 ICSI and 237 CONV cycles) cultured to the blastocyst stage in TL-monitored incubators. 536 women undergoing ART between 9/2013 and 9/2016 at a large academic fertility center were included in the analysis.

Participants/materials, setting, methods: Groups were compared in regard to time periods: i) tPN*f* to 1st-cytokinesis (P_1), ii) 2- to 3-cells (P_2), iii) 3- to 4-cells (P_3), iv) 4- to 5-cells (P_4), v) 5- to 8-cells, and vi) tPN*f* and 5-cells to early blastulation (P_{SB} and P_{5SB}, respectively). *Usable* (selected either for transfer or cryopreservation) and *discarded* embryos were sub-analyzed separately.

Generalized fixed and random effects models, Mann-Whitney *U*-, and χ^2-tests were used as appropriate. $P < 0.05$ was statistically significant.

Main results and the role of chance: Median (interquartile range, IQR) age, BMI, day-3 FSH and AMH were 34.8(32.3, 37.8) vs. 36.2(33.2-39.4) years, $p < 0.01$; 23.1(21.0-26.7) vs. 23.4 (21.6-26.6) kg/m^2, $p = 0.46$; 6.7(5.7-8.0) vs. 7.0(5.9-8.4) IU/L, $p = 0.16$; and 3.0(1.5-5.1) vs. 2.1(1.1-4.0) ng/ml, $p < 0.01$; for ICSI vs. CONV, respectively. Male factor infertility (MFI) was more prevalent among ICSI cycles (40.7% vs. 5.0%, $p < 0.01$ for ICSI vs. CONV, respectively).

When all embryos were included in the analysis and models were adjusted for age, BMI, day-3 FSH, AMH, and MFI, P_{5SB} and P_{SB} were found to be significantly prolonged in ICSI cycles by 2.4(95%CI: 0.6-4.2) hours, $p = 0.01$; and 1.8 (95%CI: 0.2-3.3) hours, $p = 0.02$; respectively. When *usable* were analyzed separately from *discarded* embryos, the impact of ICSI persisted in *usable* embryos only [*usable*: P_{5SB} and P_{SB}: 3.1(95%CI: 1.4-4.7) hours, $p < 0.01$; and 2.3(0.8-3.7), $p < 0.01$; respectively*; discarded*: P_{5SB} and P_{SB} 1.6(-0.9-4.1), $p = 0.20$; and 1.2(-0.8-3.3) hours, $p = 0.23$; respectively].

No impact of ICSI was noted on all other morphokinetic parameters, when either *all* embryos were included in the analysis, or *usable* were analyzed separately from *discarded* embryos.

Limitations, reasons for caution: This study is limited by its retrospective design. Furthermore, all aspects of sperm quality may not have been considered by adjusting for male factor infertility diagnosis.

Wider implications of the findings: ICSI did not impact significantly early cleavage times. However, time to early blastulation was prolonged in ICSI embryos with the impact becoming more prominent from the 5-cell stage and onward, a finding suggestive of a possible an interaction between fertilization method and late paternal genome activation.

Trial registration number: Not applicable.

O-228 Differences in time-lapse practice: is a consensus on standards needed?

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Study question: Do clinics differ in how they implement time-lapse technology into their processes?

Summary answer: How Time-Lapse is used varies between IVF clinics, particularly with regards to patient and cross-department involvement, communication, embryo selection and how process-efficiencies are optimised.

What is known already: Time-Lapse incubation involves culturing the embryo in an incubator that takes regular pictures of the embryos. This has allowed for embryos to be cultured uninterrupted. With increased observation, benefits from the technology started to change how embryology was practised, improving customer service whilst increasing process efficiency. However, not all clinics have experienced these benefits. To our knowledge this is the first study to assess how time-lapse technology is used in different clinics.

Study design, size, duration: In December 2017, 38 experienced time-lapse embryologists from the UK responded to an anonymous questionnaire with 20 questions assessing how far they adapted the clinic wide processes to the time-lapse technology since implementation.

Participants/materials, setting, methods: All participants were Embryoscope users, 48% with 3+ years experience. The questionnaire was presented using Mentimeter in a live presentation and anonymity of respondents was maintained.

Main results and the role of chance: 71% did not train non-laboratory departments in how the Time-Lapse technology affected their processes. 87% had time-lapse related communication processes between the embryologist and the patient, a lower proportion involving other departments (23%-51%). 73% provided the patient access to videos of embryos transferred at the time of transfer with a USB. 52% thought patients would welcome live access to videos whilst in the incubator, whilst 45% affirmed that irrespective of patient's wishes, their clinics would be against giving patients live access. 65% did not provide patients with written reports. 42% only provided access to time-lapse incubation to patients willing to pay. 76% did not adapt embryology process timings to the flexibility provided by time-lapse. 1 clinic adapted the hcg time. Embryo selection differed: 58% did not review the video. Reliance on algorithm increased with the number of years experience. Clinics prioritised morphology on day 5, followed by unusual embryo cleavage, followed by KIDScore. 46% of the clinics reviewed embryo selection consistency. 19% reviewed efficacy. 52% annotate daily, followed by morning of ET (32%), and 4 clinics did not annotate at all. Most clinics reported not having the time required to ensure the annotations are complete, accurate and standardised (56%).

Limitations, reasons for caution: Participants all came from the same country, and the answers may not be reflective of practice in other countries. Participants were all Embryoscope users, and practice may further differ with other Time-Lapse systems.

Wider implications of the findings: This study documents the diversity in the practice of the time-lapse technology between different IVF clinics, suggesting that there is a need for a formal consensus on standards of time-lapse practice to ensure patients and clinics benefit fully from time-lapse technology, irrespective of which clinic they are treated in.

Trial registration number: NA.

O-229 Deconstructing the myth of poor prognosis for fast-cleaving embryos on Day 3. Is it time to change the consensus?

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Women's Health Dexeus, Reproductive Medicine Service, Barcelona, Spain

Study question: Do fast-cleaving embryos (>8 cells at 68 ± 1hpi) have a lower potential to become euploid blastocysts and implant when compared to 8-cell embryos?

Summary answer: The chance of fast-cleaving embryos on Day 3 (D3) to give rise to euploid blastocysts and implant is comparable to the one of 8-cell embryos.

What is known already: The cell number is one of the most predictive morphological parameters for blastocyst formation, implantation and live-birth rates. The ESHRE-Alpha consensus (2011) established that embryos that cleave slower or faster than the expected rate (8 cells at 68 ± 1hpi) have a reduced implantation potential. Likewise, according to the ASEBIR criteria (2015) fast-cleaving embryos on D3 are included in B or C categories with a lower implantation potential compared to 8-cell embryos classified as A. Regarding the ploidy status, higher percentage of chromosomal abnormalities were found in fast-cleaving embryos compared to 8-cell embryos.

Study design, size, duration: A total of 4028 embryos from 513 PGT-A cycles performed between July 2014 and June 2017 were included in this observational retrospective study. Embryos were classified in groups based on the number of cells on D3 (from 2-cell to >12-cell and compacted).

Participants/materials, setting, methods: Embryos were cultured in single-step medium (Global®, LifeGlobal®; GTL™, Vitrolife) and incubated in *Time-lapse* incubators (Embryoscope®; Geri®). Embryos were biopsied at the blastocyst stage followed by vitrification (Cryotop®, Kitazato). The trophectoderm samples were analysed by a-CGH. A generalized linear mixed model adjusted for confounding factors was performed to assess the chance to become an euploid blastocyst in each and every group compared to the chance of 8-cell embryos.

Main results and the role of chance: In this study, 64.4% of 8-cell embryos on D3 (875/1358) and 59.3% of fast-cleaving embryos on D3 (863/1454) reached the blastocyst stage and then were biopsied. After the genetic test, 22.7% of 8-cell embryos (308/1358) and 20.6% of fast-cleaving embryos (299/1454) were diagnosed as euploid. The statistical analysis showed that the probability of fast-cleaving D3 embryos to give rise to an euploid blastocyst is not significantly different from the chance of 8-cell embryos. The results are valid for every analyzed fast-cleaving D3 group (from 9-cell group to compacted group) as demonstrated by the odd ratios (OR): 9-cell group OR (95%CI): 0.76 (0.57-1.01); 10-cell group OR (95%CI): 0.72 (0.51-1.01); 11-cell group OR (95%CI): 0.95 (0.60-1.51); 12-cell group OR (95%CI): 1.11 (0.75-1.63); >12-cell group OR (95%CI): 1.35 (0.85-2.15) and compacted group OR (95%CI): 1.19 (0.81-1.76). Moreover, euploid blastocysts derived from fast-cleaving D3 embryos and from 8-cell D3 embryos exhibit similar implantation potential (67% vs 55%, p = 0.25).

Limitations, reasons for caution: This is a retrospective study. The findings which originate from a specific culture environment might not be applicable to all in vitro culture conditions.

Wider implications of the findings: Recommendations published by scientific societies (ASEBIR, ESHRE-Alpha) regarding the poor prognosis of fast-cleaving embryos on D3 must be reconsidered.

Trial registration number: Not Applicable.

O-230 Pregnancy and neonatal outcomes of embryos achieving blastulation on day 7: are they of clinical value?

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Study question: Are embryos achieving blastulation on day 7 of clinical value?

Summary answer: Day 7 blastocysts resulted in a 25.2% live birth rate and had similar risk of adverse neonatal outcomes with day 5 and day 6 blastocysts.

What is known already: Advantages of blastocyst transfer over cleavage embryo transfer have led to the shift towards the former in in vitro fertilization (IVF) practice. However, published data about the pregnancy outcomes of embryos with a delayed blastulation on day 7 are scarce and controversial. Moreover, there are few data available on the neonatal outcomes of day 7 embryos. As a result, the clinical value of day 7 embryos cannot be appropriately evaluated.

Study design, size, duration: This was a retrospective cohort study including two parts, first of which involved 2,908 women undergoing 2,908 frozen-thawed embryo transfer (FET) cycles of IVF/intracytoplasmic sperm injection (922, 1,752 and 234 women in day-5, day-6 and day-7 vitrified embryo transfer groups, respectively) from January 2006 to May 2015. In study part II, 1,518 liveborn infants derived from those women were analyzed.

Participants/materials, setting, methods: Women undergoing day-5, day-6 and day-7 vitrified embryo transfers were matched into three comparing cohorts (day 5 vs. day 6, day 5 vs. day 7, and day 6 vs. day 7) by baseline characteristics using propensity score matching. Neonatal outcomes were compared among liveborn infants derived from day-5, day-6 and day-7 vitrified embryo transfers. Logistic regressions were performed to investigate risk factors for implantation failure (among single blastocyst transfers) and congenital malformations (among liveborn infants).

Main results and the role of chance: The included women were matched into 921 pairs, 227 triplets and 227 tetrads of cycles in day 5 vs. day 6 (1:1), day 5 vs. day 7 (2:1), and day 6 vs. day 7 (3:1) cohorts, respectively. In study part I, day-7 vitrified embryo transfers had clinically important but significantly lower rates of intrauterine implantation, clinical pregnancy and live birth than both day-5 (23.9% vs. 49.9%, p<.001, 31.7% vs. 58.1%, p<.001, and 25.1% vs. 46.5%, p<.001, respectively) and day-6 (24.7% vs. 42.3%, p<.001, 33.0% vs. 53.2%, p<.001, and 25.6% vs. 41.4%, p<.001, respectively) vitrified embryo transfers. But no statistically significant difference was observed among the rates of biochemical pregnancy, ectopic pregnancy and miscarriage between the matched cohorts. Female age, year of treatment, developmental stage, morphological grade, and vitrification day of blastocysts transferred were significant factors affecting implantation rate. In study part II, no statistically significant difference was observed in the rates of low birth weight, congenital malformations and early neonatal death among the 616, 932 and 68 newborns in day-5, day-6 and day-7 vitrified embryo transfer groups, respectively. Multiples were 5.70 times more likely to experience congenital malformations compared with singletons (OR: 5.70, 95% CI: 1.42-22.92; P=.014).

Limitations, reasons for caution: This was a single center retrospective study, and most of the neonatal data were extracted from parent questionnaires. Besides, the amount of day-7 vitrified embryo transfer cycles was still limited.

Wider implications of the findings: Vitrification day of embryos transferred is an independent risk factor for implantation failure. The compromised implantation potential in growth-retarded blastocysts might be due to spindle abnormities. The negative effect of potential defects underlying delayed blastulation may be mostly realized during implantation, and affects less afterwards.

Trial registration number: Not applicable.

O-231 The impact of extremely long term human embryo cryopreservation: embryonic, obstetric and neonatal outcomes

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Study question: Is extremely long term slow freezing of human embryos do harm to their embryonic, obstetric and neonatal outcomes?

Summary answer: The embryonic, obstetric and neonatal outcomes following long term cryopreserved embryo transfer were encouraging, which met the common anticipation of embryo freezing.

What is known already: Due to the mature techniques of embryo cryopreservation and thawing, the cumulative pregnancy outcomes have greatly improved.During the ultra-low temperature storage state, the embryos are also affected by the cosmic background radiation and vibration. The theoretical cryopreservation span is infinite since the mimic experiment with an equal dose of 2000 years cosmic radiation on mice embryos gave us a positive result. While considering future utilization, there is still lacking of solid clinical evidence to draw conclusions about human embryos.The longest existing storage span of successful live birth after frozen embryos transfer is 12 years.

Study design, size, duration: This is a retrospective observational study of 128 embryos of 20 patients who seek to have a second child with the utilization of their long-term cryopreserved embryos (more than 12 years) from March 2012 to April 2017.

Participants/materials, setting, methods: Participants: Patients have received IVF/ICSI treatments between February 1999 and July 2004 and had surplus embryos cryopreserved for at least 12 years in our center.

Setting:University teaching hospital.

Methods:We have closely mesured the length of cryopreservation, survival rate of cryopreserved embryos, blastocyst formation rate, implantation rate, clinical pregnancy rate, abnormal pregnancy rate, live birth rate, perinatal complications and neonatal development.

Main results and the role of chance: A total of 128 embryos of 20 patients were observed. The embryo storage duration was 12.00-17.08 yrs, with a mean of 13.90 ± 1.73 yrs. Among them, 115 embryos were thawed to transfer, with a survival rate of 73.9%. Sixty embryos were further cultured which resulted in 20 blastocysts with a blastocyst formation rate of 33.3%. There were 34 embryos transferred in a total of 23 cycles, which resulted in 1 biochemical pregnancy, 1 first trimester miscarriage, 2 ectopic pregnancies, 3 singletons and 1 case of twins, with a clinical pregnancy rate of 30.4% and live birth rate of 21.7%. Two out of 4 patients who had live birth developed gestational diabete mellitus. One out of 5 live births was pre-term delivery. The physical and psychological development of all live births matched the standard curve in the first 6 months.

Table I

	Cleavage stage	Blastocyst stage	Total
Pregnancy Rate	25%(3/12)	45.5%(5/11)	34.8%(8/23)
Implantation Rate	19.0%(4/21)	30.8%(4/13)	23.5%(8/34)
Clinical Pregnancy Rate	25.0%(3/12)	36.4%(4/11)	30.4%(7/23)
Abnormal Pregnancy Rate	66.7%(2/3)	25%(1/4)	42.9%(3/7)
Live Birth Rate	16.7%(2/12)	27.3%(3/11)	21.7%(5/23)

Limitations, reasons for caution: Due to the limited sample size, we could only present the descriptive data. No further statistical analysis was used to detect the potential correlation factors concerning the clinical outcomes.

Wider implications of the findings: The reassuring results obtained in the study show that embryos after extremely long term cryopreservation could be of great value.

Trial registration number: not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 63: MALE LIFESTYLE AND REPRODUCTIVE HEALTH

Wednesday 4 July 2018 Room 211 + 212 10:00–11:45

O-232 Has sperm quality been declining for the past 20 years?

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[2]*Central South University, Institute of Reproductive and Stem Cell Engineering- Basic Medicine College, Changsha, China*

Study question: To evaluate studies of temporal trends in sperm quality in China.

Summary answer: Sperm concentrations, motility, and morphologies demonstrated substantial changes in China between 1996 and 2017, and Geographical differences exist in various regions of China.

What is known already: In recent years, numerous reports have suggested that semen quality in normal men has been declining. Nevertheless, all of these reviews showed significant changes in sperm quality only in Western countries. There have been few studies from non-Western countries, and those that do demonstrate no trends.

Study design, size, duration: We extracted summary statistics on semen volume, sperm concentration, total sperm count, normal morphology, progressive motile sperm (A + B) and motile sperm (A + B + C), inclusion and exclusion criteria, number of samples per man, and information regarding data completeness.All data could be derived from 186 studies. Ten of these were English language studies, and the other 176 studies were not in English.

Participants/materials, setting, methods: The study was excluded if study participants were selected based on infertile men, genital abnormalities, or other diseases. We also excluded studies limited to men with exposures that may affect fertility, such as occupational exposure, post-intervention, smoking, drinking, and other unhealthy lifestyles. We applied the Cochran–Armitage trend test for trend to evaluate change over time, and linear regression was performed to evaluate change over time of semen parameters.

Main results and the role of chance: The sperm concentration declined by 1.0% per year, and overall by 21.0% between 1996 and 2017 ($P < 0.001$). A similar trend was seen for progressive motile and motile sperm (slope per year = -0.56% and -0.57%; respectively, $P < 0.05$), Sperm with normal morphology declined by 2.4% per year ($P < 0.001$). Although total sperm count declined by 3.37 million per year, there was no statistical significance. Semen volume did not change significantly over the study period (slope per year = 0.005 ml; $P = 0.69$). The Sperm concentrations and progressive motility were greater in the area of Eastern and Central China than in Northeast China.

Limitations, reasons for caution: First, our findings may not be based on a community population, since the study group did not including infertile men and had a limited age range.Second, our study population lives only in some capital cities, and does not represent all cities and geographical areas of China.

Wider implications of the findings: Whether sperm quality is actually declining in some regions and across time periods remains unknown. The debate regarding the question continues, but these findings represent a serious reproductive health warning. The concerns raised regarding the reproductive health of men remain urgent.

Trial registration number: None.

O-233 Male reproductive health is a key determinant of unhealthy aging: results from a longitudinal cohort study

E. Ventimiglia[1], P. Capogrosso[1], W. Cazzaniga[1], L. Boeri[1], F. Pederzoli[1], M. Alfano[1], N. Frego[1], C. Abbate[1], F. Chierigo[1], F. Dehò[1], F. Gaboardi[1], V. Mirone[2], E. Montanari[3], F. Montorsi[1], A. Salonia[1]

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Study question: To inquire reproductive factors associated with health status decrease in infertile men.

Summary answer: 29% of infertile men had a decrease of the overall health status. Azoospermic men were those at highest risk of health status decrease.

What is known already: A severe male infertility factor has been associated with both lower health status and increased mortality in men from infertile couples. Little is known about the evolution of the health status in infertile men over time.

Study design, size, duration: Cohort study enrolling 645 infertile men evaluated at baseline between 2003 and 2010. Patients were followed-up yearly recording any worsening in their health status until 2017.

Participants/materials, setting, methods: Infertility was defined according to the WHO definition. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI). Cox regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of CCI score increase including as covariates age, BMI, CCI, azoospermia, FSH, and duration of infertility.

Main results and the role of chance: Mean patient age was 37 years (interquartile range, IQR 34-40), median BMI 25 (IQR 23-27), 30 (5%) men had a CCI≥1, and median duration of infertility was 24 months (IQR 13-30). Median follow-up was 9 years (IQR 7-11). 186 men (29%) saw an increase of their CCI score of at least 1 point. The most frequent reason for CCI upgrade was cancer, i.e. 43 solid tumors and 5 lymphomas. Compared to patients without a CCI increase, patients with a CCI increase had a higher proportion of CCI≥1 (9% vs. 3%, $p = 0.001$) and a higher proportion of azoospermia (32% vs 20%, $p = 0.001$) at baseline. Azoospermic men had an increased risk of developing comorbidities at multivariable analysis (HR 1.88, 95% CI 1.31–2.69; $p = 0.001$); along with a basal CCI≥1 (HR 2.61, 95% CI 1.57–4.36; $p < 0.001$), and with a longer duration of infertility (HR 1.02, 95% CI 1.01-1.03; $p = 0.008$) azoospermic men were at higher risk of developing comorbidities during follow-up at multivariate analysis, after accounting for the aforementioned confounders.

Limitations, reasons for caution: The CCI does not include some conditions (e.g. hypertension) that are important contemporary comorbid conditions.

Wider implications of the findings: Infertility is a proxy of decreased health status, therefore infertile men must be strictly follow-up to prevent and early detect the onset of potentially disabling comorbidities.

Trial registration number: not applicable.

O-234 Spermatozoa from patients with seminal alterations and advanced age show lower reproductive competence in egg donation cycles

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Study question: Do spermatozoa from men with normal or abnormal semen and different age perform differently in terms of fertilization and embryo development in egg donation cycles?

Summary answer: In presence of abnormal semen, older men spermatozoa have lower fertilization and embryo development potential. In presence of normal semen, male age has no effect.

What is known already: The delay in parenthood age observed in the developed world has brought increasing attention to age-related gametes quality, embryo development and reproductive outcomes. Whereas the effect of maternal age has been extensively studied, less is known about the consequences of advanced paternal age. Moreover, no paternal age cut-off value as been widely accepted. The inverse association between male age and semen quality has been reported in literature. However little was said about the combined effect of semen quality and paternal age. Oocyte donation cycles are an ideal model to study the paternal effect on reproductive outcomes.

Study design, size, duration: We retrospectively analyzed 276 egg donation cycles (OD) performed with 1713 vitrified/warmed oocytes and fresh ejaculated semen between October 2014 and December 2017. Our population was divided into 4 groups according to sperm quality (WHO, 2010) and male partner's age. Data are shown as avarage±SD and were analyzed with Chi square test or Student-t test.

Participants/materials, setting, methods: Groups-1 and -2 included men with normal semen and respectively <45 yeras old (N = 53 OD; 383 oocytes; male age 40.5 ± 3.03; donor age 25.7 ± 3.41) or ≥45 yo (N = 50 OD; 363 oocytes; male age 48.9 ± 3.31; donor age 25.8 ± 3.68). Groups-3 and -4 included men with abnormal semen and <45y.o. (N = 84 OD; 580 oocytes; male age 41.0 ± 2.85; donor age 26.4 ± 3.60) or ≥45y.o (N = 89 OD; 616 oocytes; male age 50.4 ± 5.25; donor age 25.0 ± 3.40). Semen samples distribution was similar in groups-3 and -4.

Main results and the role of chance: The number of surviving and injected MII, fertilized oocytes and total developed embryos either transferred or frozen (EC + ET) were, respectively, in group-1, 343, 247 and 202; in group-2, 319, 233 and 198; in group-3, 505, 359 and 307 and in group-4, 546, 359 and 286. Between groups-1 and -2, no difference was observed in the fertilization rate (FR 72.0% and 73.0% respectively) and in the rate of available embryos obtained (EC + ET) calculated either per injected oocytes (58.9% and 62.1% respectively in groups-1 and -2) or per fertilized oocytes (81.8% and 85.0%). Between groups-3 and -4, there was a significant difference in fertilization rate (FR 71.1% and 65.8%, respectively, $p < 0.05$) and in the rate of embryos obtained (EC + ET) calculated per number of injected oocytes (60.8% and 52.4%, Chi square $p < 0.005$) or per number of fertilized oocytes (85.5% and 79.7%, Chi square $p < 0.05$). After fresh embryo transfer, clinical pregnancy rates (CPR) in groups-3 and -4, were 58.8% (47/80) and 38.8% (31/80) ($p < 0.05$) and implantation rates (IR) were 38.4% (58/151) and 23.7%(34/144) ($p < 0.005$). In groups-1 and -2, CPR and IR were not significantly different: CPR were 49.0% (25/51) and 45.8% (22/48); IR were 41.3% (38/92) and 29.7% (25/84), respectively.

Limitations, reasons for caution: Since partners' ages are correlated, in order to compare clinical outcomes between groups and ascribe the outcomes decrease to the father, it should be assumed that maternal ageing has no effect. Additionally, the spermiogram used to categorizes semen is not a clear indicator of male infertility.

Wider implications of the findings: Spermatozoa from ejaculated sperm with abnormal parameters (concentration, motility and morphology) show lower fertilization potential and embryo development when retrieved from older patients as compared to younger patients or patients with normal semen. More oocytes could be necessary in OD cycles with advanced paternal age and poor sperm quality.

Trial registration number: Not applicable.

O-235 Paternal lifestyle factors and its relationship to semen quality and in vitro reproductive outcomes

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Study question: Do paternal lifestyle factors influence semen parameters and the outcomes of intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: Cigarette smoking and alcohol consumption appear to reduce semen quality, fertilization and blastocyst formation rates.

What is known already: Seminal parameters have been declining over time worldwide. This decline is likely multifactorial, and a variety of lifestyle factors has been proposed to influence spermatogenesis and reproductive function, either positively or negatively. It is now known that fertilisation and preimplantation embryo development are influenced by sperm-derived factors that may impact ICSI outcomes. Lifestyle factors can be adapted to enhance well-being and are ultimately under one's own control, therefore, adjusting for their influence may yield valuable information for counselling couples submitted to ICSI.

Study design, size, duration: This prospective cohort study included 965 male patients undergoing conventional semen analysis for infertility investigation from October/2015 to December/2016. The influences of alcohol consumption, cigarette smoking, environmental and occupation exposure, medications and physical activity on semen quality were evaluated by regression analyses. For the investigation of the influence of lifestyle factors on ICSI outcomes, only couples with isolated male infertility in which female partner was ≤ 36 y-old were included in the analysis.

Participants/materials, setting, methods: The study was performed in a private university-affiliated in vitro fertilization center. Prior to semen sample collection, participants were asked to complete a detailed non-validated questionnaire containing daily cigarette smoking amount; weekly frequency of alcohol consumption; weekly exercise frequency over the past 3 months; medications used in the past 3 months; exposure to any hazardous agents in workplace.

Main results and the role of chance: Cigarette smoking negatively influenced semen volume (r: -0.417, slope: 1.5700, $p = 0.047$), sperm count/mL (r: -7.363, slope: 52.2981, $p = 0.014$), total sperm count (r: -4.43, slope: 178.165, $p = 0.023$), TMSC (r: - 1.38, slope: 100.276, $p = 0.045$), and sperm DNA fragmentation (r: 0.014, slope: 9.76741, $p = 0.033$). Total sperm motility, progressive sperm motility and sperm morphology were not significantly influenced by smoking habit. Alcohol consumption negatively influenced sperm count/mL (r: -12.527, slope: 42.2553, $p = 0.040$) and SDF (r: 5.833, slope: 9.68068, $p = 0.002$). The other investigated semen parameters were not significantly influenced by alcohol consumption. There were no significant influences of other investigated paternal lifestyle factors on semen quality. Cigarette smoking and alcohol consumption negatively influenced fertilization rate (r: -1.349, slope: 21.9506, p-value: 0.039 and r: -3.617, slope: 20.1380, p-value: 0.041, respectively), and blastocyst formation on day 5 (r: -14.244, slope: 28.8513, p-value: 0.025 and r: -34.801, slope: 30.0446, p-value: 0.042, respectively). There were no significant influences of other investigated paternal lifestyle factors on ICSI outcomes.

Limitations, reasons for caution: Information on paternal lifestyle factors was collected through self-completed non-validated questionnaires and we only assessed lifestyle factors through a few questions, which may have introduced

under-reporting and underestimation of the true association. The female lifestyle were not taken into account, which may have biased the associations with ICSI outcomes.

Wider implications of the findings: Cigarette smoking and alcohol consumption appear to reduce semen quality, fertilization and blastocyst formation rates. Therefore, it would be wise to advise male partners to abstain from smoking and drinking alcohol to avoid decreased in vitro reproduction outcomes.

Trial registration number: None.

O-236 The association between glycemic control and semen parameters

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Study question: The current study aimed to assess the impact of diabetes mellitus (DM) and impaired fasting glucose (IFG) on seminal fluid and sperm parameters.

Summary answer: Interrupted glycemic control, mainly DM, deteriorates semen volume and sperm morphology, resulting in possible reduced fecundity.

What is known already: The prevalence of diabetes mellitus (DM) and impaired fasting glucose (IFG) is increasing worldwide. DM is known to deteriorate male sexual function, and to affect the endocrine function, a crucial role player in normal spermatogenesis. The impact of abnormal glycemic control on semen parameters and consequently on male fecundity is lacking.

Study design, size, duration: A retrospective case-control study including data from semen analyses of patients undergoing fertility evaluation was conducted. Samples were collected between the years 2009-2017 in a single tertiary hospital. DM and IFG patients were detected according to diagnoses, Lab criteria and prescribed medications documented in their medical records.

Participants/materials, setting, methods: Patients who were younger than 18 or older than 65 years, had previous chemotherapy treatment or radiotherapy, and patients with a diagnosed genetic abnormality (kleinfelter/kallman etc.) were excluded. Semen analyses were conducted and stratified according to the WHO criteria. Multiple regression models were constructed to define independent predictors of abnormal semen parameters. A p value of <0.05 was considered statistically significant.

Main results and the role of chance: A total of 7,118 semen examinations of 3,724 patients were included. Of them, 423 had a valid HbA1c result obtained within ± 90 days of the semen analysis. Comparing DM (n = 336) to IFG (n = 276) and healthy patients (n = 6679), the DM patients were older (41.0 ± 10.0, 39.9 ± 9.1, 32.2 ± 7.2, respectively, $p < 0.001$) with significantly higher smoking rates (47.6%, 36.6, 45.1, respectively, $p = 0.013$) and had higher baseline FSH levels (6.6 ± 4.2, 5.5 ± 3.4 and 5.4 ± 3.9, $p < 0.001$). The BMI of DM and IFG pateints was significantly increased compared to the healthy patients group (28.8 ± 5.4 and 29.4 ± 6.0 vs. 25.9 ± 6.7 respectively, $p < 0.001$). Evaluating the semen analysis, the DM group had higher rates of low semen volume compared to the IFG and non-DM groups (24.1%, 18.1% and 15.3% respectively, $p < 0.001$), asthenozoospermia (40.2%, 39.1% and 34.2% respectively, $p = 0.035$) and teratozoospermia (65.7%, 51.5% and 25.9%, respectively, $p < 0.001$). Using multiple logistic regression models, controlling for confounders including age, BMI and smoking status, DM but not IFG were found as independent risk factors for low seminal fluid volume (OR 1.57, CI 1.18-2.1, $p = 0.002$ vs. OR 0.96, CI 0.67-1.37, $p = 0.809$) and teratozoospermia (OR 1.68, CI 1.23-2.27, $p = 0.001$ vs. OR 0.96, CI 0.7-1.32, $p = 0.791$). Nonetheless, asthenospermia was comparable between the groups.

Limitations, reasons for caution: The study is a retrospective population based study with its inherited limitation.

Wider implications of the findings: Glycemic control is a well-known factor for health in general but as shown in the current study, may alter fecundity of the male partner. Glycemic control should be advocated as part of the fertility treatment consultation.

Trial registration number: not applicable.

O-237 The effects of Mycoplasma genitalium infection on semen: A retrospective study in infertile men of our hospital in China

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Study question: *Mycoplasma genitalium* is an emerging sexually transmitted infections (STIs) that is associated with genital tract infection. Does *Mycoplasma genitalium* infection influence semen quality?

Summary answer: *M.genitalium* incidence (2.49%) in infertile men was higher than in fertile men (0%).It significantly reduced sperm concentration, count, normal morphology, and increased sperm DNA fragmentation.

What is known already: It is estimated that approximately 15% of male infertility is related to infections in the genital tract. *M.genitalium* is associated with male nongonococcal urethritis(NGU), non-chlamydial non-gonococcal urethritis (NCNGU), but evidence supporting its role in male infertility remains inconclusive. And to date there are few large infertile men-based studies of China burden of disease.

Study design, size, duration: The study was performed as a retrospective survey of 30794 semen specimens from 30094 infertile men between October 2016 and December 2017.

Participants/materials, setting, methods: *M.genitalium* was detected using a novel simultaneous amplification testing that is RNA-detection based. Macrolide and tetracycline resistance screening was introduced using PCR and Sanger sequencing from September 2017. Semen analysis, papanicolaou staining, and sperm DNA fragmentation detection were performed on semen samples.

Main results and the role of chance: The incidence (749 of 30094,2.49%;95CI, 2.31%-2.66%) in infertile men was significantly higher than in fertile men (0 of 200, 0%).0.60%(159 of 26627;95CI, 0.50%-0.69%) of infertile couples had concordant infections. There were 123 cases of treatment failure (13.4%,123/918;95%CI, 11.2–15.6). Macrolide and tetracycline resistance were detected in 54 samples (98.18%;95%CI,94.54%-101.83%),27 samples (49.09%;95%CI,35.45%-62.73%) respectively.The most prevalent infection was in oligozoospermia (5.76%). After treatment, semen volume, sperm concentration, sperm count and total motile sperm count were significantly higher than those of before treatment($p = 0.046$, $p < 0.001$,$p = 0.011$,$p = 0.024$,respectively) in 56 patients. Normal morphology in *M. genitalium* positive group(n = 50) was significantly lower than that of negative group(n = 70) (2.98 ± 0.87% vs.3.71 ± 0.91%, $p < 0.001$). Sperm DNA fragmentation (DFI) in *M. genitalium* positive group(n = 50) was significantly higher than that of negative group(n = 70) (13.72 ± 7.61% vs.8.56 ± 4.81%, $p < 0.001$). Our study suggested a low prevalence of *M. Genitalium* in the infertile men in China. >98% of macrolide resistance is an likely explanation for high rates of azithromycin treatment failure (34.48%).*M. genitalium* infection significantly led to a decrease in semen concentration, sperm count, normal morphology, and increased sperm DNA fragmentation(SDF).

Limitations, reasons for caution: A limitation of the study is that we did not study the pregnancy rates before and after antibiotic treatment. Studying the pregnancy rates might better support our findings. And the positive group sizes followed in this study were relatively small.

Wider implications of the findings: Since incidence is very low, caution is required to establish the relationship to *M. genitalium* and male fertility. These findings indicate that *M. genitalium* infection might finally affect semen quality. And there is a need to provide resistance testings, reappraisal the recommended antimicrobial options in China.

Trial registration number: Not applicable.

O-238 Effect of nut consumption on semen quality and functionality in healthy males: a randomized controlled trial

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Study question: Can a chronic consumption of a mixture of nuts improve the semen quality parameters and the sperm functionality in healthy individuals?

Summary answer: Including nuts in a regular diet significantly improved the sperm count, vitality, motility, and morphology, partly explained by a reduction of the DNA fragmentation.

What is known already: Human semen quality has declined in industrialized nations where pollution, smoking, and trends toward a western-style diet are hypothesized as potential causes. Recently, some studies described that healthy diets rich in omega-3, antioxidants (e.g. vitamin C and E, selenium and zinc), carnitines and folate could improve semen quality. Because nuts are nutrient dense foods containing some of the above-mentioned nutrients, we hypothesize that, added to a western-style diet, would beneficially affect semen quality and functionality.

Study design, size, duration: The study was designed as a 14-week randomized, controlled, parallel two-group trial. 119 healthy male aged 18-35 were allocated to either following their usual western-style diet supplemented with 60 g/day of a mix of almonds, hazelnuts and walnuts or to follow with their usual western-style diet free of nuts.

Participants/materials, setting, methods: Sperm and blood samples were collected at baseline and after 14 weeks of intervention. Dietary information was recorded in four visits distributed along the trial. Conventional semen parameters (WHO, 2010) were determined as primary outcome. To elucidate at the molecular level the effects of nuts consumption, sperm DNA fragmentation (TUNEL assay), sperm ROS (chemiluminescence using Luminol), sperm chromosome stability (FISH for chromosomes X, Y and 18) and total sperm DNA methylation (ELISA assay) were measured.

Main results and the role of chance: A total of 98 participants completed the study. General characteristics of the study population (age, weight, height, BMI, waist circumference, and systolic and diastolic blood pressure) did not differ between interventions. No significant differences were observed in conventional blood biochemical parameters (fasting plasma glucose, total cholesterol, HDL, LDL and VLDL cholesterol, triglycerides, fasting plasma insulin, C-reactive protein and folate). In nuts group, we found a significant increased intake of total lipids, MUFA, PUFA, magnesium, vitamin E, omega-3, ALA, and omega-6. We found an improvement of sperm count (P-value = 0.0043), vitality (P-value = 0.0027), total motility (P-value = 0.0093), progressive motility (P-value = 0.0207), and morphology (P-value = 0.0073) in the nut group compared to the control. Participants in the nuts group shown a significant reduction of the sperm DNA fragmentation (SDF) (P-value = 0.0018) a parameter closely related with male infertility. Negative correlations between sperm vitality and SDF (rho=-0.2252; P-value = 0.0266), and between total spermatozoa and SDF (rho=-0.3170; P-value = 0.0015) were detected. No changes between interventions were found in ROS (P-value = 0.1996), sperm chromosome (X, Y and 18) anomalies (P-value disomies = 0.3336, P-value nullisomies = 0.9386, and P-value diploidies = 0.0674), and DNA methylation (P-value = 0.8652).

Limitations, reasons for caution: By design, a limitation of this trial is that it focuses on health, apparently fertile and with western-style diet subjects, and the results cannot be extrapolated to the general population.

Wider implications of the findings: Our findings support a beneficial role of chronic nut consumption in sperm quality and explore the molecular mechanism that could explain our results. Additional efforts to identify male-specific dietary recommendations that optimize sperm quality and fertility should be encouraged.

Trial registration number: The trial was registered in ISRCTN registry with identifier ISRCTN12857940 (DOI: 10.1186/ISRCTN12857940).

SELECTED ORAL COMMUNICATIONS
SESSION 64: NOVEL INSIGHTS FOR IVF PROTOCOLS

Wednesday 4 July 2018 Room 111 + 112 10:00–11:45

O-239 Medroxyprogesterone acetate versus ganirelix in oocyte donation cycles stimulated using GnRH antagonist protocol triggered with GnRH-agonist: a randomized controlled trial (RCT)

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Study question: To evaluate the non-inferiority of oral medroxiprogesterone acetate (MPA) compared to ganirelix on the number of mature oocytes (MII) retrieved at ovum pick-up (OPU) in oocyte donation cycles.

Summary answer: MPA is comparable to ganirelix in terms of MII retrieved at OPU. However, MPA use during ovarian stimulation might decrease pregnancy rates in oocytes' recipients.

What is known already: Oral treatment with MPA inhibits the pituitary LH surge during ovarian stimulation in infertile patient. Because of its negative effect on the endometrium, MPA suppression is combined with freeze-all. Published reports indicate that both the number of MII retrieved and pregnancy rates from these oocytes are comparable to short protocol of GnRh agonists during IVF cycles with freeze-all. MPA might allow for more comfortable and cost-effective ovarian stimulation in oocyte donation cycles.

Study design, size, duration: Randomized clinical trial (RCT), open-label, conducted from June 2016 to June 2017 to assess the non inferiority of MPA (10 mg/day) versus a GnRH antagonist protocol with ganirelix (0.25 mg/day) from day 7 in ovarian stimulation cycles triggered with triptoreline. Trigger criterion was >3 follicles of diameter>18 mm.

Participants/materials, setting, methods: 280 oocyte donors were selected, 232 randomized and 173 reached OPU: 86 under MPA and 87 under ganirelix. The main outcome was number of MII at OPU. Secondary outcomes were: embryological laboratory outcomes and reproductive outcomes in oocyte recipients. The study was powered to indentify a difference ≥3 MII, with an alpha risk of 0.05 and beta risk of 0.2. Differences were tested using a two-sided Student's t-test or a Pearson's Chi^2 test as applicable.

Main results and the role of chance: All participants were in their first cycle of oocyte donation. On average, donors were 24 ± 4.5 years old and with a BMI of 23 ± 2.9 kg/m^2. Days of stimulation were similar in both groups (11.2), as well as the total gonadotropin doses up to trigger (2162 IU in MPA and 2163 IU in ganirelix). The number of MII retrieved was no different: 15.1 ± 8.3 with MPA and 14.6 ± 7.0 with ganirelix (p = 0.69).

Both recipients' and embryo transfer (ET) characteristics were similar among groups. The average age of recipients was 42 ± 4.8 years and the BMI was 24 ± 4.4 kg/m2. The mean number of MII assigned to each recipients was 6.7 ± 1.1 in MPA and 6.6 ± 1.1 in ganirelix (p = 0.58). MII were fertilized with partner sperm in 84.9% cycles overall and fertilization rate was 75.7% with MPA vs. 73.9% with ganirelix (p = 0.50). Overall, there was 55.2% of double ET and 44.8% of single ET; 39.1% of ETs were performed in D5. Surprisingly, and in spite of similar recipients and cycle characteristics, reproductive outcomes were unexpectedly lower with MPA. Biochemical pregnancy rate was 44.3% vs. 56.2% (p = 0.042); clinical pregnancy rate 29.9% vs. 42.5% (p = 0.026); ongoing pregnancy rate 27.4% vs. 36.9%, (p = 0.085) and live birth rate 15.4% vs. 26.0%, (p = 0.036).

Limitations, reasons for caution: Although oocyte recipient and ET characteristics are similar among groups, this RCT has been designed under a hypothesis of no-inferiority in the number of MII obtained in two treatment groups, therefore the reproductive outcomes in recipients should be evaluated with caution.

Wider implications of the findings: Ovarian stimulation with MPA yield comparable results compared to ganirelix in oocyte donation cycles. The unexpected finding in reproductive outcomes should be further investigated.

Trial registration number: EudraCT Number 2015-004328-73

O-240 Triggering method dramatically alters the cumulus cell transcriptome in high and normal ovarian reserve patients

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Study question: Does triggering oocyte maturation with single vs. dual trigger (GnRHa & hCG) significantly alter the transcriptome of cumulus cells?

Summary answer: Triggering protocol significantly alters the cumulus cell transcriptome; hallmark pathways affected include cell-cycle regulation, ECM remodeling, and ion-channel activity, all important for folliculogenesis and ovulation.

What is known already: Cumulus cells (CCs) support the growing oocyte and their transcriptome could potentially be a valuable non-invasive biomarker for oocyte quality and competence. The CC transcriptome is influenced by the women's age, BMI, infertility diagnosis (eg. Endometriosis, PCOS, low ovarian reserve) and stimulation protocol. Previously, using microarray, when triggering with either GnRHa or hCG, it has been shown that GnRHa enhanced expression of genes involved in steroidogenesis. Differences in the CC transcriptome may potentially affect clinical outcomes, including the number of eggs retrieved, their quality, blastulation rates, aneuploidy, and pregnancy rates.

Study design, size, duration: This study is a part of a larger ongoing RCT comparing outcomes between single trigger (ST) vs. dual trigger (DT), in different patient populations. A total of 21 consented patients (12 with high ovarian reserve and 9 with normal ovarian reserve) undergoing IVF-ICSI at the CReATe Fertility Centre (Toronto, Canada) were enrolled in the study. Patients were matched according to demographic characteristics (age, BMI, etc.), ovarian reserve parameters (AMH and AFC) and stimulation protocol.

Participants/materials, setting, methods: CCs from expanded MII oocytes were trimmed, lysed, and RNA was extracted. Sequencing libraries were prepared (Clonetech SMARTer-v4 and Nextera-XT) and sequenced using a HiSeq 2500. Sequences were aligned to hg19, differential expression was conducted, and filtered by false discovery rate (FDR < 0.05) and fold change (logFC<-2 or logFC>2). Pathway analysis using GSEA was performed to elucidate biologically significant genes involved in hallmark processes in the ovary.

Main results and the role of chance: Samples clustered separately by triggering method in patients with both high and normal ovarian reserve. Eight hundred and forty-one genes were differentially expressed between treatment groups in the high responder cohort, and 2000 genes were differentially expressed in the normal responder cohort. In the high responders, dual trigger led to downregulation of ion channel regulators and G-protein receptor binding pathways when compared with single trigger and upregulation of mesenchymal development. In the normal responder cohort, dual trigger caused downregulation of immune related pathways (MHC complex, antigen processing, etc.) and extracellular matrix remodeling, when compared with single trigger and upregulation of cell division related pathways (mitotic spindle, chromosome segregation, etc.). Furthermore, genes previously associated with live birth were enriched in the normal responders treated with DT. Blastulation rate was significantly higher in normal responders triggered by DT when compared to ST (3.0 ± 0.37;1.67 ± 0.24 $p < 0.01$). Triggering method did not affect clinical outcomes among the high responders.

Limitations, reasons for caution: Our findings are limited by the sample size and biological variability. To adjust for this, "significant DE" between the two triggering cohorts was defined with stringent criteria, increasing confidence in the validity of our findings. No potential confounding variables were deemed significant (Wilcoxon test).

Wider implications of the findings: Triggering protocol alters the transcriptome profile of CCs in both high and normal responders and blastulation rate in normal responders. Hallmark pathways involved in cellular proliferation, oocyte maturation, and ovulation are affected. This study enhances our understanding of the effect triggering has on processes involved in oocyte growth and maturation.

Trial registration number: Not applicable.

O-241 Daytime variation in serum progesterone during the mid-luteal phase in women undergoing IVF treatment

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Study question: Does mid-luteal serum progesterone (P_4) exhibit clinically significant fluctuations during a 12-hour daytime period in women undergoing *in vitro* fertilization (IVF)?

Summary answer: Large P_4-fluctuations were seen in patients with median P_4>250 nmol/l whereas patients with P_4<60 nmol/l had clinically constant P_4-levels during daytime.

What is known already: Large fluctuations in serum-P_4 levels are present in the mid-luteal phase of the natural cycle, hampering the use of serum-P_4 measurements in the evaluation of corpus luteum function. No study so far examined the possible daytime serum-P_4 fluctuations during the mid-luteal phase in a group of women undergoing IVF treatment.

Study design, size, duration: Observational daytime study of ten women undergoing IVF treatment at the fertility clinic in Skive, Denmark. Patients were prospectively enrolled between December 2014 and December 2015.

Participants/materials, setting, methods: Seven days after oocyte pick-up, patients were admitted to the fertility clinic early in the morning. They had blood samples drawn every 60 minutes for the subsequent 12 hours, and during two of these hours every 15 minutes. Patients were selected to represent different stimulation protocols, different modes of triggering of final oocyte maturation as well as different regimens of luteal phase support. Serum samples were analyzed for progesterone, estradiol and LH.

Main results and the role of chance: There was a significant positive correlation between median P_4-levels and the magnitude of P_4-variations – women with median P_4 < 60 nmol/l had clinically stable P- levels throughout the day, while patients with median P_4 > 250 nmol/l exhibited periodic P_4-peaks of several hundred nmol/l. These endogenous P4-fluctuations were observed irrespective of the type of stimulation protocol or mode of triggering of final oocyte maturation and despite the fact that LH was under the detection limit at the time of measurement. Simultaneously large fluctuations were seen in s-estradiol. There was no obvious common daytime pattern in the secretion of P_4 in the ten women examined.

Limitations, reasons for caution: Although the sample size was small, which may limit the validity of general interpretations, this is the first study to explore mid-luteal P_4-fluctuations in different types of IVF cycles.

Wider implications of the findings: When monitoring mid-luteal P_4-levels during IVF-treatment, clinicians should be aware that large P_4-fluctuations may exist when serum-P_4 exceeds 250 nmol/l. In contrast, a measured serum P_4<60 nmol/l can be regarded as a true low value – thus, the accuracy of an individual mid-luteal serum-P_4 measurement depends on the median P_4-concentration.

Trial registration number: NCT02673034 (Clinicaltrials.gov).

O-242 Effects of high progesterone in in-vitro fertilization cycle on DNA methylation of endometrium receptivity markers during implantation window

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Study question: Does high progesterone level in in-vitro fertilization (IVF) cycle affect DNA methylation of endometrium receptivity markers during implantation window?

Summary answer: The endometrium receptivity markers, MUC1, CDH1 and CTNNB1, in patients with high progesterone were highly methylated, while PAEP was lessly methylated.

What is known already: High progesterone increased endometrium DNA methylation but how methylation affect the endometrium receptivity during implantation window is still unclear.

Study design, size, duration: A cohort study including 20 women with high progesterone level (HP) and 20 women with normal progesterone level (NP) on the day of human chorionic gonadotrophin (hCG) administration after controlled ovarian hyperstimulation in an IVF cycle.

Participants/materials, setting, methods: Endometrial tissues were collected on 7th day after hCG administration from women with NP or HP on hCG day. Immunohistochemical staining of DNA methyltransferases (DNMTs) and receptivity markers (MUC1, CDH1, CTNNB1 and PAEP) was performed. Methylation level of promotor region of receptivity markers was detected by Sequenom MassARRAY and/or bisulfite-sequencing PCR. RT-qPCR was used to quantify mRNA expression levels of the receptivity markers. Then methylation level and expression levels of the receptivity markers were correlated.

Main results and the role of chance: Except for progesterone level on hCG day, no significant demographic difference exists between HP and NP group. Expression of DNMT3B, but not DNMT1 and DNMT3A, in endometrium from HP group was significantly higher than that in NP group. DNA methylation levels of promotor region of MUC1, CDH1, CTNNB1 in HP group was significantly higher, while the PAEP in HP group was significantly lower than that in NP group. On the contrary, expression levels of MUC1, CDH1 and CTNNB1 in HP group were significantly lower, while PAEP in HP group was significantly higher than that in NP group, as detected in RT-qPCR and IHC staining. The DNA methylation level was negatively correlated with the mRNA expression level of the receptivity markers.

Limitations, reasons for caution: The sample size was not sufficiently large to permit in depth analysis of confounding variables.

Wider implications of the findings: Our data demonstrated high progesterone affects DNA methylation and gene expression of endometrium receptivity markers during implantation window, which may contribute the failure of IVF treatment.

Trial registration number: No.

O-243 Implantation enhancement by elective Cryopreservation of all viable Embryos (ICE): randomized controlled trial (RCT) comparing the two safest approaches in IVF/ICSI for high-responders

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Study question: How does the freeze-all strategy compare to GnRH-agonist triggering followed by intensified luteal support and fresh embryo transfer (ET) in terms of pregnancy/safety outcomes?

Summary answer: Pregnancy rates after either fresh ET or freeze-all did not vary significantly. However, ovarian hyperstimulation syndrome (OHSS) occurred more frequently after a fresh ET attempt.

What is known already: GnRH-agonist triggering has drastically reduced the incidence of OHSS. However, the best method to perform an ET after agonist triggering is still widely unknown. Two strategies (the freeze-all approach and a fresh ET attempt using a low-dose bolus of hCG followed by intensified luteal phase support) have shown to be safer alternatives to conventional hCG triggering with comparable pregnancy outcomes. However, these two strategies have never been compared head-to-head.

Study design, size, duration: The ICE trial was an RCT designed to recruit 212 women with an excessive response after ovarian stimulation (≥18 follicles measuring ≥11 mm) for IVF/ICSI in a GnRH antagonist suppressed cycle between 2014-2017. Our primary outcome was clinical pregnancy at 7 weeks after the first ET. Secondary outcomes included live-birth and the development of moderate-to-severe early-onset OHSS.

Participants/materials, setting, methods: Following GnRH-agonist triggering, women were randomized to perform either a fresh ET attempt (after a 1500 IU hCG bolus on the day of oocyte retrieval followed by oral estradiol 2 mg bid and vaginal progesterone 200 mg tid) or to cryopreserve all good-quality embryos followed by a frozen ET in a subsequent artificial cycle.

Main results and the role of chance: The required total of 106 patients in each study arm were recruited in the study, with 3 patients (1 in the fresh ET group and 2 in the freeze-all group) later withdrawing their consent to participate in the study. One patient in the freeze-all group became pregnant spontaneously prior to the first frozen ET. The study arms did not vary significantly in terms of the number of oocytes retrieved and embryos cryopreserved/transferred. The intention-to-treat clinical pregnancy rates after the first ET did not vary significantly among the fresh ET and freeze-all study arms (49% versus 54%, $p = 0.446$). However, moderate-to-severe early-onset OHSS occurred more frequently in the group who had a fresh embryo transfer (9% versus 0%, $p = 0.002$).

Limitations, reasons for caution: These preliminary results are limited by the fact that the accrual of live-births is still ongoing. Furthermore, given that the RCT was powered for the superiority of one of the arms, smaller differences among the groups in terms of clinical pregnancy rates may have failed to reach statistical significance.

Wider implications of the findings: This study offers the first comparative analysis of the two most commonly proposed alternatives for high-responding women performing IVF/ICSI in modern-day medicine. While pregnancy rates seemed comparable, a fresh ET may increase the risk of OHSS. Thus, serious consideration should be made before recommending a fresh ET as first-line treatment.

Trial registration number: ClinicalTrials.gov identifier NCT02148393.

O-244 Oral dydrogesterone is an efficacious and safe alternative to intravaginal progesterone gel for luteal phase support in IVF: a randomized, open-label, multicenter, phase III study

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Study question: Is oral dydrogesterone 30 mg daily non-inferior to 8% intravaginal micronized progesterone gel 90 mg daily for luteal phase support in in vitro fertilization (IVF)?

Summary answer: Oral dydrogesterone demonstrated non-inferiority to intravaginal micronized progesterone gel for the presence of fetal heart beats at 12 weeks of gestation (non-inferiority margin 10%).

What is known already: Progesterone therapy for luteal phase support is an accepted treatment practice for women undergoing IVF. The intravaginal route of micronized progesterone administration is routinely used in most clinics; however, it is associated with vaginal irritation, discharge and poor patient compliance. It has been demonstrated that oral dydrogesterone is non-inferior to micronized vaginal progesterone (MVP) capsules for luteal phase support in IVF with a similar safety profile (LOTUS I trial; Tournaye et al., Hum Reprod. 2017); In the present study, the efficacy, safety and tolerability of oral dydrogesterone versus intravaginal micronized progesterone gel (Crinone® 8%) were assessed.

Study design, size, duration: Lotus II was a randomized, open-label, multicenter, phase III clinical study, performed across 37 sites in 10 countries from August 2015 until May 2017. Subjects were premenopausal women (>18 to <42 years) with a documented history of infertility who were planning to undergo IVF. A centralized electronic system was used for randomization to oral dydrogesterone 10 mg tablets three times daily or 8% intravaginal micronized progesterone gel 90 mg (Crinone®) once daily.

Participants/materials, setting, methods: In total, 1034 subjects were randomized to receive either oral dydrogesterone (n = 520) or intravaginal micronized progesterone gel (n = 514). Luteal support was started on the day of oocyte retrieval and continued until 12 weeks of gestation if a positive pregnancy test was obtained 2 weeks after embryo transfer and no miscarriage occurred. The primary outcome measure was the presence of fetal heartbeats at 12 weeks of gestation as determined by transvaginal ultrasound.

Main results and the role of chance: In the oral dydrogesterone and intravaginal micronized progesterone gel groups, 494 and 489 subjects were included in the full analysis set (FAS), and 490 and 481 subjects were included in the per protocol set (PPS), respectively. Pregnancy rates at 12 weeks of gestation for the oral dydrogesterone and intravaginal micronized progesterone gel groups were 38.7% and 35.0% in the FAS (difference 3.7%; 95% CI: -2.3–9.7%), and 36.7% and 34.7% in the PPS (difference 2.0%; 95% CI: -4.0–8.0%), respectively. Thus, in both the FAS and PPS, non-inferiority of oral dydrogesterone versus intravaginal micronized progesterone gel was demonstrated as the lower-bound CI was greater than the non-inferiority margin of -10%. Live birth rates in the FAS were 34.4% and 32.5% (difference 1.9%; 95% CI -4.0–7.8%). The number of women with multiple newborns was comparable in the two treatment groups (20.6% versus 17.6% in the FAS for the oral dydrogesterone and intravaginal micronized progesterone groups, respectively). Oral dydrogesterone was well tolerated, and the incidence of treatment emergent adverse events for mothers and newborns was comparable to that of intravaginal micronized progesterone gel in this study.

Limitations, reasons for caution: The analysis of the results was powered to consider the ongoing pregnancy rate, but a primary objective of greater clinical interest may have been the live birth rate. Conclusions relating to the treatment effects in secondary variables, such as live birth rate, should therefore be made with caution.

Wider implications of the findings: The present study confirms the findings of the Lotus I trial (Tournaye et al., Hum Reprod. 2017), which already established oral dydrogesterone as a viable alternative to vaginally administered micronized progesterone due to its efficacy and comparable tolerability in the studies.

Trial registration number: NCT02491437 (clinicaltrials.gov)

O-245 FSHR type is a risk factor for developing ovarian hyper stimulation syndrome during in vitro fertilisation as observed in vivo and in vitro

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Study question: Is the follicle stimulating hormone genotype of importance when evaluating the risk of ovarian hyper stimulation syndrome (OHSS) in women undergoing in vitro fertilisation (IVF)?

Summary answer: Women with asparagine in the amino acid 680 of the FSHR had an OR of 11 (95% CI 1.4-82; p = 0.022) for OHSS compared to women with serine.

What is known already: A serious and potentially life-treathening complication to IVF-treatment is OHSS, generally affecting 0.5-5% women undergoing controlled ovarian hyperstimulation with exogenous FSH, and there are currently no effective methods to predict the response. It is known that carriers of NN in aminoacid 680 in the FSHR require lower doses of FSH during controlled ovarian stimulation compared to women with SS in order to ovulate. Some cases with mutations in the FSHR and spontaneous OHSS have been reported, but linking this condition with polymorphisms in the FSHR have shown contradicting results.

Study design, size, duration: Retrospective study. Women undergoing IVF at Reproductive Center, Malmö hospital, Malmö, Sweden, were enrolled and genetic variants examined and compared between those who developed OHSS and those who did not. FSHR variants were studied in monkey kidney cells treated with rFSH.

Participants/materials, setting, methods: In total n = 586 women were included in the study and genotyped regarding the polymorphism N680S in the FSHR gene. A risk allele analysis regarding the association between genotype and development of OHSS was done. To study receptor activity *in vitro*, COS-1 cells were transfected with FSHR variants and response was measured as cAMP amount produced when stimulated 1 h with 1, 10 or 90 IU follitropin alpha (Gonal-f, MerckSerono). The experiments were done in duplicates and repeated 3 times.

Main results and the role of chance: The proportion of women who developed OHSS was 6% (n = 36) in the total cohort. The N680S A > G polymorphism in the FSHR was associated with OHSS, ptrend = 0.004 and pallelic = 0.038. Carriers of an N allele had significantly higher OR for OHSS compared to carriers of S (OR 1.7, 95% CI 1.0 – 2.8, p = 0.04). A higher receptor activity in cells expressing N compared to the S variant was also evident at all concentrations of rFSH tested (p < 0.05 for all).

Limitations, reasons for caution: The number of total OHSS cases is small in this study but the genotype distribution for all participants is similar to earlier reports, therefore not indicating selection bias.

Wider implications of the findings: This study strengthens previous findings regarding higher hormonal sensitivity in carriers of N in the FSHR. Genotyping the FSHR before IVF or egg donation could be a help in identifying high risk individuals and precaution could be taken regarding hormonal dose to prevent OHSS in women with this particular genotype.

Trial registration number: not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 65: IMPROVING IVF OUTCOME

Wednesday 4 July 2018 Room 113 + 114 + 115 10:00–11:45

O-246 Vaginal microbiota profile at the time of embryo transfer does not affect pregnancy in IVF cycles with donated oocytes

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Study question: What is the relationship between the vaginal microbiota profile at the time of embryo transfer and pregnancy rate in women undergoing IVF with donated oocytes?

Summary answer: The vaginal microbiota profile at embryo transfer in women receiving donated oocytes does not seem to be related with the pregnancy rate in this population.

What is known already: Vaginal microbiota, i.e. the community of bacterial species colonizing the vaginal mucosae, plays an important role in the physiology of the female genital tract. The vaginal microbiota of healthy women is dominated by lactic-acid producing bacteria, mainly *Lactobacillus* spp. Dominance of anaerobic species such as *Gardnerella vaginalis* or *Atopobium vaginae* lead to a dysbiosis known as bacterial vaginosis (BV). In some reports, such condition has been correlated with negative results in IVF, while other authors did not find any relation. It is still unclear whether vaginal microbiota status influences IVF outcome or not.

Study design, size, duration: 121 Caucasian women receiving donated oocytes were prospectively included in March-September 2017. Vaginal swabs were taken immediately before transfer of single blastocysts. Genomic DNA was extracted with QIAamp DNA mini kit (Qiagen), and bacterial load as well as the expression of 8 bacterial species was determined by qPCR. The main outcome was βhCG levels in blood 14 days after single blastocyst transfer. Univariate analysis was performed to evaluate the association between microbiota profiles and pregnancy.

Participants/materials, setting, methods: Absolute number of bacterial genomes per sample was determined by qPCR for 4 species of lactobacilli (*L. crispatus, L. gasseri, L. jensenii, L. iners)* commonly found in vaginal microbiota and 4 species related with BV (*Gardnerella vaginalis, Atopobium vaginae, Mycoplasma hominis and Prevotella spp*). Vaginal microbiota profiles for each patient were characterized as *Lactobacillus* dominated (>50% *Lactobacillus* spp), BV associated (>50% BV associated bacteria), or mixed microbiota (dominance not clear) and correlated with biochemical pregnancy.

Main results and the role of chance: Although bacterial load was variable, most samples were dominated by a single species. Overall pregnancy rate was 55.4%. A majority of samples (81.8%) were dominated by Lactobacillus spp. In this group, the pregnancy rate was 52.5%, while in non-Lactobacillus dominated profiles the pregnancy rate was 68.2% ($p = 0.238$). We further analyzed profiles including *Lactobacillus* dominated, BV associated and mixed microbiota. The prevalence of BV associated microbiota was 14.9%, with pregnancy rate of 66.7%. Mixed microbiota samples (composed by a combination of Lactobacilli and BV associated species) had a very low prevalence (3.3%). Pregnancy rates among the three groups did not show significant differences ($p = 0.391$). In addition, we determined microbiota profiles according to the relative expression of bacterial species referred to human and bacterial load. This approach gave similar results, with no statistically significant differences between groups ($p = 0.345$). Our results suggest that a vaginal microbiota dominated by BV associated species is not associated to a pregnancy decrease when high quality embryos derived from donor oocytes are transferred to a receptive endometrium, in absence of ovarian stimulation. Vaginal microbiota profile does not seem to be determinant for oocyte recipient cycles success.

Limitations, reasons for caution: Sampling variations cannot be ruled out; bacterial and human genomic DNA proportion varied slightly between samples. Our three-way group analysis needs to be confirmed using a higher number of samples for BV and mixed microbiota categories.

Wider implications of the findings: Some authors report deleterious effects of BV on reproductive outcomes. Our data suggest that BV-like vaginal microbiota at time of embryo transfer does not directly affect endometrial receptivity and pregnancy rate.

Trial registration number: Not applicable

O-247 Low ovarian response to stimulation in IVF can be related with activation of the NLRP3 inflammasome

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Study question: Does the NLRP3 inflammasome play a role in low ovarian response to stimulation in IVF?

Summary answer: The activation of NLRP3 is higher in patients with low ovarian response compared to donors. Increased oxidative stress may induce increased expression of inflammatory NLRP3

What is known already: Oxidative stress can stimulate tissue inflammation and induce pyroptosis, a process strongly regulated by inflammasomes. The best-characterized inflammasome is NLRP3, whose activation allows the recruitment of an adaptive ASC protein, which interacts with procaspase-1 and activates caspase-1, the responsible for maturing pro-IL-1b and pro-IL-18 to obtain biologically active forms. Parallel to this process, and as a consequence of caspase activation, apoptosis can be induced too.

It has been also described that large number of miRNAs can bind to coding mRNAs for proteins in the constituents of inflammasome. miR-146, has been described as one of them.

Study design, size, duration: mRNA expression of NLRP3, ASC, and Caspase-1 together with the expression of miR-146 were measured in oocyte-granulose (CGs) complexes and in follicular liquid (FL) retrieved from 8 healthy fertile oocyte donors (<35 y.o. with at least a previous cycle with pregnancy) and compared with 8 low responders patients (<35 y.o. who had ≤ 5 oocytes retrieved after gonadotropic stimulation) whom took part in a prospective observational study realized from July 2016 to December 2017.

Participants/materials, setting, methods: Participants were stimulated with the same protocol (FSHr and triggering with GnRH analogues). GCs and FLs were collected and immediately fresh-frozen. The mRNA expression of NLRP3, ASC and Caspase-1 was measured by qRT-PCR. Exosomes were extracted by ultracentrifugation from FL and miR-146 was measured by qRT-PCR. Relative changes in gene expression were calculated using the 2-ΔΔCT method. No parametric tests were used to identify any significant difference between groups. Statistical significance was set at $p < 0.05$.

Main results and the role of chance: Inflammasome constitutes a high molecular weight signaling platform that leads to the activation of inflammatory caspases. In our study, the mRNA expression of NLRP3, the best-characterized inflammasome, was significantly increased in patients with low ovarian response compared to donors ($p = 0.045$). Also, the adaptive ASC protein, whom activation depends on NLRP3, was significantly increased in the patients group compared to the donors one ($p = 0.0005$). At the same, caspase-1 was found increased in the patients group compared to the donors one ($p < 0.0001$). The activation of caspase-1 can induce the maturing of IL-1b, which has been observed to be increased in patients with low ovarian response in previous studies from our group.

Although a large number of miRNAs can bind to coding mRNAs for proteins in the constituents of inflammasome, miR-146 seems especially important because in addition to control inflammasome, can also regulate inflammation formation by interaction with RNA encoding for adaptive molecules. In our study, the expression of miR-146 observed in the exosomes collected from FL was significantly reduced in patients with low ovarian response compared to donors ($p = 0.009$).

Limitations, reasons for caution: The main limitation of this study was the relatively small sample size. Further studies are needed to elucidate the molecular and cellular mechanisms underlying the relation between the inflammasomes and the oxidative stress observed in relation with infertility as well as their biological role in follicular function and activity.

Wider implications of the findings: To observe significant differences in the activation of inflammasome in patients with low ovarian response suggests that, in these patients, there is an inflammatory state that could diminish their antioxidant defenses. These findings could open up to new therapeutic strategies for the treatment of low ovarian reserve.

Trial registration number: This study received no external funding and there were no competing interests.

O-248 Do female age and body weight help to identify women who benefit from individualised gonadotropin dosing in IVF? A secondary analysis of the OPTIMIST study

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Study question: Can patient factors, including female age or body weight, serve as markers to guide individualised gonadotropin dosing in IVF treatment?

Summary answer: In predicted hyper responders, there might be a role for female age and body weight in identifying women that benefit from a particular dosing regimen.

What is known already: The OPTIMIST study was a multi-centre prospective cohort study with embedded randomised clinical trials in 1515 women starting IVF treatment in the Netherlands between 2011 and 2014. This study revealed that for women starting IVF treatment, no substantial differences exist between individualised dosing based on the antral follicle count (AFC) (100-450 IU/day) and standard dosing (150 IU/day) in terms of cumulative live birth rates (LBR). On basis of physiological concepts, female age and body weight have been suggested as patient characteristics that might have a differential impact on the effectiveness of individualised gonadotropin dosing.

Study design, size, duration: This was a secondary data-analysis of the OPTIMIST randomised clinical trials, in which women were categorised in subgroups of predicted response according to their AFC, and then randomly allocated to either a standard dose or an AFC based individualised gonadotropin dose. The primary outcome of the OPTIMIST study was cumulative live birth, of which the ongoing status was achieved within 18 months of follow-up.

Participants/materials, setting, methods: Logistic regression modelling with interaction terms was used to investigate whether female age or body weight interact with the treatment effect of individualised dosing. The primary outcome was LBR after the first complete treatment cycle, and secondary outcomes were cumulative LBR, safety (defined as any form of OHSS and/or preventive interventions) and observed response categories. All analysis were performed separately for predicted poor (AFC < 8), low-normal (AFC 8-10) and hyper responders (AFC > 15).

Main results and the role of chance: In the predicted hyper response group (n = 521), a significant interaction was found between female age and the effect of individualised dosing on first cycle LBR (p-value for interaction = 0.002). In women under 32 years of age, individualised dosing seemed to negatively affect first cycle LBR compared to standard dosing, whereas in women above 32 years of age this effect was not present.

Also, in the predicted hyper response group, a significant interaction was observed between body weight and the effect of individualised dosing on safety (p-value for interaction = 0.003). For women with a low body weight (from 50 to 55 kg) lowering the dose did not affect OHSS risks, whereas in women with higher body weights individualised dosing seemed to reduce OHSS risks.

In the predicted poor (n = 234) and predicted low-normal response group (n = 277), no significant interactions were found between female age or body weight and the effect of individualised dosing on the predefined outcomes.

Limitations, reasons for caution: Due to the relatively small sample size of the predicted poor and low-normal response group, the power to detect small associations in these groups was limited. Lack of blinding in the original trial might have introduced performance bias, such as selective cycle cancellation, which could have influenced our findings.

Wider implications of the findings: These preliminary findings suggest that for women with a predicted hyper response, female age and body weight could be possible markers for dose selection in IVF treatment. However, as these secondary analyses were exploratory and therefore hypothesis generating, confirmation of the validity in a new study is necessary.

Trial registration number: The OPTIMIST study was registered at the ICMJE-recognized Dutch Trial Registry (www.trialregister.nl). Registration number: NTR2657.

O-249 The vaginal microbiome as predictor for in vitro fertilization with or without intracytoplasmic sperm injection outcome; a prospective study

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Study question: Could we use the composition of the vaginal microbiome as predictor for IVF/IVF-ICSI outcome?

Summary answer: Microbiome profiling with IS-pro enables correct prediction of embryo implantation failure prior to an IVF/IVF-ICSI treatment with a high predictive accuracy.

What is known already: In the last decade, research has shown that besides the known predictive factors, as duration of subfertility, women's age, body mass index, also micro-organisms have a possible impact on assisted reproduction outcome. Micro-organisms together with their genetic information and the milieu in which they interact is called the microbiome. One common genus that habitats the vagina is the Lactobacillus. Studies have shown that the presence of Lactobacillus during the ART has a positive impact on the outcome. However, the potential role of using the microbiome as a predictor for outcome has not been investigated yet.

Study design, size, duration: In a prospective study, we included 301 women from 8 different IVF centres in the Netherlands. These women were included between June 2015 and March 2016. In total 192 of the 301 women underwent an embryo transfer (ET) and had a sample that could be analysed.

Participants/materials, setting, methods: Participants were women who were eligible for their first IVF/IVF-ICSI attempt. These women provided a vaginal swab prior to the IVF/IVF-ICSI treatment. Microbiome composition was analysed with IS-pro. IS-pro is an eubacterial technique based on the detection and categorisation of 16S-23S rRNA gene interspace regions with lengths that are specific for each microbial species. The predictive accuracy of the composition of the vaginal microbiome for IVF/IVF-ICSI outcome was validated for the fresh ET.

Main results and the role of chance: The vaginal microbiome of each participant was assigned to a community state type based on the dominant microbial species. A low percentage of Lactobacillus had a strong association with embryo implantation failure. With a combined prediction model based on a small number of microbial parameters, a subgroup of women (n = 34) who did not become pregnant could be identified with a low chance of pregnancy following ET (sensitivity 26%, specificity 97%). This failure to implant was correctly predicted in 32 out of 34 women based on the vaginal microbiome, resulting in a predictive accuracy of 94%.

Limitations, reasons for caution: We analysed the vaginal microbiome to determine the predictive accuracy of the microbiome for IVF/IVF-ICSI outcome after the fresh ET. Follow up is needed to investigate whether the predictive accuracy lasts for the consectutive frozen ETs that may be part of the IVF/IVF-ICSI treatment.

Wider implications of the findings: Knowledge of their microbiome profile may enable couples to make a more balanced decision on whether to continue with treatment or not. Hence, the unnecessary physical and emotional burden of an IVF/IVF-ICSI treatment can be avoided.

Trial registration number: Not applicable.

O-250 Endometrial injury in women with previous in vitro fertilization and intra-cytoplasmic sperm injection failure: A systematic review and meta-analysis

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Study question: Dose endometrial injury (EI) in the cycle preceding ovarian stimulation improve the pregnancy outcome in women with previous in vitro fertilization/intra cytoplasmic sperm injection failure?

Summary answer: In the meta-analysis of published data, EI does not improve the pregnancy outcome in women with previous unsuccessful IVF/ICSI.

What is known already: The pregnancy outcome of endometrial injury in patients with IVF/ICSI failure is still controversial.

Study design, size, duration: A systematic review and meta-analysis were performed. Main search terms were those related to endometrial injury for the pregnancy outcome in women with previous IVF/ICSI failure. The final computerized database search was performed on 11 october 2017. No language restriction was placed on the published articles. Two authors independently performed the literature screening, scrutinized articles of potential interest, selected relevant studies and extracted data.

Participants/materials, setting, methods: A systematic review was performed in PUBMED, EMBASE, CENTRAL, WEB of SCIENCE, and CLINICALTRIALS. Overall, 11 randomized clinical trials with a total of 1013 subjects in the intervention group and 1019 subjects in the control fulfilled the inclusion criteria. The dichotomous data for live birth rate, clinical pregnancy rate and miscarriage rate were expressed as risk ratios with 95% confidence intervals and were combined in a meta-analysis using the random-effects model or fix-effects model.

Main results and the role of chance: Funnel plot reveals no evidence of publication bias. Meta-analysis demonstrated that: live birth: RR = 1.19, 95% CI 0.91 to1.55; P = 0.21; 10 RCTs, 1917 women; I^2 = 53%; moderate-quality evidence. Clinical pregnancy rate: RR = 1.25, 95% CI 0.98 to1.60; P = 0.07; 10 RCTs, 1972 women; I^2 = 59%; moderate-quality evidence. Sensitivity analysis excluding the studies at high risk of other bias and subgroup analysis showed similar estimates. Miscarriage: RR = 1.04, 95% CI 0.73 to 1.49; P = 0.83; 634 women; I^2 = 0%; moderate-quality evidence.

Limitations, reasons for caution: Although the trials we included are RCTs, there still 5 studies at high risk of other bias, 5 studies at unclear risk of allocation concealment, and 2 study at unclear risk of reporting bias. Sensitivity analysis excluding the 5 studies at high risk of bias revealed similar estimates.

Wider implications of the findings: The advantage of our systematic review is that we only included RCTs, while the most trials included in the meta-analysis before are non-randomized trials. More future RCTs trials on the association between EI and pregnancy outcome of women with previous IVF/ICSI failure are needed.

Trial registration number: not applicable.

O-251 The use of non-donor egg IVF with embryo selection by morphology is efficient for patients aged 40-43

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Study question: Can we counsel 40-43 years old female (AMA) patients to consider non-donor IVF without selection by PGT-A as their first option for infertility treatment?

Summary answer: AMA patients have a lower than optimum but not negligible 21% probability of delivery per IVF cycle with their own-oocytes and morphological embryo selection.

What is known already: IVF clinics frequently recommend AMA patients to avoid using their own eggs for the purpose of maximizing success rates. Reimbursed IVF programs often reject treatment to older patients in order to optimize the efficient use of their resources. The high incidence of diagnosed aneuploid embryos in older patients lead to the spread of strategies of routinely selecting embryos by preimplantation genetic testing for aneuploidies (PGT-A) in those patients. In conclusion, AMA patients are often derived to private clinics, submitted to costly PGT-A treatments or advised to accept egg donation as preferred option in the first place.

Study design, size, duration: We analyzed the cumulative (fresh and frozen) delivery rate per oocyte retrieval of non-donor egg, no PGT-A IVF cycles. The study group was composed of 424 oocyte retrievals carried out between 2007 and 2016 to women aged 40-43 and which produced 1729 cleaved embryos. Patients aged 30 to 39 years treated during the same period were studied as control groups: 30-34, Group A; 35-37, Group B; 38-39, Group C.

Participants/materials, setting, methods: Retrospective analysis of the delivery rate of non-donor egg IVF cycles in our clinic. Cycles with total fertilization failure were excluded. Embryos were selected either for fresh transfer or cryopreservation at day 2-3 based on classical morphological evaluation. For the calculation of embryo efficiency, the remaining frozen embryos were discounted. Data from egg donation in the Spanish Registry 2015 were used for comparison of the delivery rate.

Main results and the role of chance: Cumulative delivery rate per oocyte retrieval was 21,2% for patients aged 40-43 (90/424). In Group A the rate was 53,3% (519/973); in Group B: 44,5% (394/885); in Group C: 36,3% (181/498). This means that the study group reached 40%, 48% and 58% of the delivery rates of control groups A, B and C respectively.

The percentages of transferred embryos that resulted in a live baby born were: 26,6% (648/2439) in Group A; 22,1% (470/2125) in Group B; 16,1% (200/1245) in Group C and 10,1% (102/1013) in the study group.

The percentages of cleaved embryos obtained per cycle that resulted in a live baby born (embryo efficiency of the cycles) were: 13,6% (648/4774) in Group A; 11,9% (470/3966) in Group B; 9,0% (200/2217) in Group C and 6,3% (102/1616) in the study group.

All differences were statistically significant.

The 7 Spanish centers with better results in egg donation according to the Spanish Registry achieved a 45,7% (501/1096) delivery rate per fresh embryo transfer in recipients aged > = 40. In our study group, delivery rate per fresh embryo transfer was 18,1% (70/386) this means 40% of the maximum obtained with egg donation in same age recipients.

Limitations, reasons for caution: Retrospective observational study. Lacking a direct comparison of results between egg donation and own oocytes, the adequacy of the observed delivery rate in the study group was established by comparison with other age groups and also by benchmarking against the higher performance Spanish centers with egg donation IVF.

Wider implications of the findings: A 21,2% delivery rate makes advisable for patients aged 40-43 to use their own oocytes without PGT-A selection as first option for IVF treatment as this means 40% of the delivery rate in our good prognosis patients (aged 30-35) or after fresh-embryo transfer with egg-donation in top-quality centers.

Trial registration number: Not applicable.

O-252 Autologous stem cell ovarian transplant (ASCOT), allowed 5 pregnancies in poor responders (PR) women

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Study question: Can bone marrow (BM)- autologous stem cell ovarian transplantation (ASCOT) improve the impaired ovarian reserve in poor responder (PR) patients?

Summary answer: ASCOT improved ovarian reserve biomarkers in 81.3% of aged PR patients. The number of MII-oocytes increased allowing five pregnancies although the low euploidy rate (16.1%).

What is known already: A number of gonadotropin stimulation protocols have been tested for the clinical management of PR women; nevertheless none

have been proved to be successful, due to the absence of stimulable antral follicles.

Competent oocytes can be retrieved, even in women with premature ovarian insufficiency (POI), after providing them with an appropriate growth-supporting ovarian niche following several approaches. BM transplants in patients with POI, due to chemotherapy or radiotherapy, recovers ovarian function as demonstrated by several spontaneous pregnancies. BM-derived stem cells (BMDSC) can reactivate to growth ovarian follicles through paracrine events occurring in the ovarian niche, as described for other organs.

Study design, size, duration: This is a prospective pilot study developed at La Fe University Hospital from 2014 – 2017 in which a total of 17 PR women, according to the ESHRE criteria, were involved. The patients were ≤ 40 [SH1] years old with 3–5 years of infertility and had[AP2] 24 previous IVF cycles as PR.

BMDSC were injected in one ovary by intra-arterial catheterization, therefore the contralateral ovary served as the control.

Participants/materials, setting, methods: G-CSF was subcutaneously administered to mobilize BMDSC into peripheral blood. Stem cells were isolated on the fifth day by aphaeresis. Cellular populations and growth factors related to follicular growth were also determined in aphaeresis.

Intra-arterial catheterization was performed into one utero-ovarian artery, delivering 50×10^6 non-selected CD133+ cells. After ASCOT, serum AMH and antral follicular count (AFC) were recorded for up to 5 months. COS started on the 2nd menstrual cycle day after AFC increase.

Main results and the role of chance: Ovarian reserve biomarkers enhanced in 81.3% patients. Several patients displayed the AFC increase in both the treated and control ovary. Presence of circulating FGF-2 and THSP-1 positively correlated with both AMH and AFC parameters.

ASCOT promoted an increase in total AFC, compared to baseline, from day 2 to 43 after ASCOT, specially on Day 15 ($p = 0.031$). Some patients also had an important AMH rise during the first 4 weeks.

In the previous 24 COS initiated by 15 PR, a total of 35 MII-oocytes and 24 embryos were obtained; 13 of them were considered as good quality embryos by morphological criteria and transferred but pregnancy was not achieved.

After ASCOT, 28 COS cycles were initiated but only 4 in optimal conditions. Ovum pick-up was performed in 85.7% initiated COS, embryos obtained in 67.8%, and cancellation rate reduced compared to previous attempts (14.2 vs. 29%. Fifty-one MII were retrieved and 35 embryos obtained after ICSI (fertilization rate 75.4%) but only 16.1% were euploid. Five pregnancies were achieved, 2 by ET (1miscarriage) and 3 were spontaneous (1ongoing).

When IVF outcomes were compared with previous cycles, the number of antral follicles ($p = 0.001$), punctured follicles ($p < 0.01$) and MII ($p < 0.01$) increased after ASCOT.

Limitations, reasons for caution: A pilot study, in which COS and ovum pick up was not performed in the most favorable conditions. There were confounding variables such as the fact that these women do have antral follicles, and a small increase might not be detected. The ideal population might be women with POI.

Wider implications of the findings: ASCOT in PR women may have few applications, but this study has shown a potential use in POI women. The finding of a correlation between certain growth factors and outcomes suggests potential development of less invasive procedures in the future.

Trial registration number: NCT02240342

SELECTED ORAL COMMUNICATIONS

SESSION 66: RIF AND RM: NEW DIAGNOSTIC APPROACHES

Wednesday 4 July 2018 Room 117 10:00–11:45

O-253 Relationship between uterine natural killer (uNK) cells percentage and chronic endometritis in women with recurrent miscarriage

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Study question: Is there any significant correlation between uterine natural killer (uNK) cells count and chronic endometritis in women with recurrent miscarriage?

Summary answer: A significantly ($p < 0.01$) increased prevalence of chronic endometritis was observed in high uNK cells group, compared to normal and low uNK cells groups.

What is known already: Our previous work showed an altered number of uNK cells in peri-implantation endometrium of women with recurrent miscarriage. In addition, chronic endometritis was reported to be associated with reproductive failure. However, there has not been any study examining the relationship between uNK cells count and chronic endometritis in recurrent miscarriage.

Study design, size, duration: It was a retrospective study carried out in a university center. A total of 102 women with unexplained recurrent miscarriage were included in this study.

Participants/materials, setting, methods: Endometrial biopsies were obtained precisely 7 days after luteinization hormone surge in natural cycles. Endometrial sections were immunostained for CD56 for uNK cells and CD138 for plasma cells, respectively. UNK cell counting was performed by a standardized protocol (Lash *et al.*, 2016) and results were expressed as percentage of positive uNK cell/ total stromal cells. Chronic endometritis was diagnosed as the presence > 5.15 plasma cells/0.1mm^2 based on our recent work (Liu *et al.*, 2018).

Main results and the role of chance: Based on our published reference range for uNK cells percentage, 21 women were classified into high uNK group, while 62 women in normal uNK group and 19 women in low uNK group. The prevalence of chronic endometritis was 8/21(38.1%), 5/62(8.1%), 1/19(5.3%) in high, normal and low uNK cells percentage group, respectively. A significantly ($p < 0.01$) increased prevalence of chronic endometritis was observed in high uNK cells group, when compared with normal and low uNK cells groups.

Limitations, reasons for caution: The role of other immune cells in the peri-implantation endometrium has yet to be confirmed.

Wider implications of the findings: Observations on the relationship between uNK cells and chronic endometritis may improve the understanding of mechanisms in endometrial immune cells leading to endometrial dysfunction in recurrent miscarriage.

Trial registration number: N/A.

Funding by the Health and Medical Research Fund in Hong Kong (No. 04152786) and Hong Kong Obstetrical and Gynaecological Trust Fund in 2017.

O-254 Anti-Müllerian Hormone does not predict chance of live birth in 565 women with recurrent pregnancy loss

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Study question: Do low levels of anti-Müllerian hormone (AMH) predict live birth rate in women with unexplained recurrent pregnancy loss (RPL)?

Summary answer: In women with unexplained RPL, low AMH levels did not reduce the chance of live birth in the first pregnancy achieved after referral.

What is known already: Recurrent pregnancy loss affects 2% of all women and in most of these women no explanation can be identified. In women with RPL compared to infertile women, previous studies have shown a similar incidence of diminished ovarian reserve as assessed by FSH and estradiol levels. AMH is cycle day independent and is currently debated as not only a marker of egg reserve quantity, but also quality. With increasing age, AMH levels decreases, while the pregnancy loss rate increases. Little is known about AMH-levels in women with recurrent pregnancy loss.

Study design, size, duration: Cohort study of 565 women with RPL followed at the tertiary referral center, RPL Unit, Copenhagen University Hospital (Rigshospitalet), from 2011-2014.

Participants/materials, setting, methods: All women attending the RPL unit from 2011-2014. RPL≥3 consecutive losses. AMH drawn more than one year from referral to the RPL unit were excluded. AMH-levels were measured with ELISA Generation I (Beckman Coulter, Marseilles, France). According to our clinical cut-off, low AMH was defined as ≤5 pmol/L. Regression analyses were adjusted for age, previous number of losses, and smoking status.

Main results and the role of chance: A total of 565 women with RPL had attended the unit, of which 478 (84.6%) had a measurement of AMH-levels. Median AMH-levels were 17.5 (interquartile range (IQR), 9.0-30) and AMH levels were below 5 pmol/L in 55 (11.5%) women. In total 355 (74.3%) women had a first pregnancy after referral. These women did not differ from the women who did not conceive in terms of median AMH-level (19 pmol/L vs. 16 pmol/L, Mann-Whitney U $p = 0.44$) or proportion of women with low AMH (10.7% vs 13.8%, $p = 0.41$). In women with unexplained RPL, 301 had singleton intrauterine pregnancies with a last menstrual period of more than nine months before data extraction. In the 28 women with a low AMH level, live birth rate was 50% compared to 60.8% in women with an AMH level above 5 pmol/L ($p = 0.31$). Having a low AMH level was not a predictor for live birth in adjusted analyses (adjusted odds ratio (aOR) 0.7 95% confidence interval (CI);0.2-1.8, $p = 0.39$). Median AMH level was 20 pmol/L in women who gave live birth compared to 19 pmol/L in those who did not (Mann-Whitney U, $p = 0.89$). AMH levels did not predict live birth (aOR 1.0 95%CI:0.88-1.12, $p = 0.95$).

Limitations, reasons for caution: Although this study to our knowledge is the largest to date in women with RPL, there may be a lack of power in calculations of association between live birth rate and AMH levels.

Wider implications of the findings: AMH levels can serve as a clinical guidance to the chance of achieving pregnancy. However, women with unexplained recurrent pregnancy loss can be reassured that the chance of live birth in case of an achieved pregnancy is similar to that of women with normal AMH levels.

Trial registration number: Not applicable.

O-255 The oestro-androgen dehydroepiandrosterone sulphate (DHEAS) may be a critical intracrine regulator of implantation and early pregnancy in Eumenorrhic non-PCOS women undergoing

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Study question: To evaluate the significance of endogenous levels of circulating DHEAS during implantation in predicting implantation failure/early miscarriage in eumenorrhic non-PCOS women undergoing IVF.

Summary answer: Maintenance of a steady/balanced rise in serum DHEAS levels is an early indicator of successful implantation and predicts implantation-failure/early miscarriage in eumenorrhic women undergoing IVF.

What is known already: Decidualization of endometrial-stroma is necessary for successful implantation; which if improper, may lead to implantation-failure/early pregnancy loss. Elevated levels of DHEA are a causative factor for higher incidences of implantation-failure among PCOS women by inhibiting endometrial-stromal cell differentiation via prevention of glucose-flux through the pentose-phosphate-pathway. Contrarily, low DHEA women with diminished ovarian-reserve, when supplemented with DHEA show significant reduction in early miscarriage rates. Sulphonated-DHEA (DHEAS) is more stable than DHEA, a major oestro-androgen and most abundant circulating steroid precursor for estrogen production in humans. However, no study has yet evaluated significance of innate DHEAS during implantation in eumenorrhic non-PCOS women.

Study design, size, duration: Prospective pilot study of n = 85 non-PCOS eumenorrhic, normo-responder women undergoing conventional antagonist stimulation protocol IVF from March 2016 to March 2017. Poor-responder/premature ovarian failure and hyper-androgenic PCOS women (defined as per Rotterdam consensus) were excluded from the study. All cycles involved day5 fresh, elective single blastocyst transfer (eSBT). Luteal phase support was provided to all women. Serum DHEAS levels in baseline as well as day7 and day14 post-eSBT were measured by radio-immuno-assay using diagnostic kits.

Participants/materials, setting, methods: Serum estradiol, β-hCG and progesterone levels were also measured on day7/day14 post-eSBT. β-hCG measurement on d7 of eSBT was considered early indicator of pregnancy. Implantation rate and live birth rate were main outcome measures. Cycles were classified on the basis of live birth (LB, n = 33), biochemical pregnancy (BCP, n = 3), early miscarriage (EM, n = 2) and no implantation (NI, n = 47). Statistical analysis involved Student's-t test, One-Way ANOVA, ROC analysis, as applicable, using Graph-pad Prism VI software.

Main results and the role of chance: Overall rates of LB, BCP, EM and NI were found to be 38.82%, 3.53%, 2.35% and 55.29% respectively. DHEAS levels depicted a steady rise from baseline to d7 to d14 post-eSBT in women with LB (165 ± 11.01 vs. 390.3 ± 40.26 vs. 737.9 ± 59.47 respectively). Although a rising trend was also observed in women with EM, the rise from baseline to d7 post-eSBT was rather steep (68.5 ± 3.5 vs. 247.5 ± 7.5 vs. 255 ± 10). However, the rising pattern was disrupted in BCP cycles where the levels dropped from baseline to d7 and then increased on d14 post-eSBT (211.7 ± 31.67 vs. 120 ± 5.8 vs. 258.3 ± 10.14); and in NI cycles where a sharp rise on d7 was followed by a decrease in levels on d14 post-eSBT (201.1 ± 13.95 vs. 1321 ± 134 vs. 791.7 ± 103.4). A significant difference in the ratio of d7: baseline DHEAS levels was observed in LB vs. BCP vs. EM vs. NI cycles (2.59 vs. 0.61 vs. 3.61 vs. 9.65; $p = 0.0005$). Similarly, the ratio of d14:d7 DHEAS levels differed significantly in LB vs. BCP vs. EM vs. NI cycles (2.23 vs. 2.16 vs. 1.03 vs. 0.8; $p < 0.0001$). Thus, a twofold rise in DHEAS levels from baseline to d7 and d7 to d14 is critical for successful implantation leading to a live-birth.

Limitations, reasons for caution: This prospective pilot study significantly implicates endogenously circulating levels of DHEAS in the implantation process and as a differentiating factor for various categories of implantation. Although our study has a power of 80%, more systematic studies/RCTs are required to establish DHEAS as an efficient and robust predictor of implantation.

Wider implications of the findings: Although the role of various androgens during pregnancy have been studied, their significance in the implantation process have received less scrutiny. Previous contradictory results with DHEAS have been largely due to improper selection of patient populations. This study underlines the significance of optimum levels of endogenous DHEAS for successful implantation.

Trial registration number: Not Applicable.

O-256 Endometrial proteomic signature in recurrent implantation failure

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Study question: How endometrial proteomic profile in patients with recurrent implantation failure (RIF) differ from fertile controls?

Summary answer: 6 pathways based on proteomics profile are found to be different in RIF patients compared to fertile patients.

What is known already: Endometrial changes are important for the development of adequate endometrial receptivity and subsequent embryo

implantation. Recently, mitochondria have been found to play a vital role in fertilization and implantation. It provides ATP to the oocyte for embryo development, it affects endometrial receptivity by altering the energy metabolism in the endometrium. Some studies about Coenzyme Q10 supplement in infertility and total parenteral nutrition in patients with RIF patients showing good results may be based on mitochondria's effect.

Study design, size, duration: For this prospective cohort study, RIF was defined as failure of pregnancy in ≥ 3 consecutive IVF cycles with ≥1 transfer(s) of good quality embryo in each cycle. Women with ≥1 live birth(s) and no other comorbidities were included in the fertile control group. 24 patients were included in each groups, all 48 patients were recruited during August 2014-2015.

Participants/materials, setting, methods: From 48 patients recruited in Istanbul University School of Medicine, endometrial samples were taken during mid-secretory phase, stored in -80°C following snap freezing with liquid nitrogen. Once all tissues were obtained, a LC-MSE-based analyzes of peptides by mass spectrometry were performed. Protein expression differences were calculated with Gene Ontology enrichment analysis and DAVID functional annotation bioinformatics microarray analysis.

Main results and the role of chance: From 274 proteins found in different expression in patients with RIF compared to fertile patients, 6 representative pathways were outlined: pathways in carbon metabolism (p value = 1.63E-06), pathways in citrate cycle (TCA cycle) (p value = 5,07E-05), spliceosome metabolism (p value = 2,28E-04), protein processing in endoplasmic reticulum (p values = 3,08E-05), biosynthesis of antibiotics pathway (p values = 6,649E-06) and ribosome pathway (p value = 2,47E-06).

Limitations, reasons for caution: Endometrial samples were not taken on the same exact menstrual day for all patients. We chose to be between day 19-21.

Wider implications of the findings: Our results revealed a disturbance in energy metabolism in endometria of patients with RIF. In conjunction with previous reports, the failure of pregnancy despite good embryos and endometrium may lie in a lack of appropriate energy that would have supported an implanting blastocyst and subsequent progression to a viable pregnancy.

Trial registration number: This research received funding from the Scientific Research Projects Coordination Unit of Istanbul University (grant no. 47795).

O-257 CRISPR/Cas9 genome editing to reveal the effect of implantation related genes on specific cell types which contribute to implantation process in human and mouse models

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Study question: Can we manage to reveal the effects of silencing a single implantation related gene on different cell populations functionally related to implantation process?

Summary answer: Silencing of one gene in isolated cell populations can be achieved and the effect can be evaluated successfully in mouse and human 3D implantation models.

What is known already: Despite all developments in ART, implantation failure is considerably high due to unexplained molecular, genetic and morphological interactions affecting the crosstalk between endometrium and embryo. Complicated nature of this phenomenon still remains obscure as a result of experimental methods that never can be performed effectively to reveal the biological functions specific for each cell type which participates in implantation process. To interfere with cellular functions, CRISPR/Cas9 genome editing technology has been shown to be applicable in a wide spectrum of cells, including cells of the developing embryo. Same technology can be used to detect and manipulate molecular interactions of implantation.

Study design, size, duration: Being expressed by both blastocyst and endometrial cells, LIF was chosen to be silenced separately via transfection in mouse blastocyst, human cell-line derived trophoblastic spheroids and stromal and epithelial cells of human and mouse endometrium. Three-dimensional culture was maintained on extracellular matrix(ECM) based gel systems for 48 hours and implantation parameters related to adherence, invasion and function were evaluated quantitatively by student's t test in wild and mutant groups(n = 24/each group, a total of 192 samples).

Participants/materials, setting, methods: Human and mouse epithelial and stromal cells were isolated separately. The experimental groups (n = 24 samples/group; 2 blastocysts-spheroids/sample) for human and mouse cells separately were: i.mutant(gene-edited) epithelia+wild type(wt) stroma +wt blastocyst/spheroid, ii.wt epithelia+mutant stroma+ wt blastocyst/spheroid,iii. wt epithelia+wt stroma+mutant blastocyst/spheroid,iv. wt epithelia+wt stroma+wt blastocyst/spheroid. Three-dimensional culture was engaging epithelial cells onto and stromal cells into the ECM based gel-system. After 48 hours, implantation parameters (e-cadherin,MMP9, progesterone receptor,invasion depth) were evaluated by qRTPCR(triple-repeats), WB and IF-multiphoton microscopy.

Main results and the role of chance:

i. Both mouse and human control groups composed of wild type cells showed decrease in e-cadherin, increase in MMP9 and PR after 48 hours of culture, where results with mouse blastocysts were more significant ($p < 0.05$). Blastocysts showed a better penetration. Survival and implantation parameters were in acceptable ranges in both control groups, implicating that 3D culture was efficient enough for this experimental model.

ii. LIF was successfully knocked out in all mutant cells as shown by its absence by qRTPCR ($p < 0.01$). In blastocysts trophoblastic cells were silenced more effectively than ICM, as expected due to transfection.

iii. When mutant groups were compared, implantation parameters were significantly affected most in groups with blastocysts/spheroids ($p < 0.05$) in both human and mouse; so LIF can be accepted as primarily working through the embryonic site rather than the endometrial site.

iv. Separate silencing of LIF in epithelial and stromal cells of mouse resulted in differences regarding implantation parameters, as a deeper decrease of expression in MMP9 and PR was observed with silenced epithelial cells. Although significance was less ($p > 0.05$), still it shows the behavioral differences between two cell types. In human however, silencing of stromal cells had a more dramatic effect ($p > 0.05$) on implantation parameters.

Limitations, reasons for caution:

(i) Use of in vitro 3D culture, since a culture totally mimicking in vivo conditions wasn't maintained.
(ii) Use of human spheroids instead of human blastocysts, since spheroids are of trophoblastic cells only, ICM effect was not tested in human groups, while it was taken into account for mouse model.

Wider implications of the findings:

(i) CRISPR/Cas9 genome editing technology can be utilized for all known implantation related genes to reveal the cell specific effects of these genes during implantation process.
(ii) ECM gel based three-dimensional endometrial co-cultures created for human and mouse implantation models are successful enough to run implantation experiments.

Trial registration number: not.

O-258 T helper 1 cell level is associated with reproductive outcome of women with repeated implantation failures who received Tacrolimus treatment

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Study question: Is the peripheral blood T helper (Th) 1 cytokine producing cell level associated with the ongoing pregnancy rate in the repeated implantation failure (RIF) patients treated with tacrolimus?

Summary answer: Women with RIF and increased Th1 cell levels had lower ongoing pregnancy rate even though they were treated with low dose tacrolimus.

What is known already: The peripheral blood Th1 and Th2 cells determine immune inflammatory responses at the maternal fetal interface. Previously, we reported that women with RIF had elevated Th1/Th2 cell ratio. When they were treated with tacrolimus, immunosuppressive agent, pregnancy rate was significantly improved (*Am J Reprod Immunol 2015*).

Study design, size, duration: This is a prospective cohort study of 140 RIF women with elevated Th1/Th2 ($CD4^+IFN\text{-}\gamma^+$/ $CD4^+IL\text{-}4^+$) cell ratios (≥10.3) from November 2011 to July 2017. All received tacrolimus 1-3 mg per day based in Th1/Th2 cell ratios. Study populations were grouped according to the Th1 cell levels; Th1 cell levels <22.8 (Low group), Th1 cell levels 22.8≤Th1<28.8 (Mid group), and Th1 cell levels 28.8≤Th1 (High group).

Participants/materials, setting, methods: All patients received tacrolimus, starting 2 days before the ET to the day of pregnancy test. The daily dose of tacrolimus was depended on the Th1/Th2 ratio as follows; patients with a mild (≥10.3 and <13.0), moderate (>13.0 and <15.8), and high levels of Th1/Th2 ratio (>15.8) were treated with 1, 2, and 3 mg of tacrolimus, respectively. The clinical and ongoing pregnancy rates and miscarriage rates were compared among three groups.

Main results and the role of chance: Out of 140 patients, 65 women got pregnant (pregnancy rate, 46.4%). In the pregnant group, 59 women eventually had a clinical pregnancy, which was documented by the detection of gestational sac using ultrasound (clinical pregnancy rate, 42.1%). Forty-two patients delivered healthy babies and 8 are ongoing pregnancy (Ongoing pregnancy/delivery rate, 35.1%).

The Th1/Th2 cell ratio for the high Th1 group was 18.1 ± 9.0, and this was significantly higher than that in the Low Th1 group (14.5 ± 4.4; $p < 0.05$). The pregnancy rates for the Low, Mid and High Th1 groups were 55.3, 46.8, and 37.0 %, respectively, and there were no significant differences among the three groups. The clinical pregnancy rates for the Low, Mid and High Th1 groups were 48.9%, 44.7%, and 32.6% respectively. The clinical pregnancy rate of the High Th1 group was lower than those of the Low and Mid Th1 groups, however the difference was not significant. The ongoing pregnancy rate (≥12 weeks gestation) of the Low Th1 group was 46.8%, which was significantly higher than that of the High Th1 group (23.9%, $p < 0.05$) but not different from that of the Mid Th1 group (36.2%).

Limitations, reasons for caution: The endometrial changes or peripheral immune responses after tacrolimus treatment were not evaluated thoroughly. Further study is needed for tacrolimus effect on both systematic immune responses and local changes on the uterine endometrium.

Wider implications of the findings: Adjustment of tacrolimus dosing based on Th1 cell level may improve clinical efficacy of the treatment. The study is ongoing, which increases the tacrolimus dose (additional 1 mg) for the patients who have high Th1 cell level.

Trial registration number: N/A.

O-259 Couples' perspectives on their need for treatment, support and follow-up after recurrent pregnancy loss

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Study question: What do couples attending a recurrent pregnancy loss (RPL) clinic believe they need in terms of treatment, support and follow-up after RPL?

Summary answer: Men and women wish for treatment, support and follow-up that includes attention to the psychological impact of RPL and includes both members of the couple.

What is known already: Previous research has highlighted women's dissatisfaction with medical care provided post-pregnancy loss (PL) and their desire for medical professionals to have increased awareness about PL and recognition of the psychological impact of PL. Less is known about the needs of the male partner, the needs of those experiencing RPL (three or more PL) and if needs change depending on whether it is the first or one of several losses.

Study design, size, duration: This was a qualitative study. Over a two-month period in 2017-2018, 13 couples who were referred to the RPL program in Rigshospitalet, Copenhagen, Denmark were interviewed. Interviews were held at the Rigshospitalet and ranged between 81 and 109 minutes (average 91 minutes).

Participants/materials, setting, methods: Inclusion criteria included: heterosexual couples with at least three consecutive PL under 12 weeks gestation with no children/one child who were willing to be interviewed in English. Couples were interviewed together in a semi-structured format. Data was analyzed using thematic analysis.

Thirty invitations were sent to couples who met the inclusion criteria and indicated an interest in participating and 15 couples contacted the interviewer to schedule an interview. Due to cancellations, 13 interviews were held.

Main results and the role of chance: On average participants had experienced three PL (range 3-6) and had been on the waiting list for the RPL program for six months (range 3-9).

Both men and women described the cumulative effect of RPL with a decrease in hope and increase in pressure and exhaustion in the third and subsequent losses. With each PL their dream of having a child felt further from their reach and they felt a growing sense of dread they would not become parents or not have the family they pictured. Referral to the RPL clinic often resulted in an increase in hope. Inclusion of the male partner in consultations and treatment was seen as important. Couples emphasized medical professionals should recognize that "every loss counts" when recommending and providing treatment and comments such as "It's nature's way" are unhelpful, given it invalidates their sense of loss.

Data showed that couples desired reliable and accurate information about RPL (e.g., common physical/ emotional responses, treatment options). They desired recognition from the medical community that RPL is not only a physical experience, but has a significant psychological impact as well, and stressed that effective treatment should include attention to both physical and psychological aspects of the RPL.

Limitations, reasons for caution: Data was collected until saturation to enhance the trustworthiness of the findings. Participants were self-selected thus findings cannot be generalized to all couples who have experienced RPL. Single women and same-sex partners were not included in the study.

Wider implications of the findings: The findings suggest that medical professionals need to take a holistic approach in their treatment of RPL and include attention to the psychological impact and cumulative effect of the PL on both men and women. The results underscore the need for informational resources psychological support for couples experiencing RPL.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 67: CURRENT PERSPECTIVES ON PREIMPLANTATION GENETIC TESTING AND EMBRYO SELECTION

Wednesday 4 July 2018 Room 116 10:00–11:45

O-260 Improved embryo selection: a novel approach for simultaneous genomic and transcriptomic analysis from a single trophectoderm (TE) biopsy

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Study question: Can RNA and DNA be sequenced simultaneously from the same trophectoderm biopsy, and be an addition to the clinical preimplantation genetic screening (PGS)?

Summary answer: The addition of full RNA sequencing to standard PGS did not alter the quality of the genomic data produced and yielded high-quality RNA sequencing data.

What is known already: With elective single embryo transfer (eSET) becoming the leading strategy to avoid multiple gestations, improved embryo selection is key. PGS reduces time to pregnancy and helps avoid miscarriages, but it cannot predict the implantation potential of a euploid embryo. Previous studies attempting to identify genes which are crucial for implantation and fetal development did not control for ploidy, due to the lack of methods to evaluate both together. To accurately interrogate the transcriptome, the ability to simultaneously sequence DNA and RNA from the same trophectoderm (TE) biopsy, without impacting the clinical PGS efficiency, would be a significant advance.

Study design, size, duration: After institutional REB approval, 24 embryos were collected from consented patients undergoing IVF-ICSI treatment at the CReATe Fertility Centre. The first 3 embryos were used for the initial development and optimization of sample processing and sequencing. Thereafter, 21 embryos (3 trisomy-16, 5 monosomy-16, 3 Turner (X0), and 10 euploid, harboring a genetic mutation per PGD) were used to demonstrate the clinical safety and reproducibility.

Participants/materials, setting, methods: Two TE biopsies were taken from each embryo. The first was processed as a clinical sample destined for PGS only. The second TE biopsy was lysed and split into two fractions: 1) for genomic analysis with next generation sequencing (NGS) and 2) for transcriptomic analysis. gDNA was amplified (SurePlex), libraries constructed (VeriSeq), sequenced on a MiSeq, and analyzed using BlueFuse Multi. RNA was sequenced on a NextSeq and analyzed using a custom-built pipeline.

Main results and the role of chance: The DNA fraction from both TE biopsies from each embryo was sequenced using a validated clinical NGS workflow and determined 'clinically acceptable" when the quality control (QC) metrics met/exceeded the Illumina guidelines (reads post-filtering>250,000 and Noise (DLR) < 0.4). There was no statistical difference in NGS QC between control and split samples. All split samples met or exceeded this criteria with average post-filtered reads of 559,769 ± 31,121 and DLR of 0.20 ± 0.005. The concordance between PGS results obtained from split and control samples was 100%, underlining the reliability and safety of our method. High-quality cDNA and libraries were obtained from all split samples. This yielded sufficient sequencing data that, after filtering, met/exceeded QC metrics (average reads 21,563,094 ± 1,636,245; average depth 26.7 ± 3.5; average quality 33.5 ± 0.03) to perform principal component analysis (PCA) and differential expression (DE). All TE biopsies expressed several specific TE markers including CDX2, GATA2, and TGFBR3, further validating the accuracy of our method. Using PCA, the samples clearly clustered in euploid and aneuploid aggregates, highlighting the importance of controlling for ploidy. Euploid embryos showed downregulation of genes involved in anaerobic metabolism, oxidative phosphorylation, and fatty-acid oxidation.

Limitations, reasons for caution: This pilot study assessed the feasibility and safety of adding transcriptomic analysis to the clinical workflow of PGS. As such, clinical outcomes were not collected, impeding our ability to validate the implications of our findings. Future clinical trials will be required to correlate transcriptomic data with outcomes.

Wider implications of the findings: This is the first study to provide full genomic and transcriptomic profiles of a single TE biopsy from human embryos. The addition of RNAseq did not affect the quality of gDNA or actual results of PGS. This method has the potential of improving embryo selection in IVF.

Trial registration number: not applicable.

O-261 Pre-implantation genetic screening (PGS) of in vivo conceived and developed blastocysts recovered by lavage: Preliminary experience

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Study question: Can in vivo conceived embryos be reliably recovered by uterine lavage for diagnostic characterization?

Summary answer: Uterine lavage performed after insemination successfully recovered blastocysts. Using Next Generation Sequencing (NGS) we performed the first chromosomal characterization, where 27.5% were euploid.

What is known already: Early work performed by Buster and colleagues in 1983 recovered embryos from the uterus by lavage in natural cycles. At that time, genetic testing of embryos was not possible, although approximately 50% of normal appearing blastocysts conceived. Chromosome abnormalities are commonly found in embryos after in vitro fertilization (IVF). In contrast, data on in vivo conceived embryos does not exist. In vivo developed embryos in animal models, possess different characteristics (i.e. rate of chromosomal abnormalities, higher rates of blastocyst formation, different characteristics in development after birth, reduced genetic aberrations) compared toin vitro developed embryos of similar species.

Study design, size, duration: We performed a single site, prospective, feasibility study in 54 women to evaluate the safety and efficacy of a new uterine lavage system (Previvo Genetics, Inc., San Carlos, CA). Patients gave their written informed consent for the research protocol approved by Western Institutional Review Board. All lavages were performed in Punta Mita, Mexico during August 2017 to January 2018 by a single investigator. Subjects were followed for 30-days post-lavage to monitor for complications.

Participants/materials, setting, methods: 54 women were pretreated with oral contraceptives and stimulated with gonadotropins for 69 cycles. Ovulation was triggered with either 5,000 IU of human chorionic gonadotropin (hCG) or Lupron 4 mg and 2,500 IU of hCG, followed by insemination of donor sperm 36 hours after trigger. Uterine lavage occurred 4-6 days after the insemination. Recovered embryos underwent trophectoderm biopsy, vitrified and stored in liquid nitrogen. Biopsies were analyzed using the NGS technique.

Main results and the role of chance: This was the first study to reliably demonstrate the feasibility of recovering in vivo derived human blastocysts following ovarian stimulation. Recovered blastocysts were then successfully characterized using comprehensive chromosomal analysis.

A total of 69 uterine lavages were performed in 54 women undergoing superovulation followed by intrauterine insemination (IUI). After IUI, uterine lavage recovered embryos in 25 (36.2%) cycles. A total of 48 embryos were recovered: 18 (37%) multi-cell embryos, 7 (15%) morulas and 23 blastocysts (48%). Six (12%) morulas progressed to blastocysts, for a total of 29 blastocysts (60%). All embryos regardless of stage were biopsied, vitrified and cryopreserved. Of the 29 tested blastocysts, 27.5% were euploid (8/29), 27.5% were

complex abnormal (8/29), 21% were aneuploidy (6/29), 17% were mosaic (5/29) and 2 had no result (7%).

The data is observational and the number of participants small making it difficult to assess the impact of 'chance" on the database. However, our stated objective was to evaluate the feasibility of using uterine lavage for recovering blastocysts for analysis and it appears that this goal was met.

Limitations, reasons for caution: The sample size is small, and the lavage tool and system are not fully optimized. The ovarian stimulation protocol prescribed may influence the chromosomal profiles observed in the recovered blastocysts. Future experiments are needed to ascertain a potential role in the observed aneuploidy induced by medications.

Wider implications of the findings: This study implies uterine lavage is a feasible method to recover embryos in selected patients who desire to bank embryos or planning for genetic testing (PGS or PGD). Uterine lavage offers a nonsurgical minimally invasive approach to recovering embryos that is different from IVF.

Trial registration number: The trial has been registered at www.clinicaltrials.gov and is pending receipt of a trial registration number.

O-262 Comprehensive mitochondrial DNA (mtDNA) analysis and IVF outcome

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Study question: Do the mtDNA copy number, heteroplasmy and haplotypes affect the ongoing pregnancy?

Summary answer: Our study suggests that elevated mtDNA copy number above a threshold is associated with lower ongoing pregnancy rate, a mutational load could be an explanation.

What is known already: Mitochondria plays a vital role in cell function and mtDNA is associated with a number of traits such infertility. Recently, there has been an increasing focus on research in its potential as biomarker of embryo implantation. It has been speculated that both mtDNA-heteroplasmy and copy number contribute to the mitochondrial function but the effects of both factors in embryo implantation were discussed separately. NGS allows us to study in depth mtDNA-hetroplasmy and copy-number simultaneously. Moreover, mitochondrial genomes can be clustered into groups known as haplotypes/haplogroups. mtDNA-haplotypes are responsible for complex diseases and at the cellular level could influence expression patterns.

Study design, size, duration: A prospective non-selection study was performed. We included 159 blastocysts biopsies from 142 couples who attended to our clinic for PGT-A from January 2016 to December 2017. All embryos were biopsied on day 5 or day 6. The aneuploidy testing was performed by NGS using Veriseq® kit and the BlueFuse Multi software (Illumina). All blastocysts were diagnosed as euploid non-mosaic and were transferred. The mtDNA analysis were performed once the diagnosis was known.

Participants/materials, setting, methods: Sequencing reads mapping to the mtDNA genome were extracted from indexed bam files to identify copy number, heteroplasmy and haplotypes. The relative measure of mtDNA copy number was calculated by dividing the mtDNA reads by the nuclearDNA value to normalize for technical variants and the number of cells collected at the biopsy. All the results were subjected to mathematical correction factor according to the embryo genome. Haplogroups were assigned by HaploGrep and heteroplasmy by MitoSeek.

Main results and the role of chance: The average of mtDNA was 0.0016 + 0.0012 with an average depth of 205×. To detect heteroplasmic sites we consider a sequencing coverage >40 and a minor allele count (MAF)> = 2. According to this criterion 40 embryos were heteroplasmy carriers (26.32%). The mtDNA haplogroup of embryos was determined in 94 biopsies, we identified 8 different haplogroups and 87% belonged to the H2a2a. With respect to IVF-outcome for mtDNA copy number analysis, due to the fact that mitochondrial dysfunction is only revealed when overall mictochondrial function drops below a threshold, we set 0.003 for the following analysis. The vast majority of the embryos (142/159) are below the threshold and 17 embryos were classified as high mtDNA. We showed a reduction in ongoing pregnancy rate associated with elevated mtDNA (42.6% vs 17.6%;$p < 0.05$). This result was independent of maternal age, day of the biopsy and embryo morphology as these factors were included as confounding factors. No significance differences were reported for haplotype (43.9% vs 41.7%;$p = 0.884$) and heteroplasmy (40.5 vs 37.8;$p = 0.77$). However, a clear association between heteroplasmy and mtDNA copy number was reported (0.0013 vs 0.0025;$p < 0.001$). With the combination of all the factors a predictive performance of the embryo implantation score was calculated with 83% of specificity.

Limitations, reasons for caution: Limitation may be due to the size of the sample and the high-troughput sequencing technology that might not have detected heteroplasmy levels lower than 2% and requires high sequence depth. A clinical randomized trial will be necessary. More research in the mitochondrial function are needed.

Wider implications of the findings: Our results have demonstrated that elevated mtDNA embryos have a lower chance to produce an ongoing pregnancy. Moreover, our data suggest a link between mutation of the mtDNA and the increased mtDNA. Therefore, mitochondrial activity could be a balance between functional capacity and absolute mtDNA copy number within the cell.

Trial registration number: Conflicts of interest and source of funding none declared.

O-263 Improved concordance rates for aneuploidy detection in spent culture media compared to trophectoderm biopsies: a step forward towards non-invasive preimplantation genetic testing (niPGT-A)

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Study question: Is the embryonic cell-free DNA in the spent culture media representative of the chromosomal constitution of the blastocysts?

Summary answer: Increased knowledge on the dynamics of embryonic cell-free DNA has improved aneuploidy detection in blastocysts by the analysis of spent culture media.

What is known already: Current techniques used for preimplantation genetic testing of aneuploidies (PGT-A) enable the analysis of the full chromosome content of a single cell with high sensitivity and specificity. Recent studies have reported the existence of embryonic cell-free DNA, opening a new era of possibilities for niPGT-A. However, the percentages of informative samples vary widely. These discrepancies, could reflect the existence of mosaicism and presence of DNA from granulosa cells or polar bodies in the spent culture media. Thus, efforts should focus on identifying embryonic cell-free DNA and rule out maternal DNA contamination to translate this non-invasive technology into clinical use.

Study design, size, duration: This is a pilot prospective study that includes analysis of 64 samples of spent culture media from embryos included in a PGT-A program. These samples correspond to 22 patients with ages ranging from 35-45 years (39.1 ± 2.5), with advanced maternal age, recurrent miscarriage, or repetitive implantation failure as PGT-A indications. Collection and analysis of samples were carried out from November 2017 to January 2018. Analysis of both types of samples from each embryo was performed blindly.

Participants/materials, setting, methods: In the PGT-A cycle, chromosome diagnosis from each blastocyst stage embryo was obtained from the trophectoderm biopsy and the spent culture media. Both, spent culture media and trophectoderm biopsies have been analysed by Next Generation Sequencing (NGS). NGS was performed using the Ion ReproSeq PGS Kit (ThermoFisher Scientific) using Ion Chef™ plus the Ion S5™ XL Sequencer™. For the analysis of the spent culture media, the standard protocol has been modified to improve amplification efficiency.

Main results and the role of chance: Informative results were obtained from all 64 trophectoderm biopsies and from 60 spent culture media (93.7% successful amplification in spent culture media). Concordance rate between trophectoderm biopsies and spent culture media in terms of blastocyst diagnosis as aneuploid or euploid was 75.0% (45/60). Concordance rate for autosomes was 80.0% (48/60). Considering the 15 discrepancies, 3 of them corresponded to euploid diagnosis in both types of samples, but with discordant gender (5% gender mismatch); 11 discrepancies were classified as false positives, with the spent culture media diagnosed as aneuploid and the trophectoderm biopsy as euploid (18.3% false positive rate); and only 2 discrepancies were false negatives, with spent culture media diagnosed as euploid and trophectoderm biopsy as aneuploid (3.3% false negative rate). In 4 of the false positive samples, the spent culture media was diagnosed as chaotic (40% of the false positives), with multiple chromosomes altered in a pattern reflecting low input of DNA or poor DNA quality. In the 2 false negative samples, the result in the trophectoderm biopsy was a single uniform monosomy in one case and, a single low-degree mosaic monosomy in the other one, raising the question of which sample better represents full blastocyst chromosome content.

Limitations, reasons for caution: This is a study with limited number of samples. There is a need to fully discard maternal DNA traces in spent culture media, to understand the impact of mosaicism in the accuracy of the diagnosis in PGT-A and niPGT-A and, to unravel why some embryos release more DNA than others.

Wider implications of the findings: In this study, the improvements in methodology and concordance rates prompt us to foresee the possibility of including niPGT-A in the clinical practice. The niPGT-A approach would help patient's access to aneuploidy testing by avoiding the need of invasive biopsy techniques and by reducing the cost of the procedure.

Trial registration number: Not applicable.

O-264 Concordance rates among multiple trophectoderm samples and ICM remain high proving the efficacy of NGS for preimplantation genetic screening

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Study question: Are human blastocysts homogeneous for chromosomal copy number between multiple samples of the trophectoderm and does trophectoderm ploidy accurately predict ICM ploidy?

Summary answer: Upon re-biopsy, trophectoderm homogeneity for full gain or loss was 97%, while ICM concordance was 100%. Human blastocyst ploidy proved consistent between cell samples.

What is known already: Blastocyst biopsy and preimplantation genetic screening (PGS) allows for ploidy status determination before implantation without adversely impacting embryo competence or baby health and well-being. The efficacy and invasive nature of the trophectoderm biopsy, as well as resultant NGS reports of mosaicism, have been scrutinized. The importance of mosaic profiles on PGS outcomes is not well defined and the effect to the offspring not fully understood. Importantly, the uniformity of aneuploidy within the trophectoderm and ICM remains unclear. It has been shown that increased mosaicism decreases implantation, while low-level mosaics can result in viable pregnancies, questioning the ICM concordance with trophectoderm.

Study design, size, duration: Nine research consented embryos were thawed and a minimum of 3 additional trophectoderm samples were taken. Samples were tubed according to standard NGS amplification protocols and sent blinded to two separate reference labs, including the original testing lab. Furthermore, both labs were unaware of the experimental plan or cell types being tested (i.e, double-blinded design). The geneticists performed NGS and profile examination to produce standard euploid/aneuploid results in accordance with their own established lab guidelines.

Participants/materials, setting, methods: Research consented embryos possessing single/dual chromosomal aneuploidies were selected and blindly sampled. MicroSecure vitrified blastocysts were warmed (100% intact) and cultured in LifeGlobal medium (37°C, tri-gas humidified conditions) for a minimum of 4 hours. Each blastocyst was biopsied 3-5 times according to standard protocols. A final biopsy per embryo attempted to isolate only ICM cells as a separate sample. A total of 28 re-biopsies were performed for a total of 37 samples.

Main results and the role of chance: A full gain or loss was determined if the NGS profile reached >95% gain/loss for each chromosome. This cut-off is contrary to standard calls of ~30%. Of the original samples,7 of the 9 had a single full gain or loss, 1 embryo had a full loss and a mosaic loss of >50%, and another sample had only a mosaic gain of 65%. A total of 36 samples, or 97% were concordant for the same chromosomes called. A total of 7 ICM samples achieved 100% concordance with the original trophectoderm sample result. The one sample not producing concordance was the original 65% mosaic embryo with all re-biopsies resulting in euploidy. Unfortunately, this embryo did not have an ICM tubed to determine relevant mosaicism. Additional low-level mosaics occurred on 3 samples (8%). It is incredibly encouraging that so many of the profiles exhibited mirror images proving the efficacy of NGS repeatability. Our laboratory has previously reported the importance of biopsy technique, so every attempt was made to sample healthy cellular masses free of degeneration. Of note, all mosaic profiles had a documented fair to poor cellular mass result from the biopsy, reinforcing the need for proper technique.

Limitations, reasons for caution: Trophectoderm biopsy with NGS is heavily reliant on proper technique and geneticist interpretations. Genetic calling policies and definitions of euploidy/aneuploidy and mosaic embryos can differ from lab to lab, adding to the growing uncertainty of PGS. Understanding result interpretation is imperative to improving patient education on the risks of mosaicism.

Wider implications of the findings: This study supports that full gain/losses are extremely reproducible among all cells/types of the human blastocyst. Mosaic profiles are potentially detrimental or possibly just artifact. Further research is needed help alleviate the ambiguity of mosaic profile risks, but this study has proven NGS efficacy when full aneuploidy is present.

Trial registration number: None.

O-265 What is the genetic significance of blastomeres excluded from the formation of a morphologically normal blastocyst?

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Study question: Is there a correlation between the ploidy of the blastocyst and that of the blastomeres excluded from the formation of the blastocyst?

Summary answer: Morphologically normal blastocysts with excluded blastomeres were diagnosed with aneuploidy affecting maximum three chromosomes whereas excluded blastomeres were chromosomally chaotic.

What is known already: Blastomeres that have arisen throughout cleavage-stage are sometimes excluded from the formation of the blastocyst and are sequestered either between the zona pellucida and the perivitelline space or remain internally during blastocyst formation and are sequestered to the blastocoel cavity. In both ways, once isolated, those cells do not take part in preimplantation development. It has been demonstrated that excluded cells have poor gap junction communication with the embryo *(Hardy et al., 1996)*. It was hypothesized that cell surface markers promoting ingestion by neighboring cells were absent from those isolated cells since phagocytosis is possible in the blastocyst-stage *(Hardy, 1997)*.

Study design, size, duration: This observational study was based on 25 good or top-quality blastocysts (at least 4BB) with blastomeres excluded from the formation of the blastocyst and sequestered between the zona pellucida and the perivitelline space. They were belonging to 20 patients applying for pre-implantation genetic screening (PGS) due to advanced maternal age (>37). Patients with structural abnormalities or a history of abnormal fetal karyotype were not included in the analysis.

Participants/materials, setting, methods: The 25 trophectoderm biopsies were prospectively analyzed and the corresponding excluded blastomeres (2-5/ blastocyst) samples were in parallel diagnosed either by next generation sequencing (NGS) (PGM platform, ThermoFisher) or by array Comparative Genomic Hybridization (aCGH, Illumina). Assisted hatching was done on day-3 and blastocysts having excluded blastomeres in the axis of the zona opening were included in the analysis to decrease manipulation and most importantly, not to harm the blastocyst.

Main results and the role of chance: The PGS analysis of the trophectoderm biopsies revealed 11 monosomies, 11 trisomies, 3 mosaicisms and 4 euploidies, showing that only 16% of the blastocysts with excluded blastomeres were euploid. In 11 blastocysts one chromosome was involved in the aneuploidy whereas for 8 blastocysts two chromosomes were implicated. Only two blastocysts were aneuploid for three chromosomes. None but one of the excluded blastomeres had the same diagnosis with the corresponding embryo (trisomy 4). However, in half of the embryos (12/25) one chromosomal aneuploidy was shared by both samples; the excluded blastomeres having a more complex diagnosis with a mean of four chromosomes involved in the aneuploidy [for example, a diagnosis of monosomy 7, 14 for the blastocyst and a diagnosis of trisomy 1q, monosomy (3, 4, 7, 14, 16, 19) for the corresponding excluded blastomeres]. Only one sample of excluded blastomeres was euploid although the corresponding blastocyst was aneuploid (monosomy 1). It may be hypothesized that either the embryo attempts to exclude blastomeres with chaotic aneuploidies from the blastocyst or that the excluded blastomeres have become chromosomally chaotic because of apoptotic cell death mechanisms that have been already initiated in the aneuploid embryo.

Limitations, reasons for caution: Results were obtained in a small group of blastocysts and in a defined IVF setting. Increasing the number of the study cohort will undeniably give more reliable results.

Wider implications of the findings: In the light of the strong association showed by Munné and colleagues (2017) between center-specific ART treatment practices and the incidence of chromosome abnormality in human embryos, it would be interesting to check the validity of the results in an independent IVF clinic.

Trial registration number: Not applicable.

O-266 Human in vitro post-implantation culture models reveal the depletion of aneuploid cells in chromosomally mosaic embryos 12 days post fertilisation

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Study question: To what extent does genomic instability at the blastocyst stage affect early post-implantation development *in vitro*, 12 days post fertilisation (dpf)?

Summary answer: While aneuploid blastocysts showed reduced developmental potential during extended culture, mosaic embryos generated euploid outgrowths 12 dpf, indicating no preferential lineage allocation of abnormal cells.

What is known already: Embryo aneuploidy is associated with implantation failure and early pregnancy loss. Yet, a proportion of blastocysts are chromosomally mosaic, containing genomically distinct cell populations. Although mosaic embryos may lead to live births, the clinical implications of chromosomal mosaicism on early pregnancy, including foetal and placental development, remain unknown. The complex nature of genomic instability coupled with the inaccessibility of research material has inherently limited our understanding. Newly established embryo culture systems allow blastocyst attachment, outgrowth formation and extended culture for up to 14 days *in vitro*, providing a unique opportunity for investigating chromosomal instability during early human post-implantation development.

Study design, size, duration: A total of 30 chromosomally abnormal human blastocysts were included in the study. Embryos were donated following preimplantation genetic screening (PGS) and selected based on their original diagnosis. Twenty embryos presented with either one (n = 13) or multiple aberrations (n = 7), while 10 blastocysts showed evidence of mosaicism. The latter were diagnosed with either one mosaic abnormality (n = 5) or both a uniform and a mosaic aberration (n = 5). Vitrified blastocysts were warmed and cultured to 12 dpf.

Participants/materials, setting, methods: We applied established culture protocols to generate embryo outgrowths. Outgrowth viability on day 12 was carefully assessed and correlated to the genomic status of the plated blastocysts. We further selected 13, day 12 embryos for genomic analysis. Of these, 7 outgrowths were separated into two portions, corresponding to inner cell mass (ICM) and trophectoderm (TE) derived lineages. Taken together, whole genome amplification (WGA), followed by next generation sequencing (NGS) was performed on 20 samples.

Main results and the role of chance: Of the total embryos plated, 46.7% remained attached during the extended culture (n = 14). Viable day 12 outgrowths were predominantly generated from blastocysts diagnosed with trisomies, structural duplications or mosaic aberrations (13 out of 14, 92.9%). Conversely, monosomies, deletions and complex aneuploidies significantly impaired *in vitro* embryo development to 12 dpf (1 out of 14, 12.5%; p = 0.01). Interestingly, all embryos originally diagnosed with a single mosaic aberration remained viable at 12 dpf, regardless of the degree of mosaicism originally reported. Remarkably, of these, all sequenced outgrowths presented with normal profiles (n = 4), including 2 embryos that were separated into ICM and TE derived portions. All segments were euploid, suggesting a depletion of aneuploid cells in both embryonic lineages. Moreover, we could not confirm mosaicism in any day 12 outgrowths generated from blastocysts diagnosed with both a uniform and mosaic aberration. Here only uniform abnormalities were detected at 12 dpf. However, within this group, embryos with a higher percentage of mosaicism were more likely to detach during culture. Finally, we determined 100% concordance for uniform aberrations diagnosed at the blastocyst stage and at day 12. This applied to whole outgrowths and those for which lineage-specific profiles were obtained.

Limitations, reasons for caution: To thoroughly evaluate genomic instability and correlate the degree and type of mosaicism to developmental potential, expanding our study to include more outgrowths is currently ongoing. Established models omit the requirement of endometrial tissues, hence do not account for trophoblast-uterine interactions. The known methodological artefacts of WGA warrant careful interpretation.

Wider implications of the findings: Our findings echo current prenatal screening data and further demonstrate the normal developmental potential of mosaic embryos. Further insights into the impact of chromosomal mosaicism will contribute to a better understanding of its clinical significance. This will inherently benefit clinical management, particularly when no euploid embryos are available for transfer.

Trial registration number: not applicable.

INVITED SESSION

SESSION 68: INFERTILITY AROUND THE GLOBE

Wednesday 4 July 2018 Room 211 + 212 12:00–13:00

O-267 Infertility and assisted reproduction in the muslim world: Social, cultural, and religious considerations

M. Inhorn

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Abstract text

Assisted reproductive technologies (ARTs) to overcome infertility are now widely available across the Muslim world. Islamic *fatwas* (religious decrees) emanating from a variety of Islamic countries have permitted ARTs as a viable solution to overcome marital infertility. However, in the Sunni Muslim world, which spans from Morocco to Malaysia, third-party reproductive assistance (egg, sperm, and embryo donation, plus surrogacy) is religiously disallowed for a number of reasons having to do with kinship, marriage, and inheritance. Having said that, religious rulings emanating from Shia Muslim-dominant Iran have created unique avenues for infertile Muslim couples to overcome their infertility through third-party reproductive assistance. The opening of Iran, followed by Lebanon, to gamete donation has led to reproductive travel across international borders, especially among Sunni Muslim couples in search of donor eggs. Having said that, sperm donation remains unpopular among both Sunni and Shia Muslim men, meaning that they must turn to intracytoplasmic sperm injection (ICSI) to overcome their male infertility. ICSI has become a "masculine hope technology" in the Muslim world, despite its many practical and ethical challenges. In addition, ARTs such as preimplantation genetic diagnosis (PGD) for sex selection and multi-fetal pregnancy reduction (MFPR) for high-order multiple pregnancies (HOMPs) are leading to complex bioethical conundrums. This presentation reviews the key social, cultural, and religious issues facing ART-seeking infertile Muslim couples. Despite complex challenges, ARTs are being used widely and ethically negotiated in novel and unexpected ways in the Muslim world—a region that has embraced ARTs along with other forms of high-tech medicine.

O-268 The burden of infertility and childlessness in Sub-Saharan Africa: how can it be reduced?

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Abstract text

Infertility is highly prevalent in Sub-Saharan Africa, in some areas affecting more than 15 % of couples of the reproductive-age population, which is mainly due to a high rate of sexually transmitted infections (STIs) and HIV/AIDS, postpartum and iatrogenic infections, and post-abortive complications. Often, infertility has a devastating impact on the people concerned, as in this context parenthood is 'culturally mandatory'. Infertility does not only have an emotional impact at the personal level (leading to grief and depression), but it also affects marital relationships and may result in disrespectful treatment by husbands and/or in-laws, domestic violence, abandonment, divorce and/or polygamy. Childless women and men may also suffer from social exclusion and stigmatization and their social security might be affected as they do not have children to take care of them at old age or when sick. Further, infertility may lead to economic hardship, as childless couples often spent their incomes on series of (ineffective) traditional and/or biomedical treatments. Finally, infertility may lead to additional health risks because people with fertility problems may be at greater risk of exposure to HIV and other STIs when they attempt to conceive extramaritally. Despite the high prevalence of infertility in Sub-Saharan countries and the huge impact on the daily lives of people concerned, it receives little attention from the public health system, neither to prevent, nor to treat it. Availability, access and quality of interventions to address infertility remain largely insufficient. Even basic interventions such as counselling, medical examinations and information provision are often scarce. Assisted reproductive technologies are - with a few exceptions - only offered in private clinics. De-stigmatization of the condition is also not addressed.

This presentation first provides insight in the burden and social suffering of infertility in the Sub-Saharan African context and subsequently present and discuss concrete recommendations to reduce this suffering. Clearly, this topic needs more attention – and funding - of various stakeholders and organizations, at national and international levels, including also NGOs and donor parties working in the field of Sexual and Reproductive Health and Rights (SRHR).

INVITED SESSION

SESSION 69: CUMULATIVE LIVE BIRTH RATES AFTER UTILIZATION OF ALL FRESH AND FROZEN EMBRYOS IN IVF/ICSI - THE OPTIMAL ENDPOINT

Wednesday 4 July 2018 Room 111 + 112 12:00–13:00

O-269 How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos?

S.K. Sunkara

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Abstract text

Traditionally, IVF success has been reported as live birth rate (LBR) per cycle, per oocyte retrieval or per embryo transfer. However, advances in controlled ovarian stimulation (COS) strategies and significant improvements in cryopreservation techniques have introduced cumulative live birth rate (CLBR) as a more robust outcome measure in defining success in ART. There have been steady increases in frozen-thawed embryo transfers (FET) and resulting live births globally. Cumulative live birth rate is defined as live births resulting from one initiated or aspirated ART cycle, including fresh and/or resulting frozen embryo transfers, until a live birth occurs or until all embryos are used, whichever occurs first.

Cumulative live birth rate is a measure of the efficiency of COS regimens and effectiveness of cryopreservation programmes in ART. The aim of COS in ART is to optimise the number of oocytes to maximise success, efficiency, safety and cost-effectiveness. Ovarian response and number of oocytes retrieved is a surrogate outcome for ART success because of important associations with both pre-clinical and clinical outcomes. There is a strong relationship between oocytes retrieved and live birth following IVF, with several studies demonstrating a non-linear association with the number of oocytes and LBR in fresh IVF cycles. These studies have demonstrated an initial linear increase in LBR with increasing number of oocytes up to a consistent threshold of ~15 and a decline in LBR following fresh transfers with excessive oocyte numbers, >20. Current evidence of higher LBR following frozen versus fresh embryo transfers in women with PCOS indirectly concurs with this finding as these women are likely to have excessive response. The assumption for this decline is the very high oestradiol levels affecting the endometrial milieu and implantation in fresh cycles. This finding cannot be extrapolated to frozen transfers where hormone levels are close to physiological range.

Cumulative live birth rate reporting has extended the threshold of oocyte numbers towards increasing ART efficiency. Initial single centre-based studies reported a positive linear association between number of oocytes and CLBR. Subsequent multi-centre and registry based studies have demonstrated patterns of association with CLBR increasing up to 20-24 oocytes and further. This topic continues to be researched currently. In addition to CLBR, other innovative measures of success have also been introduced such as the one-and-done approach. This approach aims to tailor COS to result in successive live births with embryos from one cycle. Although achieving a live birth is dominant with ART programmes, safety is paramount and eliminating OHSS is a continued focus. Large cohort studies have indicated significant increases in clinical OHSS cases with >15, >18, >20 oocytes respectively. However, such studies with historical data are likely to involve a considerable proportion of GnRH agonist cycles associated with higher incidence of OHSS. This notion of an OHSS free clinic could reach near reality with current approaches to COS using the GnRH antagonist regimen, the GnRH agonist trigger and freeze all strategy. These strategies could potentially extend the safety limits for oocyte numbers. Another aspect of safety is the perinatal risks with ART pregnancies and addressing the contribution of ovarian stimulation, ovarian response and number of

oocytes is of interest. There is a higher incidence of preterm birth (PTB) and low birth weight (LBW) with excessive response (>20 oocytes) following fresh transfers. Whilst this could be due to an innate risk for women with PCOS within this category, high peak oestradiol levels have been shown to be associated with risk of LBW.

Understanding important clinical associations is useful for planning COS regimens. Whilst higher oocyte numbers are positively correlated with higher CLBR, maximising CLBR and planning COS should give consideration to safety.

O-270 Cumulative live birth rates after utilization of all fresh and frozen embryos in GnRH antagonist versus agonist protocol

INVITED SESSION

SESSION 70: PRECONCEPTION GENETICS IN CLINICAL FERTILITY PRACTICE: HOW MUCH INFORMATION DO WE NEED?

Wednesday 4 July 2018 Room 113 + 114 + 115 12:00–13:00

O-271 Increasing use of mutation screening in fertility clinics

O-272 Genetic carrier screening in gamete donor programs

A. Veiga, M. Boada, A. Abuli

Reproductive Medicine Service, Dexeus Women's Health, Barcelona, Spain

Abstract text

The data from the ESHRE-EIM Consortium for year 2011 show that more than 15,000 oocyte donation (OD) cycles have been performed in Spain, representing 52% of the OD European cycles. Donor screening has always been an issue that has received utmost attention in Spanish ART centers. European regulations clearly states that genetic screening for autosomal recessive genes known to be prevalent in the donor's ethnic background and assessment of the risk of transmission of inherited conditions known present in the family must be carried out according to international scientific evidence.

Traditionally, in OD programmes, genetic screening has been performed through karyotype analysis and the screening of diseases most prevalent in the donor's country of origin. In the last few years, genetics has experienced important technological progress allowing the screening for multiple genetic diseases at a reasonable cost. Expanded carrier screening (ECS) panels have now been implemented to allow a much deeper analysis of the risk of disease transmission during donor evaluation and better matching between donors and recipients shifting from the screening of limited number of conditions with a high prevalence in specific groups to the pan-ethnic or universal screening of multiple recessive conditions.

Since 2010, different panels have been commercially available, with different numbers and types of diseases. With the aim to provide with up-to date risk assessment of oocyte donors and optimised matching between donors and recipients, we have developed our own ECS panel (qCarrier) in collaboration with Q Genomics, a technological company that deals with genomics in human health. For gene selection, consideration has been given to geographic prevalence, neonatal screening programs and a selection of mutations associated with severe genetic diseases. The panel has been based on Next Generation Sequencing (NGS) technology, including complete sequencing of the coding region of genes associated with the most prevalent diseases and targeted mutational analysis of rare diseases. Complementary techniques for Fragile X syndrome and some deletions have been performed.

The results describe the implementation of a carrier screening test for autosomal recessive and X-linked diseases in our OD Programme. Donors and male partners of the recipients were screened for risk assessment and matching. The ECS test covered 200 genes (68 genes by complete sequencing of the coding region and 132 sequenced by a targeted mutation analysis) associated with 314 diseases (277 recessive autosomal diseases and 22 x-linked). Among participants, 56.4% (761/1350) were identified as carriers of at least one gene mutation. Eleven (1.9%) of oocyte donor candidates were identified as carriers of X-linked diseases, and as such, were excluded from the OD programme. The mean carrier burden was 0.84 mutations per sample. With respect to the matching between oocyte donors and recipients, 26 (3.4%) preassigned matches were at high risk of transmitting a severe autosomal-recessive genetic condition to their offspring (cystic fibrosis, classical congenital adrenal hyperplasia, autosomalrecessive non-syndromic sensorineural deafness, alpha thalassemia, familial Mediterranean fever, Niemann-Pick disease, thyroid dyshormogenesis type 6 and cartilage-hair hypoplasia). The final matching between donors and recipients was performed taking into account the ECS results. A carrier state for an autosomal recessive condition was not an exclusion criterion for oocyte donors, although it implied the assignation of the donor to a recipient whose male partner was not a carrier for the same recessive condition. When performing ECS in an OD, an appropriate genetic counselling is essential for donors and recipients in order to understand the results, to decrease anxiety and to give recommendations about own reproductive risk or that of relatives, when necessary.

INVITED SESSION

SESSION 71: LABORATORY INVITED SESSION: EMBRYO CULTURE: CHANGING THE ATMOSPHERE

Wednesday 4 July 2018 Room 117 12:00–13:00

O-273 Epigenetic changes by stimulation and culture

A. Sunde

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Abstract text

Epigenetic changes means that there is a change in the phenotype of a cell or an organism without a simultaneous change in the genotype. It is the expression of genes that are changed. One mechanism of regulation gene expression is trough adding a methyl-group to a Cytosine in the binding site of a transcription regulator or by methylation or acetylation of histones. These chemical modifications of DNA or chromatin can change the likelihood that a gene or sets of genes are expressed.

Assisted reproduction is associated with shifts in the phenotype of the embryos, of the placenta and the children born. Interventions like ovulation induction, *in vitro* fertilization, embryo culture and cryopreservation of embryos are associated with changes in gene expression profiles methylation patterns in the placenta and the birth weight of the offspring. In general, the mechanisms behind the changes in phenotype are unknown, but it is reasonable to assume that this is mediated epigenetically by modulating gene expression.

From epidemiological data, it is well documented that the nutritional status of the mother in early pregnancy may have lasting effects on the health of the offspring. In human IVF, different culture media was shown to induce different gene expression profiles in embryos and a significant difference in birth weight of the children born Similarly, the oxygen concentration in the incubator will influence the gene expression in human embryos and this effect was dependent on the type of culture media used.

Commercially available culture media for ART differ considerably in their chemical composition and it should not come as a big surprise if embryos show different epigenetic responses to these differences in the culture environment.

The consequences of these small shifts in phenotype after IVF and embryo culture is to a large extent unknown, and it is unclear whether this will have an effect on the long-term health of the ART-offspring. Follow-up studies on ART-children in relationship to the intervention done both *in vivo* and *in vitro* are

important in order to understand if there is certain interventions that is associated with an increase in long term health problems.

O-274 Impact of temperature on embryo culture

R. Janssens

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Abstract text

In ART, the pre-implantation development of human embryos – and their subsequent ability to implant - depends heavily on the *in vitro* culture conditions. There is now an increasing understanding that the embryo is highly adaptive to its environment, the embryo is able to develop to morphologically normal blastocysts even in sub-optimal conditions. These culture conditions are set to mimic the *in vivo* conditions as good as possible and mainly include the culture media, the type of culture system and the incubator. The primary function of the incubator is to maintain an appropriate microenvironment (composition of gas phase) and temperature. Temperature can impact gamete and embryo function and meiotic spindle stability [1]. Although the developmental plasticity of embryos permits them to develop over a range of temperatures, from 36.0 to 37.0°C[2], it is know that the micro-environment maintenance ability of incubators significantly influences the formation of good embryos [3]. Most commercial IVF incubators have important temperature variations [4],[5] and statistically significant associations between incubation shelf and clinical pregnancy have been reported[6]. Due to these technical limitations, accurate determination of optimal incubation temperature has not yet been possible. Recent improvements in incubator technology however now allow accurate temperature regulation and determination of optimal incubation temperature. Until now, constant incubator settings matching the core body temperature (37°C) are used, but the optimal culture temperature is yet undefined. Available data on incubation temperature will be reviewed.

Although modern incubators can now regulate incubation temperature precise and stable, the temperature control out of the incubator can be problematic. Limited decrease in temperature can alter the cytoskeleton and spindle of oocytes and there is limited recovery after cooling and rewarming. Temperature issues can occur during follicle puncture, during manipulations on heated stages on stereo microscopes and injection microscopes, in incubators and during embryo transfer. Temperature should be measured in culture dishes under oil and in tubes with calibrated probes. The choice of measuring probe is important. Thin, fast responding non shielded type T thermocouples can be used to detect small temperature gradient and are excellent to detect hot spots on heating stages while more precise, small probes, fixed in culture dishes are more suitable for precise temperature measurements in incubators. While the most stable and accurate sensors are resistance temperature detectors (Pt100, Pt 1000), they are not easily available in small sizes which allow them to be fixed inside a culture dish. For this purpose thermistor probes are probably a better choice. Thermistors with 0.1°C accuracy are now widely available and at a very reasonable price. They have a fast response time and because of their high sensitivity are ideal for detecting temperature changes in culture dishes. Of course, accurate temperature measurement is only possible through the use of suitably calibrated sensors and instruments and the accuracy of these measurements will be meaningless unless the equipment and sensors are correctly used. Good knowledge of measurement science is a basic requirement, often lacking in many laboratories.

SELECTED ORAL COMMUNICATIONS
SESSION 72: NEW HORIZONS IN IVF
Wednesday 4 July 2018 Forum (Auditorium) 14:00–15:15

O-275 Using artificial intelligence (AI) and time-lapse to improve human blastocyst morphology evaluation

M. Meseguer Escriva[1], N. Zaninovic[2], F.G. Nogueira Marcelo[3], O. Oliana[4], T. Wilkinson[5], L. Benham-Whyte[5], S. Lavery[4], C. Hickman[4], J.C. Rocha[6]

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[5]*Oxford University, Reproductive Biology, Oxford, United Kingdom*
[6]*Sao Paolo State University-UNESP, Applied Mathematics Laboratory, Sao Paolo, Brazil*

Study question: Following from a bovine study presented at ESHRE 2017, we compared AI *versus* five distinct experienced embryologists in the capabilities to grade human blastocysts.

Summary answer: As observed in the bovine model, AI outperformed embryologists who graded human blastocysts using time-lapse images.

What is known already: Morphological grading of blastocysts is clinically used for embryo selection. Our group demonstrated that blastocyst grading by embryologists lead to wide inter- and intra-operator variation. We demonstrated (bovine model) that an image analysis by AI system can reduce variation in blastocyst grading and acquire additional parameters not detectable by operator assessment. Human blastocysts present additional challenges for AI image recognition compared to bovine, given the increased classification categories, diverse trophectoderm thickness, diverse ICM shapes, and lower contrast. Until now, AI has not demonstrated to improve operator blastocyst grading in human embryos, which was the focus of the current study.

Study design, size, duration: 394 human embryo time-lapse images taken at 111.5 hpi were graded for ICM, trophectoderm and expansion using Gardner grading system by 5 different embryologists from 4 different countries. Of these, 171 were excluded as blastocysts that were too early (expansion 1 or earlier), hatched, out of focus or ICM not visible. The mode of the remaining 223 images were used as output for the AI system (70% training, 15% validation and 15% blind test).

Participants/materials, setting, methods: 29 independent mathematical variables were extracted from each time-lapse image taken at precisely 111.5 hpi from the central Z-stack and inputted into the AI system. The agreement was assessed using confusion matrices, ROC curves and Kappa Index. Embryologists originally trained in the same lab were compared to embryologists trained in different labs to assess inter- and intra-clinic agreement variation.

Main results and the role of chance: Agreement among the 5 embryologists was low (Kappa agreement decreasing from Expansion 0.4 to trophectoderm and ICM 0.3). There was no difference between the kappa agreement of embryologists trained in the same clinic to embryologists from different countries. The low inter-operator agreement is likely to be due to the fixed time central stack image selection. Previously, we demonstrated that when images were selected according to focus on ICM, the operator agreement was considerably higher. Improved agreement was observed using AI to predict the mode of the embryologists with substantial agreement with Expansion (Kappa agreement 0.7) and ICM (0.7) and moderate agreement with trophectoderm (0.4). The AI's overall accuracy was almost perfect for prediction of blastocyst expansion (training 93.9% and validation 81.5%) and substantial for prediction of ICM (training 93% and validation 78.8%) and trophectoderm (training 78.8% and validation 78.3%). The AI system was considerably more predictive of Expansion (AUC 0.888-0.956) compared to ICM (AUC 0.605-0.854) and trophectoderm (AUC 0.726-0.769).

Limitations, reasons for caution: Our data suggests that AI is able to cope better than operators with the challenge of grading three dimensional embryos from a single fixed two-dimensional image. This technology has now been demonstrated in two independent centres. Further independent studies are required to demonstrate reproducibility before establishing its clinical application.

Wider implications of the findings: Applying AI to human blastocyst grading is inexpensive, non-invasive, and more reliable than grading by an operator. Instead of a human looking at thousands of images, AI faster assesses them and

continuously learns and quantifies additional information. As demonstrated, this technology can inherently enhance our capabilities of assessing embryo viability.
Trial registration number: not applicable.

O-276 Optimising Machine Learning Algorithms for the Non-Invasive analysis of the embryo secretome to enhance informed decisions imediately prior to embryo transfer

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Study question: Is it possible to mathematically analyse the secretome of blastocysts by non-invasive mass spectrometry of spent culture media to predict viability for embryo selection.
Summary answer: A simple, direct and rapid analysis of mass spectra from spent blastocyst culture media quickly identifies optimal embryos with the best chance of viable pregnancy.
What is known already: All available technologies to evaluate successful implantation, such as PGS, require embryo biopsy. MALDI-ToF MS is successfully exploited in microbiology, but has largely been dismissed in assisted reproduction. Proteins in media from embryos have been detected using MALDI and were consistent with gene expression studies and correlate well with positive pregnancy; however, the findings were not correlated with all pregnancy outcomes. This study represents the first to use mathematical modeling to correlate the spectral analysis of peptides in the embryo secretome with all pregnancy outcomes.
Study design, size, duration: A prospective analysis of 500 culture fluid samples collected at the time of embryo transfer over a three year period which were compared with eventual outcomes of ongoing viable pregnancy, SAB, Biochemical pregnancy, negative pregnancy test or evaluation following PGS.
Participants/materials, setting, methods: Over 500 samples of spent blastocyst culture media were collected from embryos of at the time of transfer from a single clinic in the US. Just 1 µL of media was analyzed using MALDI ToF mass spectrometry in a laboratory in the UK. Quantitative characteristics within the 2000 to 15000 m/z mass range were examined using multiple supervised machine learning algorithms to predict pregnancy outcome.
Main results and the role of chance: Mathematical analysis of secretome spectra following MALDI ToF MS analysis shows distinct differences between high grade embryos which go onto achieve successful pregnancy and those which do not, either as an SAB, Biochemical Pregnancy, negative pregnancy test or aneuploidy PGS result. Some analysis of algorithms used for embryos with negative PGS outcomes samples were able to distinguish different aneuploidies.
Limitations, reasons for caution: More samples tested in a real-time environment using MALDI ToF in a clinic at the time of embryo transfer would help validate this study which is limited by the freezing and shipping of samples to the UK laboratory.
Wider implications of the findings: Many cycles result in negative outcomes due to aneuploidies or implantation failure when embryos appear otherwise viable. Checking for aneuploidies is time consuming, invasive and requires vitrification. Implementation of a quick and simple non-invasive mass spectral analysis could simplify and dramatically improve embryo selection and increase live births.
Trial registration number: Not Applicable.

O-277 Automation in the IVF laboratory: preliminary results with a new device able to do both vitrification/rewarming of mice and bovine oocytes and embryos

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Study question: Can both vitrification and rewarming be standardized using a unique automated device capable to do both procedures?
Summary answer: The automatic vitrification/rewarming device (Sarah) produces high survival rates of oocytes/embryos. This novel device will become indispensable for standardization and automation in the IVF laboratories.
What is known already: Clinical demand for oocyte vitrification has been steadily increasing for women electively postponing motherhood or at iatrogenic risk of losing their reproductive ability. However, the lack of standardization of vitrification/rewarming protocols produces variable ranges of survival, pregnancy and live birth rates among ART centers. Currently, most vitrification/rewarming protocols require manual moving of oocytes or embryos one at time between various solutions (often 4 or 5) before completing the task.
Study design, size, duration: Experimental research, using mice (40 oocytes and 242 cleaved embryos) and bovine (234 oocytes and 55 cleaved embryos) samples in a new automatic vitrification/warming device (Sarah, FertileSafe, Ltd).
Participants/materials, setting, methods: Mice oocytes (n = 40) and embryos (8 cells, n = 35 and blastocysts, n = 165) and bovine embryos (2Pn, n = 35) and MII oocytes (n = 84) were vitrified using the automated device (Sarah). A total of 42 (2 cells) mice embryos, 20 (2Pn) bovine embryos, and 150 MII bovine oocytes were used as fresh controls and grown to blastocysts. Upon rewarming with the same device, all were assessed for viability, cleavage, blastocyst and hatching rates.
Main results and the role of chance: Up to 5 oocytes/embryos were loaded into 0.25 ml straws end-closed by special capsules and connected to the robotic arm. For the mice experiments, the samples were exposed to three ES (30, 60, 100%) followed by VS (100%). For the bovine experiments, samples were exposed to six ES (10, 20, 40, 60, 80, 100%) followed by VS (75, 100%). For rewarming straws were plunged into temperature-controlled 5 ml tubes containing WS 100, 50, 25 and 12.5%, at RT for 2.5 minutes each, before arrival to the holding station. 95% (38/40) of the mice MII oocytes regained isotonic volumes and all (95/95, 100%) were viable. Rewarmed 8 cell mice embryos had 95% (33/35) blastulation rate and 80% (28/35) hatched. Rewarmed mice blastocysts had 97% survival rate (160/165) and 81% (135/165) hatched. Fresh control mice embryos had 100% blastulation rate (42/42) and 73% hatching (21/42) (p = ns). Bovine embryos survival was 100% with 54 % (19/35) cleavage and 9% (3/35) reaching blastocyst stage. Fresh control bovine embryos had 65% (13/20) cleavage and 20% (4/20) blastulation rate. Vitrified/warmed bovine oocytes had 100% survival, 73% (61/84) cleavage and 7% (6/84) blastocysts rates; fresh control had 83% (125/150) cleavage and 11% (17/150) blastocyst rate (p = ns).
Limitations, reasons for caution: These successful preliminary results in animal models need to be replicated in the recently started clinical human IVF settings.
Wider implications of the findings: Automation of both vitrification and rewarming of oocytes and embryos will drastically reduce the differences in survival and success rates between ART centers worldwide. Simplification and standardization of protocols will be of great value both for patients requiring fertility preservation or elective cryopreservation and the whole ART industry.
Trial registration number: N/A

O-278 Single-cell RNA-seq reveals distinct dynamic behavior of sex chromosomes during human early embryogenesis

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Study question: For human in vitro fertilization embryos, are there differences between male and female on the gene expression level during early development?

Summary answer: There are imbalanced expression of sexual chromosomes between gender from the embryonic genome activation (EGA), with a dosage compensation firstly occurring in female trophectoderm cells.

What is known already: Several animal studies have demonstrated that the gender has an effect in the kinetics and metabolism of early embryo development. At the stage before implantation, sex-specific differences in gene expression become apparent. In rodents, this period of double X chromosome activation occurs between 4-cell and 16-cell stages, but the equivalent timing is uncertain in human embryos. Failure to accomplish dosage compensation normally results in early miscarriage and embryonic lethality. In human, the dosage compensation is started by silencing the expression of both X chromosomes in blastocyst embryos, while the exact procedure remains unclear.

Study design, size, duration: We performed global expression analysis between males and females using single-cell RNA-sequencing data of 1585 individual cells from 98 human preimplantation embryos, covering development stages from 8-cell to late blastocyst.

Participants/materials, setting, methods: We classified all the cells from E3 to E7, corresponding to their developmental stages from 8-cell to late blastocyst. An average of 20 embryos were analyzed per embryonic day. We determined the sex of each embryo after assessing the expression level of Y-linked genes for each cell. Then we performed the comparison of expression between gender for each stage.

Main results and the role of chance: We provide a comprehensive comparison of transcriptional atlas between sex of human preimplantation embryos and reveals the dynamics of sex chromosomes during embryogenesis for the first time. Consistent chromosome-wide transcriptomic level of autosomes was observed in all stages, while sex chromosomes showed significant differences after EGA. We observed a significant enrichment of differentially expressed genes on X chromosome at the 8-cell stage, on both X and Y chromosomes at the morula stage, and on Y chromosome at the blastocyst stage($P < 0.001$). Two of the DE genes on Y chromosome, RPS4Y1 and DDX3Y, achieved abundant expression in all male cells immediately beyond the completion of EGA and could be used as a gender marker in male embryos. For X chromosomes in female, over 100 significantly higher expressed genes were defined in 8-cell stage and both alleles of most heterozygous sites could be detected in our allelic analysis. Contrary to constant pattern of Y chromosome, the expression of X chromosome distinctly dropped at late blastocyst stage, especially in trophectoderm cells, revealing the exact time point and details of dosage compensation.

Limitations, reasons for caution: Lack of genomic data to verify the gender of each embryo is the limitation of this study. In addition, in the allelic analysis, we still need the variants of embryonic genome to get more informative sites.

Wider implications of the findings: Studying the sex differences during human embryogenesis, as well as understanding the procedure of X inactivation and its correlation with early miscarriage, will build upon the capabilities of assisted reproductive technology to improve the treatment of infertility and reproductive health.

Trial registration number: not applicable.

O-279 Parallel single-cell genome and transcriptome sequencing to study chromosomal instability during human embryonic preimplantation development

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Study question: Is there any transcriptional signature of aneuploidy at single cell level during human preimplantation embryonic development?

Summary answer: The largest transcriptomic differences between cells are due to embryonic genome activation. Differences due to aneuploidy become detectable comparing cells of the same developmental stage.

What is known already: More than 70% of IVF human cleavage stage embryos are mosaic. Nevertheless, some can potentially develop in healthy babies after transfer. The causative mechanisms of chromosome instability as well as the mechanisms of selection and/or correction of aneuploid cells remain largely unexplored and speculative in the embryo.

Study design, size, duration: All single cells from 31 preimplantation embryos were subjected to parallel single-cell whole genome and transcriptome sequencing. Namely, 8 embryos at Day1 (17 cells), 19 embryos at Day2 (62 cells), 2 embryos at Day3 (17 cells) and 2 embryos at Day4 (23 cells). From each single cell, genome-wide DNA copy number (CNV) and whole transcriptome gene expression levels were determined. The data from this ongoing cross-sectional study was generated between October 2016 and November 2018.

Participants/materials, setting, methods: Thirty-one zygotes donated for research from 14 couples were cultured in a time-lapse incubator after surviving cryopreservation. At a specific cell stage, embryos were mechanically dissociated into 119 single blastomeres, which were subjected to on-bead separation and amplification of DNA and RNA. Single-cell amplification products were subsequently sequenced with Illumina platforms. Single-cell DNA genome-wide CNVs were determined by focal read depth analysis. Single-cell gene expression data was subjected to clustering and principal component analysis (PCA).

Main results and the role of chance: Single-cell DNA CNV analysis revealed frequent missegregations of whole chromosomes as well as segmental rearrangements in all embryonic cell stages. Specifically, 22 out of 31 (70%) embryos contained at least one genetically abnormal cell. At single cell level, 74 out of 92 cells (80%) that passed sequencing quality control contained at least one segmental or whole chromosome aneuploidy. The remaining 18 cells (20%) were normal euploid. Furthermore, using embryonic cell cleavage phenotype and the genomic profile of its single cells, we could deduce the origin of the abnormalities and construct cell lineages.

Single-cell gene expression analysis, classified the cells according to the embryonic cell stage and the embryonic genome activation (EGA). Moreover, we could benchmark our dataset by recapitulating previously described EGA gene expression patterns. Interestingly, two Day3 blastomeres with heavily rearranged genomes clustered separately from the rest of the Day3 cells regardless of the embryo of origin. These results suggest that transcriptomic differences due to abnormal genomes might be subtler between cells from different embryo stages while more detectable when comparing embryos within the same developmental stage.

Limitations, reasons for caution: This is a preliminary analysis of an ongoing study. The current analysis includes a low number of embryos and unevenly distributed across the preimplantation embryonic stages. Further RNA-seq computational analysis in a higher number of samples are required to unravel the effect of CIN in the transcriptome.

Wider implications of the findings: We deliver proof-of-principle that this novel, state-of-the-art method for simultaneous genome and transcriptome sequencing (G&T-seq) of the same single cell can be successfully applied in human blastomeres. To our knowledge, these are the first results of G&T-seq in human embryos. We foresee a great scientific potential to study embryo development.

Trial registration number: not applicable.

SELECTED ORAL COMMUNICATIONS
SESSION 73: ART-RELATED COMPLICATIONS

Wednesday 4 July 2018 Room 211 + 212 14:00–15:15

O-280 Oocyte retrieval complications: an update after 23,833 procedures in an academic tertiary care ART referral center

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Study question: The aim of the present study was to assess complications encountered on the largest single centre series of transvaginal oocyte retrievals (OR).

Summary answer: A significant correlation between complications and women age, BMI, the number oocytes, mean time to complete oocyte retrieval, clinician experience and presence of risk factors.

What is known already: Ultrasound guided transvaginal OR represents the most commonly used technique in ART cycles. Several observational studies evaluating the rates of complications associated with this procedure have shown it to be safe, with very low rates of serious complications.

Study design, size, duration: This is a retrospective analysis including all the oocytes retrievals performed at a large teaching hospital from June1996 to November 2016. The study has been approved by our Institutional Review Board, all patients signed an informed consent to the use of their data, as long as their anonymity and confidentiality were assured. February 28th 2016. All the patients' data were extracted from the Fertility Center external-audit-anonymized electronic research query system.

Participants/materials, setting, methods: 23,833 procedures were included. We did not exclude any patient who performed retrievals during the period in exam. Complications requiring hospital admission for at least 24 hours were considered severe complications. Transvaginal ultrasound–guided OR was performed 34/36 hours after the trigger injection, under deep sedation in an operating theatre, using a 35 mm, 17-gauge aspiration needle. Antibiotic prophylaxis was not routinely used except in situation with surgical risk factors.

Main results and the role of chance: A total of 96 out of 12,615 patients (0.76%) suffered complications, with hospital admission necessary for 71 patients (0.56%). Mean hospital stay was 2.77 ± 2.5 days. When calculated per retrieval, the overall complication rate was 0.4% while 0.29% was the admission rate. A surgical procedure was necessary for 24 patients (0.1% per retrieval and 0.19% per patient). Multivariate analysis showed a significant correlation between complications and women age, BMI, the number oocyte retrieved and mean time to complete oocyte retrieval. The incidence of complications was significantly higher for physicians who had performed fewer than 250 retrievals as compared to those who had completed more than 250 (OR 0.63; 0.40 – 0.99 – p = 0.0472).

In general, the most important risk factors for the occurrence of a complication can be identified in: a) high number of oocytes retrieved; b) a longer duration of operative time; c) inexperience of the surgeon; d) a low BMI and e) a history of prior abdominal or pelvic surgery or pelvic inflammatory disease. Interestingly, sixteen of the fifty-four retrievals (29.63%) with hemoperitoneum had surgical risk factors.

Limitations, reasons for caution: Although the great majority of complications were treated in our institution (94/96 complications), others could have been treated in other hospital and not unreported. This could reflect a possible bias with an underestimation of the incidence of complication related to oocyte retrieval, severe ones are usually reported.

Wider implications of the findings: OR can be considered a safe procedure, although patients and physicians should understand that this technique is not without risks. We found that complications were consistently less when the operator had performed an average of 250 ORs. We can therefore suggest referring to more experienced surgeons OR at higher risk.

Trial registration number: NCT03282279

O-281 Transfer of two non-top quality embryos increases the risk of ectopic pregnancy after fresh and frozen-thawed embryo transfer

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Study question: Is embryo quality associated with ectopic pregnancy (EP)?

Summary answer: The odds for EP are elevated after the transfer of two embryos, even if both are of non-top quality.

What is known already: The incidence of EP after IVF/ICSI varies from 1.6% to 8.6%, and can be up to four times higher than the rate in spontaneous pregnancies. Tubal factor infertility and the transfer of multiple embryos are known to increase the probability of EP. Nowadays, top quality embryos are mostly transferred in single embryo transfers (SET). Double embryo transfers (DET) are therefore performed with non-top quality embryos. The effect of embryo quality on EP has not been investigated until now. Furthermore, there is no consensus on whether the transfer of fresh vs. cryopreserved embryos has a different effect on EP rates.

Study design, size, duration: Retrospective cohort study of 16,891 clinical pregnancies (diagnosed by ultrasound at 6-8 gestational weeks) after non-donor IVF/ICSI with fresh (n = 10,255) or frozen-thawed transfer (FET) (n = 6636), performed in 2000-2017 in Finland (LUMI database). In all cases, there were up to two embryos transferred with known morphological quality. Presence or absence of tubal factor infertility was also recorded. Heterotopic pregnancies were classified as ectopic.

Participants/materials, setting, methods: A single top quality embryo was transferred in 6634 (39.3%) and a single non-top one in 4206 (24.9%) cycles. In DET cycles, there were no (3721 cycles, 22.0%), one (1485 cycles, 8.8%) or two (845 cycles, 5.0%) top quality embryos transferred. We analyzed the effects of female age, the number of embryos transferred, embryo quality, type of cycle (fresh vs. FET) and tubal factor infertility on EP by logistic regression.

Main results and the role of chance: EP was observed in 384 (2.3%) cycles. There was no significant difference in EP rate after fresh embryo transfer or FET (225/10,255, 2.2% vs. 159/6636, 2.4%, P = 0.7). Compared to treatments in which only top quality embryos were transferred (141/7479, 1.9%), EP rate in cycles with only non-top quality embryos transferred was higher (219/7927, 2.8%, P < 0.0001). Tubal factor infertility was diagnosed more often in EP than in intrauterine pregnancies (19.5% vs. 10.2%, P < 0.0001).

Logistic regression revealed that the odds of EP after fresh transfer were similar to those after FET (odds ratio (OR) for FET 1.06, 95% confidence interval (CI) 0.86-1.23, P = 0.6). DET increased significantly the chance of EP, compared to SET (OR 1.45, 95% CI 1.18-1.78, P < 0.0001). Presence of tubal factor infertility was associated with EP (OR 2.06, 95% CI 1.59-2.67, P < 0.0001). In a separate logistic regression analysis, transfer of two non-top quality embryos (OR 1.49, 95% CI 1.13-1.95, P = 0.004) but not of one (OR 0.79, 95% CI 0.60-1.05, P = 0.1) or two top quality embryos (OR 1.31, 95% CI 0.83-2.07, P = 0.3) increased the odds of EP, compared to transfer of a single non-top quality embryo. Female age was not associated with EP (P = 0.3).

Limitations, reasons for caution: The effect of the hormonal environment in FET (spontaneous vs. hormonally-substituted cycles) was not analized due to the limited availability of complete data.

Wider implications of the findings: Transfer of non-top quality embryos decreases the chances of live birth after IVF/ICSI. The present study found that DET with two non-top quality embryos is also associated with increased odds of EP. This is particularly important in tubal factor infertility patients who are already at risk of EP.

Trial registration number: Not applicable

O-282 Hospitalizations due to OHSS after IVF is not diminished during the last 15 years despite presumed improvements in ovarian stimulation - National data from Denmark

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Study question: Do we observe a recent reduction in prevalence of severe OHSS after change towards antagonist protocols, use of response predictive factors and agonist triggering?

Summary answer: National Danish Hospital discharge register data identified 2.564 OHSS cases after 160.803 ART-cycles and no significant prevalence reduction of severe OHSS from 2001 to 2015.

What is known already: OHSS is a known risk of ovarian stimulation, but it is known that the numbers directly reported by ART professionals to the registers are grossly underreported, whereas more accurate numbers may be obtained from hospital discharge registers. Antagonist protocols that lowers the risk of OHSS are more commonly used in recent years, agonist triggering may reduce the OHSS rate, and ovarian response predictive factors like AHM and AFC have been developed. All these changes in IVF should reduce the OHSS risk.

Study design, size, duration: This is a retrospective register based study including 160.803 IVF/ICSI stimulated cycles from all public and private fertility clinics in Denmark from 2001 to 2015.

Participants/materials, setting, methods: From 2001 to 2015, a total of 2.564 women had at least one hospital admission with an OHSS diagnosis. Data on OHSS was obtained from the Danish National Discharge Register and the annual number was compared to the annual number of stimulated IVF/ICSI cycles, obtained from the ART register of the Danish Fertility Society (www.fertilitetsselskab.dk). Changes in the prevalence were estimated by Chi-square test and Cochran-Armitage test for trend.

Main results and the role of chance: The annual number of OHSS ranged from 151 cases in 2001 to 203 cases in 2009 and 2010 and 169 cases in 2015. During the same period the number of stimulated IVF/ICSI cycles increased from 8.805 in 2001 to 12.328 in 2015. The prevalence of OHSS among all stimulated cycles in 2001 was 151/8805 = 1.7% (CI: 1.4 to 1.9) and in 2015 169/12.328 = 1.4% (CI: 1.2% to 1.9%), with a marginal reduction of 0.3%. The prevalence varied with the highest figure in 2005 with 178 /9541 = 1.9% (CI: 1.6 to 2.0), and the lowest in 2007 with 153/11035 = 1.4% (CI: 1.2 to 1.9) and in 2015 with 169/12.328 = 1.4% (CI: 1.2 to 1.9). We found no significant changes between the annual prevalence of OHSS in Denmark (p = 0.126) as well as no trend for changes in the prevalence over the 15 years (p = 0.158).

Limitations, reasons for caution: The present analysis was unable to identify cases of OHSS that may have occurred after ovulation induction for anovulation or intrauterine inseminations. Extensive data sets on those IVF cycles that did and did not result in OHSS would be needed to analyze why OHSS was not reduced.

Wider implications of the findings: We have increased knowledge of risk factors for OHSS and may adapt treatment regiments towards more antagonist protocol, agonist triggering, individualized FSH dosing or a freeze all strategy. However, on a National basis we have not yet seen that the results of such efforts diminish the risk of OHSS.

Trial registration number: 'not applicable'

O-283 Pregnancy after frozen-thawed embryo transfer during hormonal replacement cycle is associated with hypertensive disorders of pregnancy and placenta accreta

K. Saito[1,2,3], A. Kuwahara[4], T. Ishikawa[3], T. Nakasuji[3], M. Miyado[1], K. Miyado[5], M. Fukami[1], N. Miyasaka[3], M. Irahara[4], H. Saito[2]

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Study question: Is there any difference in obstetrical risk between patients who conceived after frozen embryo transfer (FET) during a hormone replacement cycle (HRC-FET) and during a natural ovulatory cycle (NC-FET)?

Summary answer: The patients who conceived by HRC-FET had an increased risk of hypertensive disorders and placenta accreta compared with those who conceived by NC-FET.

What is known already: Previous study has shown that the rate of Caesarean section in pregnancies conceived through HRC-FET is higher than those conceived through NC-FET. Little has been clarified regarding an association between obstetrical complications and endometrium preparation methods.

Study design, size, duration: A retrospective cohort study of patients who conceived after HRC-FET and those who conceived after NC-FET was performed, based on the Japanese assisted reproductive technology registry in 2014.

Participants/materials, setting, methods: Pregnancy outcomes were compared between the cases of NC-FETs (n = 29,760) and HRC-FETs (n = 75,474). Multiple logistic regression analyses were performed to investigate the potential confounding factors for mode of delivery and obstetrical complications.

Main results and the role of chance: The pregnancy rate was significantly lower in HRC-FET cycles than in NC-FET cycles (32.1% vs. 36.1%, $p < 0.001$). Among pregnancies, Caesarean section rate was higher (44.5% vs. 33.7%, $p < 0.001$) for pregnancies conceived through HRC-FET than those conceived through NC-FET. A multiple logistic regression analysis showed that pregnancies after HRC-FET had increased odds of Caesarean section (adjusted odds ratio [aOR] 1.736, 95% confidence interval [CI] 1.632–1.847), hypertensive disorders of pregnancy (aOR 1.443, 95% CI 1.231–1.692), and placenta accreta (aOR 6.817, 95% CI 3.662–12.692) compared to pregnancies after NC-FET.

Limitations, reasons for caution: This study is retrospective, and some cases were excluded due to missing data. The possible role of residual confounding factors and bias must be considered.

Wider implications of the findings: Pregnancies following HRC-FET have higher risks of hypertensive disorders of pregnancy and placenta accreta. The association between endometrium preparation method and these placenta related obstetrical complications merits further attention.

Trial registration number: not applicable.

O-284 Pregnancy complications following assisted reproductive techniques - disentangling the role of multiple gestation

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[3]*Karolinska Institutet, Department of Epidemiology & Biostatistics, Stockholm, Sweden*

Study question: Is there a risk of pregnancy complications following ART among infertile couples, and to what extent is this independent versus mediated by multiple gestations?

Summary answer: Multiple gestations are responsible for a large proportion of common pregnancy complications following use of ART, but nearly none of the risk of placenta previa.

What is known already: Several of the pregnancy complications observed after use of ART are also common complications of multiple gestation, and since multiple gestations are more frequent following ART many studies choose to assess ART risks in singletons and multiples separately. This approach can induce bias if attention is not paid to potential common causes of the multiple gestations mediator and the pregnancy complications outcome, and it does not provide insight into how much of the effect is through the different pathways. Assessing the influence of intervention to reduce multiple gestations should be informative for both clinical practice and public health.

Study design, size, duration: Linkage of national registers allows population-based cohort studies based on prospectively collected data. The Medical Birth register consistently covers 96-99% of all births in Sweden. From the nearly 1.8 million births recorded in the register between 1996 and 2013 we selected the 9.9% (N = 174 067) pregnancies that occurred to couples with known trouble conceiving (clinical infertility).

Participants/materials, setting, methods: Information regarding infertility and fertility assistance was available from antenatal self-reports, medical records, and procedural information from fertility clinics. We used logistic

regression to estimate odds ratios (OR) and their 95% confidence intervals (CI), and decomposing the total effect into direct and mediated effects to estimate the proportion mediated by multiple gestations. Sweden's 2003 implementation of single-embryo transfer (SET) policy further allowed direct observation of an intervention to curb the occurrence of multiples following ART.

Main results and the role of chance: Although the prevalence of multiples from ART was substantially reduced by SET recommendations (13% in 1996-2003 to 6% in 2004-2013) it remained 4 times higher than in non-assisted pregnancies. Prior to the intervention, pregnancies achieved with ART had higher odds of all studied complications except gestational diabetes when compared with non-assisted pregnancies to infertile couples. Associations with placenta previa (OR 2.91, 95% CI 2.31–3.68) and placental abruption (OR 2.57, 95% CI 1.99–3.33) were found almost entirely independent of multiple gestations. In contrast, the majority of the associations with preterm birth (OR 2.79, 95% CI 2.28–3.42), cesarean delivery (OR, 1.79, 95% CI 1.54–2.08) and pre-eclampsia (OR 1.28, 95% CI 1.12–1.46) were mediated by multiple gestations (87%, 84% and 100% of the effect mediated, respectively). Both direct and mediated pathways contributed to the remaining positive associations with chorioamnionitis, labor induction, and postpartum hemorrhage. While ART remained associated with all noted complications also after the intervention (2004-2013), the excess risk dropped markedly for the complications found largely mediated by multiple gestations (preterm birth, cesarean and pre-eclampsia). In contrast, the excess risk of placenta previa was largely unchanged.

Limitations, reasons for caution: Some complications may be more likely detected or recorded in women having undergone ART and/or carrying multiples. Reassuringly, one of the more susceptible such diagnoses, gestational diabetes, was not more common in treated pregnancies, nor in pregnancies with multiples (among infertile) in our study population.

Wider implications of the findings: Interventions to curb the occurrence of multiples may greatly reduce the risk of several pregnancy complications following use of ART. Finding some risk independent of multiple gestations, especially for severe placental complications, demonstrates that pregnancies achieved after ART may be considered high-risk pregnancies irrespective of number of fetuses.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 74: FACTORS AFFECTING FERTILITY POTENTIAL PCOS WOMEN

Wednesday 4 July 2018 Room 111 + 112 14:00–15:15

O-285 Endometrial expression of stanniocalcin-1 (STC-1) during the menstrual cycle in healthy women and in women with polycystic ovary syndrome (PCOS)

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Study question: How is stanniocalcin-1 (STC-1) expressed in normal human endometrium and is it altered in women with polycystic ovary syndrome (PCOS)?

Summary answer: STC-1 was dysregulated in PCOS endometrium exerting lower expression in secretory phase and blunted response to 8-bromo-cyclic adenosine monophosphate (8-Br-cAMP) and hypoxia compared with controls.

What is known already: STC-1 has been shown to be one of the receptivity markers but also involved in cancer development. Endometrial STC-1 was recently reported to be dysregulated in endometriosis, a condition with endometrial progesterone resistance and inflammation that characterize PCOS endometrium. Given that women with PCOS present with subfertility, pregnancy complications and increased risk for endometrial cancer, we investigated STC-1 expression in PCOS endometrium.

Study design, size, duration: Prospective case-control study.

Participants/materials, setting, methods: The material consisted of prospectively collected endometrial tissue biopsies from healthy controls (n = 65) and in women with PCOS (n = 38). This sample set included endometrial biopsies from two clinical trials; 8 endometrial samples from control women using hormonal contraceptives for nine consecutive weeks and 14 endometrial samples from over-weight women with PCOS before and after 3-month life style intervention. The samples were investigated by immunohistochemistry, *in situ* hybridization, quantitative RT-PCR and *in vitro* cell culture.

Main results and the role of chance: In normal human endometrium, STC-1 was upregulated in glandular epithelium (GE) in early and mid-secretory phase (ESE and MSE) compared with proliferative phase (PE). Through all cycle phases, endometrial stromal cells showed the highest STC-1 expression in late secretory phase (LSE) ($P = 0.01$) whereas the expression was quiescent during the use of hormonal contraceptives. Interestingly, in non-PCOS women obesity seemed to increase the STC-1 expression in secretory phase ($P = 0.047$). Women with PCOS had comparable STC-1 expression in proliferative phase (PE) as controls but the rise in expression towards the secretory phase (SE) was insignificant. During induced decidualization *in vitro*, estrogen or progesterone (alone or in combination) were not able to trigger STC-1 expression in cultured stromal cells. However, decidualization with 8-Br-cAMP induced high STC-1 response in control stromal cells, whereas in women with PCOS the expression was significantly lower at mRNA ($P < 0.0001$) and protein ($P < 0.01$) levels, despite of the normal levels in classic decidualization markers. Furthermore, hypoxia triggered high STC-1 protein expression in control stromal cells that was blunted in stromal cells from women with PCOS ($P < 0.001$).

Limitations, reasons for caution: The study consists of rare endometrial tissue samples from several cycle phases and from clinical trials. However, the *in vitro* testing did not consider the role of hyperandrogenism or hyperinsulinemia, common in PCOS. Further studies are also warranted to elucidate the mechanisms behind the altered endometrial STC-1 expression in PCOS.

Wider implications of the findings: According to our findings, despite of successful decidualization in women with PCOS, aberrant STC-1 expression was observed possibly reflecting impaired and desynchronized endometrial function during implantation process. Moreover, blunted STC-1 in PCOS endometrium may promote endometrial cancer through defective rescue mechanism related to hypoxia.

Trial registration number: The study of clinical trial testing hormonal contraceptives: SYLVI ClinicalTrials.gov Identifier: NCT02352090, Karolinska Institutet ethical approval number is: IRB 2008/865-32

O-286 Clomiphene citrate or gonadotrophins in women with WHO type II anovulation and CC failure; a role for EMT?

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Study question: Is endometrial thickness (EMT) a biomarker to select women for clomiphene citrate (CC) or gonadotrophins in women with WHO type II anovulation and CC failure?

Summary answer: EMT measured in the sixth ovulatory CC cycle can be used as biomarker to decide on continuing CC or to switch to gonadotrophins.

What is known already: CC has been a long standing first line treatment in women with normogonadotropic anovulation. We recently showed that in women with normogonadotropic anovulation and CC failure, a switch to gonadotrophins increases the chance of live birth over continued treatment with CC with 10%. This may be due to a thinner EMT after CC compared to gonadotrophins. It is unclear if EMT during ovulation induction with CC is a marker to identify women that benefit from one or the other treatment.

Study design, size, duration: Between Dec 8, 2008, and Dec 16, 2015, 666 women with CC failure (not pregnant after 6 ovulatory cycles) were randomly assigned to receive an additional six cycles with CC (335) or six cycles with gonadotrophins (n = 331). The primary outcome was conception leading to live birth within 8 months after randomisation. In 380 women EMT (n = 190 CC, n = 190 gonadotrophins) was measured before randomisation during their sixth cycle.

Participants/materials, setting, methods: We randomised women aged 18 years and older with normogonadotrophic anovulation not pregnant after six ovulatory cycles of CC from 48 Dutch hospitals. EMT was determined in the sixth cycle of CC before randomisation. An EMT of 8 mm was used as cut-off value. We evaluated if EMT at baseline had interaction with the treatment effect. To account for multiple cycles over time we used cox proportional hazard analysis to calculate hazard rates (HR).

Main results and the role of chance: The EMT of the last CC cycle before randomisation was available in 380 women, of whom 190 were allocated to CC and 190 to gonadotrophins. Among 169 women (44%) with EMT < 8 mm, the live birth rates were 33% with CC versus 54% with gonadotrophins, respectively (RR 0.64, 95% CI 0.47 – 0.87), while in 211 women (56%) with EMT > 8 mm, the live birth rates were 53% with CC versus 48% with gonadotrophins, respectively (RR 1.11, 95% CI 0.83 – 1.50).

Our analysis showed interaction between EMT and treatment success (p = 0.02). The hazard rate (HR) for a live birth in women with CC was 1.37 (95% CI 1.01–1.84) for EMT > 8 versus EMT < 8. Mean time to live birth were 5.4 and 5.8 cycles for EMT > 8 and EMT < 8 respectively (log rank test p = 0.03). The HR for live birth in women treated with gonadotrophins was 0.85 (95% CI 0.66–1.10) for EMT > 8 versus EMT < 8. Mean time to live birth were 5.2 and 4.9 cycles for EMT > 8 and EMT < 8 respectively (log rank test p = 0.2).

Limitations, reasons for caution: This was a post-hoc analysis, and EMT measurements at baseline were not available for all included women. There were many different doctors that performed the ultrasound measurements.

Wider implications of the findings: In women with CC-failure and EMT < 8 mm in the sixth cycle, a switch to gonadotrophins improves fertility outcomes over continuing CC, while in women with CC-failure and EMT > 8 mm continuing CC and switching to gonadotrophins are equally effective. This knowledge can inform clinical decision making.

Trial registration number: Netherlands Trial Register, number NTR1449

O-287 The expression and regulation of inflammatory factor interleukin-18 in the proliferative phase endometrium of PCOS

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Study question: How does the inflammatory factor IL-18 be up regulated in the endometrium of patients with PCOS and the regulatory pathway that mediates this relationship?

Summary answer: SIRT1 interacted with C/EBP-β to activate the SIRT1/C/EBP-β/IL-18 signaling pathway, resulting in the up-regulation of IL-18 expression in the endometrium of patients with PCOS.

What is known already: Polycystic ovary syndrome (PCOS) is a multisystem, reproductive-metabolic disorder characterized by polycystic-appearing ovaries, hyperandrogenism, and irregular menstruation. Recent studies have indicated that patients with PCOS exhibit chronic inflammation, which might be correlated with the pathogenesis of the disease. According to our previous studies, the inflammatory factor IL-18 is increased both in the serum and the endometrium of patients with PCOS. CCAAT enhancer-binding protein (C/EBP) beta is an important transcription factor, and it may up-regulate the expression of IL-18 in the endometrium. However, the regulatory effects of C/EBP-β on IL-18 and the signaling pathway mediating these effects have not been examined.

Study design, size, duration: Endometrial tissue samples of eighteen women with PCOS and eighteen healthy women were obtained during hysteroscopic surgery. In the present study, we explored the expression of C/EBP-β and SIRT1 in the proliferative phase of the endometrium in women with and without PCOS. We investigated whether C/EBP-β regulates IL-18 expression and explored the underlying signaling mechanism that IL-18 was up-regulate in the endometrium of PCOS patients.

Participants/materials, setting, methods: Endometrial tissue samples were obtained during hysteroscopic surgery.IL-18 protein expression was analyzed using immunohistochemistry and western blotting, and IL-18 mRNA expression was analyzed using quantitative real-time polymerase chain reaction. C/EBP-β and Sirt1 protein combination was verified with co-immunohistochemistry. The correlation of C/EBP-β and IL-18 was demonstrated with Dual-luciferase assay. SIRT1 and C/EBP-β mRNA expression was analyzed using quantitative real-time polymerase chain reaction.

Main results and the role of chance: IL-18 was significantly increased in the endometrium of women with PCOS compared with normal groups. In overweight women, IL-18 was obviously over-expressed in the PCOS group compared with the healthy group. However, in normal weight women, there was no statistically significant difference between the two groups, and there was no significant difference in IL-18 expression in PCOS patients with or without insulin resistance. We demonstrated the interaction of CEBP-β and IL-18 by Dual-luciferase assay, and we used CO-IP assay demonstrated the combination of SIRT1 and CEBP-β. Sirt1 and C/EBP-β proteins were over-expression in the endometrium of PCOS patients.

Limitations, reasons for caution: As we known, PCOS patients have irregular menstruation, so it's difficult to obtain the secretary phase endometrium. We only detected and analysed proliferative phase endometrium and larger sample size was needed to carry on.

Wider implications of the findings: We detected the up-regulation of C/EBP-β and SIRT1 in the proliferative endometrium of patients with PCOS. Additionally, SIRT1 interacted with C/EBP-β to activate the SIRT1/C/EBP-β/IL-18 signaling pathway, resulting in the up-regulation of IL-18 expression, and which may related with endometium receptivity abnormality of PCOS patients.

Trial registration number: None.

O-288 Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome - A Cochrane Systematic Review

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Study question: Are aromatase inhibitors (letrozole) superior to other agents for ovulation induction for subfertile women with anovulatory PCOS?

Summary answer: Letrozole improves live birth and pregnancy rates in subfertile women with anovulatory PCOS, compared to clomiphene citrate.

What is known already: Polycystic ovary syndrome (PCOS) is the most common cause of infrequent periods (oligomenorrhoea) and absence of periods(amenorrhoea). It affects about 4% to 8% of women worldwide and often leads to anovulatory subfertility. Aromatase inhibitors (AIs) are a novel class of drugs that were introduced for ovulation induction in 2001. Over the last ten years clinical trials have reached differing conclusions as to whether the AI letrozole is at least as effective as the first-line treatment clomiphene citrate (CC).

Study design, size, duration: We searched the following sources from inception to November 2017 to identify relevant randomised controlled trials (RCTs): the Cochrane Gynaecology and Fertility Specialised Register, the Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, PsycINFO, Pubmed, LILACS, Web of Knowledge, the WHO clinical trials register and Clinicaltrials.gov.

We included all RCTs of aromatase inhibitors used alone or with other medical therapies for ovulation induction in women of reproductive age with anovulatory PCOS.

Participants/materials, setting, methods: Two review authors independently selected trials, extracted the data and assessed trial quality. Studies were pooled where appropriate using a fixed effect model to calculate pooled odds ratios (ORs) and 95% confidence intervals (CIs) for most outcomes and risk differences (RDs) for ovarian hyperstimulation syndrome (OHSS). The primary outcomes were live birth and OHSS. Secondary outcomes were pregnancy, miscarriage and multiple pregnancy. The quality of the evidence for each comparison was assessed using GRADE methods.

Main results and the role of chance: We included 42 RCTs (7935 women). The aromatase inhibitor letrozole was used in all studies.

Live birth rate was reported in 13 RCTs, including 2954 women. The birth rate was higher for letrozole compared to clomiphene (with or without adjuncts) followed by timed intercourse (OR 1.68, 95% CI 1.34 to 1.99, I^2 = 0%, NNT = 10).

There was no evidence of a difference in OHSS rates when letrozole (with or without adjuncts) was compared with clomiphene citrate (with or without adjuncts) followed by timed intercourse (RR 0.00, 95% CI -0.01 to 0.00, twelve RCTs, n = 2536).

Pregnanc rate was reported in 25 RCTs, including 4629 women. The pregnancy rate was higher for letrozole compared to clomiphene (with our without adjuncts) (OR 1.56, 95% CI 1.37 to 1.78, I^2 = 1%).

Miscarriage rate was reported in 18 studies, including 3754 women. There was no evidence for a difference between letrozole or clomiphene with or without adjuncts (OR 0.94, 95% CI 0.70 to 1.26, I^2 = 0%).

Multiple pregnancy rate was reported in 16 studies, including 3519 women. There was insufficient evidence to show a difference between letrozole or clomiphene with our without adjuncts (OR 0.73, 95% CI 0,43 to 1.24, I^2 = 0%).

Limitations, reasons for caution: The quality of the evidence was moderate for live birth and pregnancy outcomes. The reasons for downgrading the evidence were possible publication bias and the tendency for studies that reported live birth to report higher clinical pregnancy rates in the letrozole group than studies that failed to report live birth.

Wider implications of the findings: Our findings suggest that letrozole is superior to clomiphene citrate for the treatment of subfertility in women with PCOS who have had no previous treatment for ovulation induction or are resistant to clomiphene citrate.

Trial registration number: not applicable.

O-289 Higher probability of pregnancy after frozen-embryo transfer using a freeze-all policy compared to fresh-embryo transfer in high, but not in normal responder patients: a meta-analysis

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Study question: Is frozen embryo transfer (ET) using a freeze-all policy associated with a higher probability of pregnancy when compared to fresh ET?

Summary answer: A higher probability of pregnancy after frozen ET using a freeze-all policy compared to fresh ET is present in high, but not in normal-responder patients.

What is known already: It has been hypothesized that freezing all embryos in a fresh in-vitro fertilization (IVF) cycle and deferring embryo transfers in subsequent cycles may provide a more physiological endometrial environment for embryo implantation when compared to fresh ET. Several relevant randomized controlled trials (RCTs) have recently been published, evaluating the effectiveness of frozen ET using a freeze-all policy compared to fresh ET either in high or in normal responder patients without, however, leading to solid conclusions.

Study design, size, duration: A systematic review and meta-analysis was performed aiming to identify RCTs comparing frozen ET, using a freeze-all policy, to fresh ET. For this purpose, a relevant literature search was carried out until January 2018. Primary outcome measure was achievement of pregnancy, expressed as ongoing pregnancy/live birth rate after the first ET, while severe ovarian hyperstimulation syndrome (OHSS) was evaluated as a secondary outcome.

Participants/materials, setting, methods: Six eligible RCTs were identified including 4831 patients that evaluated frozen ET using a freeze-all policy versus fresh ET either in high responder patients (n = 3), or in normal responder patients (n = 3). Meta-analysis of weighted data using random and fixed effects model was performed. Results are reported as relative risk (RR) with 95% confidence interval (CI).

Main results and the role of chance: The eligible RCTs were published between 1999 and 2018 and the number of patients included in the study ranged from 122 to 2157 patients (median 460). Three studies were conducted in Asia (4447 patients), two studies in USA (259 patients) and a single study in Europe (125 patients). In high responder patients, a significantly higher ongoing pregnancy/live birth rate was observed after a frozen as compared to a fresh ET (RR: 1.16, 95% CI: 1.05 to 1.29; fixed effects model; heterogeneity: I^2 = 0%; n = 1755 patients, respectively). This difference was not present in normal responder patients (RR: 1.06, 95% CI: 0.90 to 1.23; random effects model; heterogeneity: I^2 = 51%; n = 3076 patients, respectively), or overall (RR: 1.09, 95% CI: 0.98 to 1.22; random effects model; heterogeneity: I^2 = 49%; n = 4831 patients, respectively). The risk of moderate/severe OHSS was significantly lower after a frozen as compared to a fresh ET in high responder patients (RR: 0.19, 95% CI: 0.10 to 0.35; fixed effects model; heterogeneity: I^2 = 0%; n = 1633 patients, respectively), in normal responder patients (RR: 0.39, 95% CI: 0.19 to 0.80; fixed effects model; heterogeneity: I^2 = 0%; n = 2939 patients, respectively) as well as overall (RR: 0.25, 95% CI: 0.15 to 0.40; fixed effects model; heterogeneity: I^2 = 9%; n = 4572 patients, respectively).

Limitations, reasons for caution: A considerable heterogeneity was present in the cryopreservation protocols used in the eligible studies. Moreover, gonadotrophin-releasing hormone(GnRH) agonist was not used for triggering final oocyte maturation in the freeze-all arm in the eligible RCTs, which might have further increased the difference in safety between the freeze-all and fresh ET policies.

Wider implications of the findings: The results obtained might be explained by an increased deterioration of endometrial receptivity due to the more intense ovarian response in high as compared to normal responders. Concomitantly, the availability of more embryos in high as compared to normal responders might counteract whatever adverse effect cryopreservation may exert on embryos.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 75: WHAT'S NEW IN THE FIELD OF IMPLANTATION AND EARLY PREGNANCY
Wednesday 4 July 2018 Room 113 + 114 + 115 14:00–15:15

O-290 Endometrial receptivity assessment using a transcriptomic approach for personalized embryo transfer

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Study question: The aim of this study was to optimize pregnancy outcome using personalized embryo replacement according to the evaluation of endometrial receptivity.

Summary answer: Individual evaluation of endometrial receptivity allows a personalized patient care management and improves pregnancy outcome for RIF patients.

What is known already: Many approaches for human endometrial receptivity including microarray has been previously reported. However, efficiency of the tests according to the clinical results is still debated. The aim of this study is to evaluate the endometrial receptivity (ER) using the **Win**dow implantation **Test** (Win Test) in RIFs patients who have had at least 3 implantation failures. The principal of concept which consisting for screening for 13 specific genes of implantation window.

Study design, size, duration: Endometrial biopsies are performed during the implantation windows 7-9 days after the LH surge or 6-9 days after progesterone administration under natural cycles and hormone replacement therapy (HRT), respectively. Then, according to the Win-test result, the transfer strategy was: transfer at the blastocyst stage at the specific day where endometrium has been diagnosed as 'receptive'; transfers at day 2/3 post-fertilization when endometrium is said 'partially receptive'. For non-receptive samples, a second evaluation is proposed later.

Participants/materials, setting, methods: 665 RIF patients with a number of previous failed attempts and number of non-implanted embryos after fresh or frozen embryo transfer cycles was 4.3 ± 2.5 and 7.3 ± 4.2, respectively, were included. RNAs from biopsies were extracted and determination of the mRNA expression levels of 13 genes predictive of endometrial receptivity referred in the Win-Test by qRT-PCR. Clinical pregnancy was defined by visualization of a gestational sac with a positive foetal heart beat.

Main results and the role of chance: Analyses of endometrial receptivity status (n = 1237 biopsies) in a large cohort of 665 RIF patients (age mean ± SD: 37.5 ± 4.6 years) revealed that a strong inter-patient variability i) of the moment of the opening of the implantation window with a delay for the majority of RIF patients between 1 to 4 days, as well as ii) of the duration of this implantation. Then, as soon as the cycle day where endometrium was said receptive, a personalized embryo transfer can to be perform during a subsequent attempt in the respect of the synchronization of the fœto-maternal dialogue. 75 % of personalized embryo transfer have been performed under HRT and 25 % under natural cycles. The implantation rate before and after personalized embryo transfers was 5.5 *vs* 22.7 %, respectively ($p < 0.0001$). Using this strategy, the positive b-hCG and clinical pregnancy rate per cycle in this group of patients with previous RIFs were 39.4 and 25.5 %, respectively (65 and 27 % with blastocysts and day-3 embryo transfers).

Limitations, reasons for caution: The benefits of this innovative strategy will be analyzed in PGS patients.

Wider implications of the findings: This finding demonstrated that for the majority of RIF patients, a delay in their opening implantation window was responsible for the implantation failure. Personalized embryo transfer according to the specific cycle day where endometrium is said receptive improves both implantation rate and pregnancy outcome in RIF patients.

Trial registration number: Not applicable

O-291 Live birth rate after frozen-thawed embryo transfer according to endometrial preparation: mild gonadotropin ovarian stimulation leads to increased success compared to artificial cycle

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Study question: Which endometrial preparation allows a better outcome after frozen-thawed embryo transfer (FET) between mild stimulated (OS) and artificial cycles (AC)?

Summary answer: Despite a similar pregnancy rate, live birth rate (LBR) was significantly higher with mild OS than with AC preparation, even after adjusting for potential confounders.

What is known already: The best protocol to prepare the endometrium for FET is still debated. A recent meta-analysis concluded that outcomes with natural cycles (NC) were similar to those with AC, but that mild OS may be promising. Some authors described a higher pregnancy rate in AC versus NC, but no significant difference in LBR was found due to higher pregnancy loss rate in AC. A deficient luteal phase in AC group could explain an asynchronised window of implantation leading to pregnancy losses. With OS preparation, a secreting corpus luteum leads to more constant progesterone level. Moreover, hCG triggering can sustain luteotropic effect.

Study design, size, duration: We conducted a retrospective study including all 1021 FET(issued from 1313 started cycles) performed in one French academic fertility centre from January 2013 to December 2016. We compared clinical pregnancy rate (PR), ongoing PR at 12 weeks of gestation (WG), and LBR, according to mild OS or AC endometrial preparation.

Participants/materials, setting, methods: In OS group, gonadotropins (37.5 to 75 IU/day) were initiated between Day 2 and 10 followed by r-hCG triggering. Vaginal micronized progesterone (200 mg/day) was given for 6 WG if pregnancy. In AC group, estradiol orally (4 or 6 mg/day) or transdermally (200 µg/3days) started on Day 1. Vaginal micronized progesterone (600 mg/day) was added to E2 for 12 WG if pregnancy. FET was performed according to embryos stage. Data were analysed using a multiple regression model.

Main results and the role of chance: Among 1021 FET, 357 (35%) patients received OS and 664 (65%) AC preparation. No difference in cycle cancellation rates was observed between both groups. They were comparable for maternal ages (at transfer and at freezing), body mass index, history of recurrent pregnancy losses, viral status and smoking habits. As expected, patients in AC group suffered more from endometriosis (18.5% vs 12.9%; p = .021) and Polycystic Ovarian Syndrome (PCOS) (21.7% v 10.9%; p<.0001) than patients in mild OS group in which masculine infertility was more frequent (49.3% vs 41.1%; p=.012). There was no difference between groups concerning endometrial thickness, number of embryos transferred, development stage at FET, cryopreservation technique, duration of cryopreservation, number of intact blastomeres post thawing for cleaved embryos, embryos' survival. Despite a similar clinical PR (24.4% vs 20.8%; p=.189), the ongoing PR at 12 WG was significantly increased in the OS compared to AC group (17.9% vs 11%; p=.002), leading to an increased LBR (17.1%vs 9.8%; p<.001). After adjusting for parameters usually linked to early pregnancy losses (woman age at freezing, smoking status, PCOS, endometriosis and previous miscarriages), the results remained significant.

Limitations, reasons for caution: Retrospective design of the study may potentially have introduced bias, and the size of the cohort, although more than 1000 cycles, could induce a lack of power to analyze subgroups.

Wider implications of the findings: In the light of LBR results and specific concerns about effects of extended hormonal treatment on periconceptional environment, the first intention endometrial preparation for FET should be OS in most cases. Nevertheless, if AC, a potential defect of luteal phase must be tracked, and treatment adapted to avoid pregnancy losses.

Trial registration number: Not applicable.

O-292 Early stop of progesterone supplementation after confirmation of pregnancy in IVF/ICSI fresh embryo transfer cycles of poor responders does not affect pregnancy outcome

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Study question: Does early stop of progesterone supplementation after confirmation of pregnancy affect reproductive outcomes of patients with poor ovarian response (POR) in IVF/ICSI-embryo transfer (ET) cycles?

Summary answer: Women with POR using hCG as luteal phase support (LPS) in fresh ET cycles can safely discontinue progesterone supplementation after positive pregnancy test.

What is known already: Some studies found no significant differences in miscarriage, ongoing pregnancy and live birth rates between general IVF patients who underwent early progesterone cessation and those who received progesterone until gestational age of 7-9 weeks. However, the usual practice of most physicians is to continue progesterone supplementation rather than to take a risk of miscarriage from an earlier stop.

Study design, size, duration: A retrospective cohort study was conducted for patients in IVF/ICSI and fresh ET cycles in single center between January 2010 and June 2016 (N = 4,320 cycles). Patients with POR using hCG as LPS were investigated (N = 480 cycles). Those had negative pregnancy test, ectopic pregnancy or loss of follow-up were excluded (N = 324 cycles). We used the Bologna criteria to define POR.

Participants/materials, setting, methods: The pregnant women of POR were included in this study (N = 156 cycles). They were divided into two groups: one stopped LPS from day of positive pregnancy test (N = 100 cycles), and the other kept progesterone supplementation until 7-9 gestational weeks (N = 56 cycles). We compared implantation, clinical pregnancy, ongoing pregnancy and live-birth rates between the two groups. The data were analyzed with independent t-test, Chi-square test and multivariable logistic regression.

Main results and the role of chance: There were no significant differences in age, body mass index, gravida, parity, causes of infertility, stimulation protocols, hormone data, number of embryos transferred and endometrial thickness between the early stop group and control group. The adjusted odds ratios (AORs) with 95% confidence intervals (CI) were calculated by adjusting for the confounders of stimulation duration and number of oocytes retrieved. After adjustment with multivariable logistic regression analysis, the implantation percentages (81.0% vs. 73.2%, AOR = 1.44, 95% CI: 0.64-3.22), clinical pregnancy rates (55.0% vs. 57.1%, AOR = 1.72, 95% CI: 0.35-1.45), ongoing pregnancy rates (47.0% vs. 46.4%, AOR = 0.86, 95% CI: 0.43-1.71) and live-birth rates (44.0% vs. 46.4%, AOR = 0.75, 95% CI: 0.37-1.50) were not statistically different between early stop group and the control group.

Limitations, reasons for caution: Some limitations included the retrospective nature and somewhat small sample sizes. Whether the early stop of progesterone can be applied for every patient or not for patients of POR using progesterone without hCG for LPS is not studied in this work. This condition may deserve further research.

Wider implications of the findings: We may suggest that an early stop could be based on endogenous activity of corpus luteum by detection of serum progesterone and estradiol levels on the day of pregnancy test.

Trial registration number: Not applicable

O-293 CSRP2BP is a novel marker in trophoblast development and preeclampsia pathogenesis

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Study question: What is the potential function of Cysteine-Rich Protein 2-Binding Protein (CSRP2BP) in the pathogenesis of preeclampsia(PE).

Summary answer: CSRP2BP plays an important role in the pathogenesis of PE by influencing the function of the trophoblast cells.

What is known already: PE is the leading cause of maternal and perinatal mortality and morbidity. The underlying mechanism is still not completely elucidated. Epigenetic abnormalities might be closely related to the pathogenesis of PE. CSRP2BP is a newly defined histone acetyltransferase, however, the research of its function in human cells is very limited. It will be interesting to explore the potential role of CSRP2BP in the process of early trophoblast development and pathogenesis of PE.

Study design, size, duration: The relationship of CSRP2BP expression and PE was explored in clinical samples. Meanwhile, Lentivirus systems and CRISPR/case9 technology were used to generate loss and gain of function models with trophoblast cell line-HTR8 to study the role of CSRP2BP in the pathogenesis of PE.

Participants/materials, setting, methods: We examined the expression of CSRP2BP in placentas from PE patients and normal controls. CSRP2BP loss and gain of function models were generated in HTR8 cells by using lentivirus and with CRISPR/case9 technology. Transwell assays, scratch-wound assays, EDU, cell apoptosis assays and cell cycle assays were used to examine the function of CSRP2BP in HTR8 cells model. RNA-seq was used to examine the gene expression panel which might be regulated by CSRP2BP.

Main results and the role of chance: CSRP2BP was enriched in the nucleus of trophoblast cells from early pregnant stage. It was significantly decreased in placental tissues of PE patients compared with those in control group by qPCR, western blotting and immunohistochemistry. The current data demonstrated that knockdown of CSRP2BP in HTR8 dramatically decreased cell proliferation, cell migration and invasion. It also showed a significantly lower plate clone formation rate in CSRP2BP knockdown -HTR8 cells compare with the WT HTR8 cells.

Limitations, reasons for caution: Although we show that CSRP2BP play an important role in the function of HTR8 by shRNA, the study of working mechanism of CSRP2BP in PE is still very limited. More studies will be performed to explore its underline mechanism.

Wider implications of the findings: This study was the first time to explore the role of CSRP2BP in PE. It will help us to better understand the pathogenesis of PE, which might be helpful to discover novel therapeutic targets in diagnosis and treatment of PE.

Trial registration number: not applicable.

O-294 Reproductive outcome following hyaluronic acid gel application after D&C in women with at least one previous curettage; long-term outcomes of a multicenter, prospective randomized trail

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Study question: Does intrauterine application of auto-crosslinked hyaluronic acid (ACP) gel following dilatation and curettage (D&C) for miscarriage in women with at least one previous D&C improve reproductive outcome?

Summary answer: Reproductive performance was similar in both groups, with a tendency to a higher pregnancy and live birth rate in women who received ACP gel.

What is known already: Intrauterine adhesions (IUAs) are reported in 19% of women after miscarriage; women with more than one D&C have statistically significant more IUAs compared to women with one D&C. Intrauterine application of ACP gel following D&C for miscarriage in women with at least one previous D&C significantly reduces the incidence and severity of IUAs. Data on reproductive performance following application of ACP gel are lacking.

Study design, size, duration: In the Prevention of Adhesion Post Abortion (PAPA) trial, a multicentre, prospective randomised controlled trail 152 women with an (incomplete) miscarriage or retained products of conception (RPOC) with at least one D&C in history were randomised to D&C plus ACP gel or D&C alone (control group). The study was conducted between December 2011 and July 2015 in 8 centers in the Netherlands. Written and signed informed consent was provided by all participants at inclusion.

Participants/materials, setting, methods: 149 Participants, 77 assigned to the intervention group and 72 to the control group received questionnaires three, six and twelve months after the D&C-procedure. The questionnaire consisted of questions regarding baseline characteristics, current health, interventions received and complications, menstrual pattern, contraceptive use, conception and reproductive outcomes. In women willingly to conceive, miscarriage, ongoing pregnancy and live birth rates were assessed. non-responders were comtacted to minimize follow-up.

Main results and the role of chance: The overall response rate for the questionnaires was 94% at twelve months. There was no significant difference in the menstrual characteristic of the responders in both groups. In the intervention group 84.9% (62/73) versus 88.6% (62/70) women in the control group attempted to conceive. After twelve months 71% conceived in the intervention group compared to 59.7% in the control group, $p = 0.56$. There was no statistical difference in the cumulative miscarriage, ongoing and live birth rate between the two groups. The median time to conception was 5.5 months in the intervention group versus 7.1 months in the control group, hazard ratio (HR): 1.40, 95% Confidence interval (CI): 0.92 – 2.14, $p = 0.12$). Time to conception that resulted in life birth did not reach statistical significance (HR: 1.40, 95% CI: 0.76 – 2.57, $p = 0.28$).

Limitations, reasons for caution: IUAs encountered during follow-up were treated, it was considered unethical not to perform adhesiolysis because of the possible negative impact on fertility and pregnancy outcomes. This RCT was powered to detect differences in occurrence of IUAs between the groups. No power analysis was performed for reproductive outcomes.

Wider implications of the findings: Intrauterine application of ACP gel immediately following D&C for miscarriage reduces the incidence and severity of IUAs. In this study, we did not find any statistical significant, probalby due to the removal of existing IUAs during the routinely planned postoperative hysteroscopy and insufficient powerto detect relevant differences.

Trial registration number: NTR3120 (Dutch Clinical Trail Registry).

SELECTED ORAL COMMUNICATIONS

SESSION 76: ART SUCCESS AND ITS FACTORS

Wednesday 4 July 2018 Room 117 14:00–15:15

O-295 Transmission of oocyte DNA damage to preimplantation embryos after in vivo mouse exposure to daunorubicin and cytarabine

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Study question: Does oocyte DNA damage induced by a previous in vivo mouse exposure to chemotherapy agents is transmissible to preimplantation embryos?

Summary answer: DNA damage was observed in preimplantation embryos issued from mice previously exposed to daunorubicin and cytarabine.

What is known already: In acute leukemia, the emergency to start a chemotherapy don't allow a fertility preservation at the time of diagnosis. Some authors have proposed to cryopreserve mature oocytes or embryos after a controlled ovarian stimulation applied shortly after the induction chemotherapy, which is mainly composed by daunorubicin and cytarabine, and reputated to be less gonadotoxic than alkylant agents. We previously observed DNA damage on mouse oocytes issued from antral follicles exposed in vivo to daunorubicin and cytarabine. Little is known about the risk of transmission of oocyte DNA damage to preimplantation embryos after fecundation of oocytes recently exposed to chemotherapy.

Study design, size, duration: By three time, two groups of mice ($n = 11$) were exposed for four days to cytarabine (10 mg/kg IP) or every two days to daunorubicin (1 mg/kg IV). Each group was compared with a negative control group ($n = 11$) and with a positive control group ($n = 11$) injected with cyclophosphamide (75 mg/kg IP). Females were mated one week after exposure and preimplantation embryos were collected by flushing the oviducts.

Participants/materials, setting, methods: 4 weeks female CD1 mice were mated one week after exposure for studying embryos conceived from oocytes exposed to chemotherapy at late pre-antral stage of follicular development. Cytotoxicity has been assessed by ovulation and fertilization rates and by embryo morphology. DNA embryonic damage was assessed by: (i) alkaline comet assay to quantify the tail DNA (ii) fluorescent immunohistochemical staining in blastomeres to quantify accumulating γH2AX foci.

Main results and the role of chance: In mouse, a recent exposure to daunorubicin and cytarabine did not alter the ovarian response to controlled ovarian stimulation with no adverse impact on the fertilization rate and the number of embryo conceived. Ovulation and fertilization rates in mice previously exposed to daunorubicin and cytarabine were similar to those in our negative control group. One week after exposure, we observed with the comet assay a significant increase of embryonic DNA damage after exposure to daunorubicin (16.57 ± 1.3, $p = 0.0003$) and cytarabine (16.46 ± 1.4, $p = 0.0003$) Vs 26.16 ± 2.5 after cyclophosphamide exposure ($p < 0.0001$) and $7{,}01 \pm 1{,}1$ in negative control group exposed to an injection of sterile saline solution. The analysis γ-H2AX on embryos showed a significant increase of foci corresponding to DNA double-strand breaks, after exposure to daunorubicin (7.97 ± 1.1; $p = 0.001$), cytarabine (6.47 ± 0.7, $p = 0.0039$), cyclophosphamide (5.92 ± 0.9; $p = 0.0148$) compared with negative control group ($2{,}8 \pm 0{,}7$).

Limitations, reasons for caution: Mouse oocyte DNA is not exactly similar to human oocyte DNA, and would be more sensitive to genotoxic effects of chemotherapy agents. After chemotherapy, the kinetic of DNA repair before and after fertilization has to be studied by further assays in exposed oocyte and in embryos.

Wider implications of the findings: DNA damage in preimplantation embryos conceived from oocytes exposed to chemotherapy at late pre-antral stage of follicular development lead us to hypothese a transmission of oocyte DNA damage to preimplantation embryo. In acute leukemia, we strongly advise to not cryopreserve mature oocytes or embryo early after induction chemotherapy.

Trial registration number: Experimental protocols and animal handling procedures were reviewed by the French National Ethics Committee on Animal Experimentation (N° 2017033010523688).

O-296 Dynamic regulation of germline-soma communication during oocyte meiotic maturation

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Study question: What happens to the structures coupling the oocyte to the surrounding follicular cells during meiotic maturation? If there are changes, which molecular mechanisms regulate them?

Summary answer: TZPs are lost during the oocyte meiotic maturation under the regulation of EGF-ERK MAPK-ROCK pathway in the cumulus-cells as well as nuclear maturation in oocyte.

What is known already: During the oocyte in mammals, cytoplasmic extensions termed transzonal projections (TZPs) emanate from the granulosa cells, traverse the zona pellucida, and contact the oocyte plasma membrane, enabling transmission via gap junctions of essential signals and metabolites from the granulosa cells to the growing oocyte. During maturation, gap junctional communication is shut off, and TZPs no longer couple the mature metaphase II egg to the adjacent granulosa cells. These results suggest that a mechanism is activated during maturation that leads to the loss of TZPs, thus freeing the oocyte from somatic regulatory signals.

Study design, size, duration: Cumulus-oocyte complexes (COCs) were obtained from antral follicles of mice and incubated under conditions that promote maturation. TZP-numbers were assessed at different time-points. Drugs targeting specific signaling pathways and developmental progression of the cumulus granulosa cells and oocyte were used to identify the to identify molecular mechanisms and relative role of the two cell types in regulating these changes.

Participants/materials, setting, methods: TZPs were stained using fluorochrome-conjugated phalloidin, an actin filament-binding protein, and imaged using high-resolution confocal microscopy. TZPs numbers were quantified using customized mathematical function. Pharmacological manipulation of oocyte meiotic maturation, and ERK MAP kinase and Rho-associated protein kinase activities was used to identify mechanisms that trigger TZPs loss during oocyte maturation. All experiments were performed a minimum of three times.

Main results and the role of chance: When COCs were incubated in the presence of epidermal growth factor (EGF) to reproduce the physiological mechanism of maturation, TZPs underwent retraction, forming large foci of actin in the cumulus granulosa cells, such that fewer than 10% remained by 8 hr after maturation initiation. Strikingly, although β-catenin and E-cadherin were lost from the oocyte plasma membrane during maturation, EGF-stimulated retraction of TZPs was unaffected when oocyte maturation was prevented using inhibitors targeting either cyclin-dependent kinases or phosphodiesterase 3 A, indicating that retraction does not require oocyte maturation. In contrast, it was almost completely prevented by U0126, an inhibitor of the ERK MAP kinase signalling pathway. Moreover, cumulus cell-oocyte gap junctional communication was retained in the presence of U0126, indicating that the TZPs remained functional. EGF-driven loss of TZPs was also blocked by Y-27632, an inhibitor of Rho-associated protein kinase (ROCK), suggesting that EGF induces TZPs loss via Rho kinase activity in the cumulus granulosa cells. Altogether, our findings suggest that activation of the EGF-ERK MAPK-ROCK signaling pathway in cumulus granulosa cells triggers the loss of TZPs during oocyte maturation under physiological conditions.

Limitations, reasons for caution: Mechanisms controlling meiotic maturation and TZP dynamics may differ among species. In vitro studies using drugs may not recapitulate the conditions of oocyte development in vivo.

Wider implications of the findings: Inappropriate activation of ERK signalling in the granulosa cells surrounding growing oocytes may trigger precocious uncoupling between the two cell types prior to the oocyte completing preovulatory development, resulting in oocytes incapable of being fertilized and giving rise to a healthy embryo.

Trial registration number: not applicable

O-297 Plasma microRNAs identified as novel markers of successful implantation

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Study question: Can plasma miRNA expression be used as biomarker for 'receptivity' to predict IVF treatment outcome?

Summary answer: 3 distinct miRNAs were found to have a statistically significant expression between the subsequently pregnant and non-pregnant groups.

What is known already: MicroRNAs (miRNAs) are small non-coding RNAs critical for regulating cellular functions. There is growing evidence supporting a role for microRNAs in embryonic and endometrial cell function. miRNAs are present within cells and are exported into plasma where their stability makes them potential biomarkers for health and disease.

Study design, size, duration: This was a prospective cohort study conducted at the IVF unit at Hammersmith Hospital over 6 months. A cohort of 12 patients was used for miRNA analysis selected to exclude confounding factors. Selected patients i) underwent a fresh cycle of IVF, ii) had AMH 5-25, ii) had no endometrial disease and iv) had a single top quality blastocyst transferred. Pregnancy outcomes were recorded.

Participants/materials, setting, methods: Blood samples were collected at two time points, first prior to the start of the IVF treatment cycle and secondly on the day of embryo transfer prior to the procedure. miRNA was extracted using Norgen Biotek kit. miRNA expression profile was obtained using nCounter™ assay. Analysis was performed separately on matching samples before and after the start of treatment and data from women who went to to be pregnant was compered to those who didn't.

Main results and the role of chance: 15 mRNAs were identified to have statistically significant differential expression following IVF treatment compared with matching samples before treatment. 3 distinct miRNAs were found to have a statistically significant expression between the subsequently pregnant and non-pregnant group. Pathway analysis strongly indicated that the identified miRNAs are essential for immune and inflammatory processes. The results have been replicated using PCR.

Limitations, reasons for caution: Due to stringent selection criteria we could only include 12 patients. a replication study is underway and the results have been replicated using PCR.

Wider implications of the findings: To our knowledge, this is the first study that has identified plasma miRNAs as biomarkers of implantation. Once utility is confirmed in replication cohorts these biomarkers have the potential to improve IVF success rates by identifying the cycle with best endometrial receptivity.

Trial registration number: not applicable.

O-298 Progesterone/estradiol (P4/E2) ratio in hormone replacement cycles can predict ongoing pregnancy rates: a prospective study

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Study question: Is progesterone/estradiol (P_4/E_2) ratio useful for predicting ongoing pregnancy rates?

Summary answer: Our data indicate that the P_4/E_2 ratio in hormone replacement cycles can predict ongoing pregnancy rates.

What is known already: P_4/E_2 ratio has been suggested as a marker for prediction of clinical pregnancy rates, but the evidence is conflicting. Furthermore, no prospective study evaluating the clinical value of P_4/E_2 ratio in hormone replacement cycles has been published.

Study design, size, duration: This prospective study evaluated infertile patients (n = 500, median age, 37.8 years old) at our clinic in Japan between July 2016 and December 2017. We evaluated P_4/E_2 ratio in patients undergoing a hormone replacement regimen (Estrogel®: 2.16 mg/day estrogen conversion after 2nd day of menstruation) for thawed blastocyst transfer.

Participants/materials, setting, methods: P_4/E_2 ratio was calculated as P_4 (ng/ml) × 1,000/E_2 (pg/ml). Receiver operating characteristic curve analysis was performed for P_4/E_2 cut-off values detrimental to *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI)-embryo transfer (ET) outcome. Ongoing pregnancy rate was the primary outcome. Mann-Whitney and chi-square tests were used for statistical analyses. Furthermore, multiple logistic regression was used to study associations between variables and the P_4/E_2 ratio. $P < 0.05$ was considered significant. All patients gave informed consent.

Main results and the role of chance: The optimal cut-off value for P_4/E_2 ratio in hormone replacement cycles was 14.75. Ongoing pregnancy rates were significantly higher at a cut-off value >14.75 than at ≤14.75 (48.6% vs. 22.4%, P < 0.001). Furthermore, the P_4/E_2 ratio in hormone replacement cycles was an independent predictor of ongoing pregnancy rates (P = 0.001), regardless of patient's age (P = 0.53), body mass index (P = 0.17), and endometrial thickness (P = 0.06) in multiple logistic regression analysis. Moreover, sensitivity was 81% and specificity was 70%.

Limitations, reasons for caution: Findings in the present study are limited to a hormone replacement regimen used in our patient population.

Wider implications of the findings: P_4/E_2 ratio in hormone replacement cycles would be useful as a predictor of ongoing pregnancy rates.

Trial registration number: UMIN000016919

O-299 Progesterone levels but not endometrial thickness influence the effectiveness of freeze-only versus fresh embryo transfer: a prespecified subgroup analysis of a randomized clinical trial

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Study question: Can progesterone level or endometrial thickness be used to predict whether a freeze-only or fresh transfer strategy will result in a higher ongoing pregnancy rate?

Summary answer: Women with a serum progesterone >1.14 ng/mL undergoing stimulated IVF will likely benefit from use of a freeze-only approach over a fresh embryo transfer.

What is known already: Both progesterone level and endometrial thickness are potential predictors of outcomes during IVF cycles. Higher progesterone levels at trigger have been associated with lower implantation and ongoing pregnancy rates in fresh embryo transfer cycles, and this might be prevented by using a freeze-only strategy. In addition, thinner endometrium might decrease the chances of successful pregnancy. However, data on the usefulness of both biomarkers are not based on randomized comparisons of the two embryo transfer strategies. We evaluated whether progesterone levels and endometrial thickness can be used as biomarkers to guide a decision about whether to use fresh or frozen transfer.

Study design, size, duration: We present a prespecified secondary analysis of data from a randomised controlled trial (RCT) performed in a large IVF unit in Ho Chi Minh City, Vietnam including 782 women undergoing IVF (Vuong LN, et al. NEJM 2018;378:137-147). The primary endpoint was ongoing pregnancy after the first transfer.

Participants/materials, setting, methods: Infertile women scheduled for IVF with ≤1 previous IVF cycle were randomised to a freeze-only strategy (n = 391) or fresh embryo transfer (n = 391). IVF was performed using a gonadotropin-releasing hormone antagonist protocol. Progesterone level was measured on the day of trigger and endometrial thickness (transvaginal ultrasound) on day 3 after ovum pick-up. We explored the impact of both markers by testing for interaction, and by assessing the treatment effect in four quartiles.

Main results and the role of chance: The initial comparison of all patients showed the interaction between serum progesterone and treatment group for ongoing pregnancy after the first embryo transfer was of borderline statistical significance (p = 0.051). When serum progesterone levels were in the third quartile (Q3, 1.14–1.53 ng/mL), ongoing pregnancy rate was significantly higher in the freeze-only versus fresh embryo transfer group (42.9% vs 24.7%, p = 0.01), while in Q4 the ongoing pregnancy rate was numerically, but not statistically significantly, higher in the freeze-only versus fresh transfer group (35.7% vs 26.9%, p = 0.21). Conversely, when serum progesterone was low (Q1, 0.03–0.82 ng/mL), there was a trend towards a lower ongoing pregnancy rate with freeze-only compared with fresh embryo transfer (33.3% vs 46.6%, p = 0.078). The ongoing pregnancy rate after first embryo transfer did not differ significantly in the freeze-only and fresh embryo transfer groups across all quartiles of endometrial thickness, and there was no significant interaction between endometrial thickness and treatment group for ongoing pregnancy rate. In women with an endometrial thickness below the 10th percentile (<10 mm), ongoing pregnancy rates were 10/30 (33.3%) in the freeze-only group versus 11/40 (27.5%) in the fresh transfer group (relative risk 1.21, 95% confidence interval 0.59–2.47).

Limitations, reasons for caution: Although this analysis was included in the parent RCT protocol, it is a secondary analysis of data. In addition, division of patients into subgroups based on serum progesterone level or endometrial thickness quartiles meant that the sample size in each group was reduced, which affects the power of this analysis.

Wider implications of the findings: Use of a freeze-only strategy could maximise ongoing pregnancy and live birth rates in women with a high serum progesterone level undergoing stimulated IVF. Based on the current findings, treatment decisions regarding a freeze-only strategy do not need to be modified based on endometrial thickness.

Trial registration number: NCT02471573 (clinicaltrials.gov)

SELECTED ORAL COMMUNICATIONS

SESSION 77: FEMALE FERTILITY FROM GENOMIC STUDIES TO NOVEL THERAPIES

Wednesday 4 July 2018 Room 116 14:00–15:15

O-300 A comprehensive two-centre RNA-seq study reveals changes in endometrial and blood miRNome at mid-secretory phase in fertile women and in patients with recurrent implantation failure

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Study question: Does an RNA sequencing study from endometrium and blood reveal new endometrial receptivity-related microRNAs in healthy women and in patients with recurrent implantation failure (RIF)?

Summary answer: Several annotated and one novel microRNA were found as differentially expressed between early secretory *vs.* mid-secretory phase samples in addition to microRNAs dysregulated in RIF.

What is known already: The involvement of miRNAs in mid-secretory endometrial functions has been shown and aberrant expression of miRNAs in RIF has been identified in a few previous studies, but not from whole blood samples. However, as the analysis settings and platforms have been different and a small number of samples has been analysed, there is no consensus about the miRNAs involved in endometrial receptivity and dysfunction.

Study design, size, duration: Our current study recruited 39 fertile women and 38 infertile RIF patients from Estonia (EST) and Spain (ESP). Paired endometrial and blood samples were collected at early secretory and mid-secretory phases from fertile women and at mid-secretory phase from infertile RIF patients. This is the largest miRNA endometrial receptivity study to date with

novel aspects of combining matched samples of endometrium and blood from two different population cohorts.

Participants/materials, setting, methods: Samples were subjected to Illumina small RNA sequencing. Annotated and novel miRNAs were identified from sequencing reads by miRDeep2 algorithm. miRNAs with significantly altered levels between the study groups in both EST and ESP datasets were considered as differentially expressed. Ingenuity Pathway Analysis was applied for identifying miRNA target genes and canonical pathways from matched mRNA data. Custom TaqMan Small RNA assay was used for the validation of novel miRNA expression levels.

Main results and the role of chance: Among fertile women, 91 differentially expressed miRNAs were identified in mid-secretory *vs.* early secretory endometrium, while no differentially expressed miRNAs were found in the corresponding blood samples. The comparison of mid-secretory phase samples between fertile women and RIF patients revealed 21 differentially expressed miRNAs from endometrium and one from blood samples. miRNA target gene prediction analysis using our mRNA sequencing data from the matched samples detected several canonical pathways to be involved in mid-secretory endometrial functions, including JAK/STAT signalling, leptin signalling and growth hormone signalling. Moreover, JAK/STAT signalling pathway was also revealed in miRNA target analysis of mid-secretory endometrium from RIF patients when compared to fertile women. Further, our study results highlight the involvement of miR-30 and miR-200 families in endometrial receptivity, and miR-424-5p in the development of endometrial dysfunction. We identified several novel miRNAs, of which miRNA chr2_4401, being 37-fold up-regulated in mid-secretory phase, has a potential role in the mid-secretory endometrial functions. From blood samples we identified miR-30a-5p as a possible marker of infertility related to RIF.

Limitations, reasons for caution: Our stringent analysis approach of matching two cohorts and analysing only the common miRNA and mRNA lists could have resulted in missing some true-positive results. Functional experiments to determine the role of differentially expressed miRNAs were not performed.

Wider implications of the findings: In addition to novel findings, our study results agree with previously published data implicating that miR-30 and miR-200 family members are involved in endometrial transition from pre-receptive to receptive state. Our results together with previous studies indicate that miRNAs play an important role in the regulation of endometrial receptivity functions.

Trial registration number: not applicable.

O-301 Sirolimus as a new drug for treatment of RIF patients with elevated Th17/Treg Ratio: a randomized, double-blind, phase II clinical trial

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Study question: Can we improve clinical pregnancy and ongoing pregnancy in recurrent implantation failure (RIF) patients with increased Th17/Treg ratio by immunosuppressive agent Sirolimus?

Summary answer: Sirolimus treatment in RIF patients can decrease Th17/Treg ratio and improve clinical pregnancy rate and ongoing pregnancy rate.

What is known already: RIF is clinically defined as the failure of good quality embryos to implant into the uterus following at least three cycles of IVF/ET. During pregnancy, a genetically different fetus is allowed to survive within the uterus despite the maternal recognition of fetal alloantigens. Compared with normal pregnancy early loss of embryo is associated with systemic lower levels of Treg cells in IVF. Several lines of evidence have indicated that differentiation of naive T cells into Th17 is deleterious for normal pregnancy and may cause implantation failure. Sirolimus as the most common mTOR inhibitor is able to effectively prevent allograft rejection.

Study design, size, duration: A total 121 patients with a history of at least 3 RIF after IVF/ET cycles that referred to Eastern Azerbaijan ACECR ART center, Alzahra Hospital of Tabriz University of Medical Sciences and Infertility Treatment center ACER Qom from July 2016 to June 2017 were selected and enrolled in this multicenter, randomized, double-blind, phase II study.

Participants/materials, setting, methods: Blood was drawn between cycle day 5 and 10 of a cycle prior to the index IVF/ET cycle for then assessment of a Th17 and regulatory T cells ratios and functions by flow cytometry, real-time PCR and ELISA. Normal ranges for Th17/Treg ratios were established 0.75 by using 50 normal fertile women. In 76 patients with elevated Th17/Treg ratios, 43 of them were treated with Sirolimus (Rapamune®; Pfizer, UK) and 33 patients were not treated.

Main results and the role of chance: Our results demonstrated a significantly higher clinical pregnancy rate (55.81%) in Sirolimus-treated patients compared with control group (20.93%) ($P = 0.0005$). We also found a significantly increased ongoing pregnancy rate (44.18%) in RIF women who received Sirolimus compared with control group (16.26%) ($P = 0.0005$).

Limitations, reasons for caution: We need more research to describe molecular mechanism of sirolimus in Th17/Treg ratio by focusing to mTOR signaling pathway.

Wider implications of the findings: The findings of the current study revealed the fact that Sirolimus is able to improve reproductive outcome in RIF women with imbalanced Th17/Treg ratio, thus representing a new approach for the potential treatment of patients with embryo implantation failure.

Trial registration number: ClinicalTrials.gov Identifier: NCT03161340

O-302 No evidences that implantation of vitrified euploid blastocysts is influenced by ovarian stimulation conducted in luteal vs follicular phase:interim analysis of a prospective multicentre study

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Study question: Is there any difference in ongoing pregnancy rate after single embryo transfer (SET) of vitrified euploid blastocysts obtained after luteal-phase-stimulation (LPS) vs follicular-phase-stimulation (FPS)?

Summary answer: To date, FPS-derived and LPS-derived vitrified euploid blastocysts did not show any evidence of a different reproductive competence.

What is known already: Multiple follicular waves can arise during a single menstrual cycle in humans, thereby highlighting a novel folliculogenesis pattern which overtakes the classic theory. Preliminary studies showed that oocytes obtained from anovulatory waves seem developmentally-similar to those obtained from conventional approaches (namely FPS) in terms of fertilization, blastulation and euploidy rates. These observations led to the introduction of novel protocols for ovarian stimulation: random-start, luteal phase-only stimulation and DuoStim (FPS plus LPS in the same menstrual cycle).

Study design, size, duration: Multicenter prospective study. 278 poor-prognosis women (AMH≤1.5 ng/ml and/or AFC≤6 and/or ≤5oocytes retrieved from a previous cycle and/or ≥35 yr) completed a DuoStim approach combined with preimplantation-genetic-testing (PGT) between October2015–July2017. To date, 174 patients obtained and transferred at least 1 euploid blastocyst either from FPS and/or LPS. Only the first SET performed was included in this study. The primary outcome was the ongoing implantation rate (>20weeks). Biochemical pregnancy loss (BPL), miscarriage and obstetrical/perinatal outcomes were also monitored.

Participants/materials, setting, methods: Both FPS and LPS were performed with gonadotrophins in an antagonist protocol. After the first retrieval from FPS we waited five days before starting LPS. All embryos were cultured to blastocyst, underwent trophectoderm biopsy and vitrification. The samples from FPS and LPS were analyzed in the same run. In presence of euploid blastocysts from both FPS and LPS, the first embryo to be transferred was randomly-chosen. Frozen-SETs were performed in a modified-natural or artificial cycle.

Main results and the role of chance: To achieve 80% power ($\alpha = 0.05$) to rule out a 15% difference in ongoing implantation rate between FPS-derived and LPS-derived euploid blastocysts, we require 174 first SETs per arm (348 overall). In this interim analysis we reached 50% of this sample size. The positive pregnancy rates were 57.0% (n = 49/86) and 55.7% (n = 49/88) from FPS-derived and LPS-derived euploid blastocysts, respectively. The BPL rates were 8.2% (n = 4/49) and 2.0% (n = 1/49), respectively. The miscarriage rates were 11.1% (n = 5/45) and 12.5% (n = 6/48), respectively. The ongoing pregnancy rates were 46.5% (n = 40/86) and 47.7% (42/88), respectively. To date, 30 FPS-derived and 32 LPS-derived babies have been delivered. Gestational age (38.1 ± 1.1 weeks, range 36-40 versus 38.0 ± 2.2 weeks, 36-41) and birthweight (3308 ± 880 g, 2200-4030 versus 3217 ± 584 g, 2010-4152) were similar so far between study arms. One FPS-derived euploid blastocyst underwent spontaneous embryo twinning and resulted in a multiple pregnancy. One and 2 gestational diabetes were reported in the two study groups, respectively. A FPS-derived pregnancy showed polidramnios and neonatal respiratory distress, which involved 7 days in the neonatal intensive care unit after birth. No neonatal issues have been reported for LPS-derived pregnancies up to now.

Limitations, reasons for caution: This is an interim analysis; therefore, we are yet underpowered to draw clear conclusions from these data, especially dealing with obstetrical and perinatal outcomes. Moreover, embryo derived from LPS are obtainted only after DuoStim approach.

Wider implications of the findings: LPS in a DuoStim-approach is promising for poor-prognosis (or oncological) patients that need to collect the highest number of oocytes in a short timeframe.Although, any stimulation protocol which exploits anovulatory waves needs a thorough investigation. Here, we produced clinical data to define the safety of the pregnancies achieved after LPS.

Trial registration number: None.

O-303 Age-related gene expression profiles of immature human oocytes

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Study question: What is the difference in gene expression profile in human germinal vesicle (GV) oocytes from women of different ages?

Summary answer: There were no significant differences in gene expression profiles of human GV oocytes from women of different ages (range 25-43).

What is known already: It is well established that reproductive capacity declines as women age. This age-related decline is almost entirely attributed to oocyte quality, since this decline is counterbalanced in older women receiving young donor oocytes. Multiple molecular mechanisms explaining the decrease in oocyte quality with increasing female age have been suggested, but none have been firmly demonstrated. Altered gene expression of human oocytes at different stages of development in relation to female age is one of the suggested mechanisms for age-related fertility decline.

Study design, size, duration: Between 2012 and 2014, 40 human GV oocytes of 40 women were obtained during follicular aspiration as part of routine ICSI treatment. Gene expression profiles were determined in four different age groups: 25-30, 31-35, 36-38 and 39-43 years of age.

Participants/materials, setting, methods: GV oocytes were snap-frozen shortly after follicular aspiration. Gene expression profile analyses were performed using 4×180 K Agilent arrays containing ~42000 probe-sets. Gene expression profiles were visualized by hierarchical clustering and multidimensional scaling. Transcripts were analyzed in a class comparison between the four age groups and for indicators of biological age: antral follicle count (AFC) and the total dosage of follicle stimulating hormone (FSH) used for ovarian hyper stimulation. Individual transcripts were analyzed using linear regression.

Main results and the role of chance: Visualization of gene expression profiles of GV oocytes with hierarchal clustering and MDS demonstrated no clear clustering of samples based on female age, AFC or FSH dosage. The gene expression profile of GV oocytes classified in four age groups revealed no significantly differentially expressed genes between the four different age groups (FDR < 0.05). There were also no significantly differentially expressed genes in the linear regression analysis for individual transcripts analyses per age year (FDR < 0.05).

Limitations, reasons for caution: Immature (GV) oocytes obtained after follicular aspiration in ICSI cycles were used. Findings may be different for in vivo matured oocytes at other developmental stages, under physiological conditions. Due to our relatively large, but still limited study sample, we cannot exclude that there might be smaller age-related gene-expression differences.

Wider implications of the findings: We did not find an effect of age on gene expression profiles of individual human GV oocytes. Other studies have suggested that gene-expression profiles are affected in mature oocytes, which might implicate that female age affects oocyte maturation. Alternatively, other mechanisms in human oocytes might affect age-related fertility decline.

Trial registration number: not applicable.

O-304 Age related differences in the translational landscape of mammalian oocytes

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Study question: How global translational program differs in mammalian oocytes after aging of females?

Summary answer: We have identified several genes with potentially altered expression in the oocytes of aged females which might contribute to defective meiosis I and consequent aneuploidy.

What is known already: Oocyte aneuploidy is the result of abnormal chromosome segregation during meiosis, giving rise to a ready to be fertilized oocyte which possesses an aberrant number of chromosomes. These anomalies are inherited by the embryo drastically reducing its developmental potential. Importantly, aneuploidy is not infrequent in mammalian oocytes but rather a common feature which increases in correlation with female age.

Furthermore, maturing oocytes are transcriptionally silent and rely on the utilization of mRNAs synthesized and stored during the growth period. To our knowledge a global analysis of translation in oocytes related to the maternal age has not yet been performed.

Study design, size, duration: Mouse model for aging: Study group - 1 year old mouse females / Control 2 months old mouse females.

Samples: 200 oocytes at nuclear envelope break down stage.

Participants/materials, setting, methods: We aim to study age-related aneuploidy in mammalian oocytes by comparing and analyzing the active transcriptome in oocytes from young and aged mouse females. We have developed a polysome fractionation protocol to isolate the RNA population involved with the translational machinery from a minute amount of cells/oocytes. These have been sequenced by Illumina NextGen to be able to identify differences in the translational program of the oocytes from young and aged females.

Main results and the role of chance: We have successfully developed a polysomal fractionation protocol to be used with as low as only 200 oocytes. Thanks to this method, we have been able to obtain database of actively translated mRNAs using Illumina NextGen sequencing. After studying the obtained data, we have found several genes with differences in expression between

oocytes from young and aged females. These might therefore contribute to the increased aneuplody characteristic in oocytes from aged females.

Limitations, reasons for caution: Our study samples are oocytes from aged female mice. This material is scarce due to the time and resources required to age females. Moreover, these produce only around 5 viable oocytes.

We used polysome fractionation which is generally performed with high amounts of material. We reduced the input to 200 oocytes.

Wider implications of the findings: This project can shed some light upon the reasons behind the loss of oocyte quality from women of advanced age. It may help to improve assisted reproduction techniques with a potential development of gene therapy to treat the high aneuploidy rates in oocytes of women of advanced age.

Trial registration number: Not applicable

Poster viewing

POSTER VIEWING

Andrology

P-001 Sperm donors' opinion towards donation and the release of identifying information in the Belgian context

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Study question: What is the opinion of sperm donors towards the release of identifying information in the Belgian context of strict anonymous donation?

Summary answer: One in five (20,1%; n = 30) of the respondents indicated they would continue to donate if the legislation would change from anonymous towards identifiable donation.

What is known already: Belgian legislation restricts clinic recruitment advertising. It allows only strictly anonymous gamete donation and known donation (donation to a recipient known by the donor). Recently an amendment of the legislation was proposed which would grant donor offspring, as of 18 years old, the right to claim the identifying information of their donor. International studies have shown that in countries with anonymous donation programmes, 11% to 50% of the donors would be willing to continue donating upon donor anonymity abolishment. In two Belgian studies, 26% and 28,8% of the candidate donors and donors would continue donating if legislation would change.

Study design, size, duration: The survey was composed of questions derived from the international literature. Eight experts validated the survey via a Delphi procedure. In addition, the survey was examined by two donors to ensure clarity of wording.

The survey was online from October 27th until December 8th, 2017. Questions (n = 36) were asked about motivation to donate, attitude towards anonymous donation and social demographics.

Participants/materials, setting, methods: Al men who were accepted as sperm donor (n = 242) by Jan Palfijn Hospital (Ghent, Belgium), from a period of 2009 to 2017 were contacted by e-mail and were invited to complete an anonymous online survey. Two reminder e-mails were sent to the study population to maximize the response rate. All statistical analyses were performed using SPSS. The response rate was 65,5% (150 donors completed the survey; 13 men were not reachable).

Main results and the role of chance: At the time of the survey, the sperm donors had a mean age of 32 years (20-46; SD 5,97). Half of the respondents (51,7%; n = 77) also were blood donors. Two thirds of the men (66,4%; n = 99) did not have children of their own. Most (86,6%; n = 129) of them were motivated by altruism although the majority (59,1%; n = 88) was (also) motivated by the compensation fee and nearly half (45,0%; n = 67) by access to blood and fertility tests. One in five (22,8%) would continue to donate without a compensation fee.

79,2% (n = 118) of the respondents were non-active sperm donors. Of those men, 68,6% (n = 81) said they were non-active because they thought that there was enough donor sperm in the sperm bank. The others (31,4%; n = 37) were non-active for personal reasons, mainly related to lack of time to donate or a non-supportive partner.

One in five (20,1%; n = 30) would continue sperm donation upon a legislation change towards identifiable donations whereas 53,7% (n = 80) would no longer donate. 26,2% (n = 39) were undecided.

No significant relationship was observed between the willingness to continue donating in an identifiable system and education (p = 0,724), age (p = 0,725), having children (p = 0,918), being a blood donor (p = 0,822) or sexual orientation (p = 0,156).

Limitations, reasons for caution: A limitation of this study is that the data originate from a single centre and may not represent the opinion of all Belgian sperm donors.

Wider implications of the findings: Currently, 40% of donor sperm used in Belgian ART is of indigenous origin. Consequently, the tendency that only 20,1% of the Belgian sperm donors would continue to donate in an identifiable system, could be detrimental for the future availability of donor sperm in Belgium.

Trial registration number: The study was approved by the Ethics Committee of the University Hospital of Ghent.

Belgian Registration Number: B0201733518.

P-002 The effect of unilateral tubal block on clinical pregnancy rate in intrauterine insemination cycles: Systematic review & meta-analysis

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Study question: What are pregnancy rates after intrauterine insemination (IUI) among infertile women with unilateral tubal block (UTB) diagnosed by hysterosalpingogram (HSG) compared to women with unexplained infertility and bilateral patent tubes?

Summary answer: Infertile patients with proximal UTB diagnosed by HSG can expect good pregnancy outcomes after IUI while patients with distal UTB have lower odds of pregnancy.

What is known already: Fallopian tube occlusion is a common cause of infertility. For normal fallopian tube function, the tube must be patent, freely mobile, and in close proximity to the ovary. However, several pathologies are implicated in distorting this normal physiology, including pelvic infections, endometriosis, and surgical adhesions. Depending on the etiology, tubal pathology may involve the proximal, distal, or entire tube, and may be transient due to obstruction or permanent due to occlusion. While bilateral tubal block confers poor pregnancy rates (PR) after controlled ovarian hyperstimulation and intrauterine insemination (COH-IUI), the effect of unilateral tubal block (UTB) remains controversial.

Study design, size, duration: We systematically searched the Cochrane Library, EMBASE and MEDLINE databases from inception to September 2017. Key words included IUI, intrauterine insemination, tubal block, tubal occlusion, fallopian tube diseases. Original research articles including randomized and non-randomized controlled trials, cohort studies, patient series and case reports were included. All included studies reported either pregnancy rates (PR) per cycle and/or cumulative pregnancy rates (CPR). Additional studies were extracted from the references in the full text articles.

Participants/materials, setting, methods: Two authors independently selected trials and extracted study characteristics. Methodological quality was

assessed using Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. Clinical pregnancy data were pooled using a random-effects model to calculate estimated average Odds Ratios (OR). In order to provide greater weight to larger studies, OR were weighted by the inverse variance. The I^2 metric was calculated to assess study heterogeneity. Publication bias was assessed by visual inspection of funnel plots.

Main results and the role of chance: 10 observational studies investigating pregnancy outcomes after IUI in women with unilateral tubal block (UTB) compared to those with bilateral patent tubes and unexplained infertility (control) were included. All included studies provided diagnostic criteria for assessing tubal block and screened for appropriate male and female factors through detailed reproductive history and infertility evaluation. Furthermore, subgroup analyses were performed among studies that differentiated proximal and distal tubal blocks. The following comparisons were evaluated based on pregnancy rate (PR) per IUI cycle and cumulative pregnancy rate (CPR): unilateral tubal block vs. control, proximal tubal block vs. control, distal tubal block vs. control, and proximal vs. distal tubal block. Among 2965 patients and 5,749 IUI cycles across 10 studies, no significant difference in PR/cycle (OR = 0.88, CI = 0.69-1.12) and cumulative PR (OR = 0.80, CI = 0.62-1.04) was observed. Patients with proximal UTB demonstrated similar PR/cycle (OR = 1.06, CI = 0.68-1.66) and cumulative PR (OR = 1.10, CI = 0.75-1.62) compared to controls while patients with distal UTB had significantly lower cumulative PR (OR = 0.49, CI = 0.25-0.97, p = 0.04). Patients with proximal block also demonstrated significantly improved cumulative PR compared to patients with distal block (OR = 2.41, CI = 1.37-4.25, p = 0.002).

Limitations, reasons for caution: All included studies were retrospective and hence susceptible to confounding. Differences in screening/exclusion criteria and lack of standardized IUI techniques between studies also bias their comparability. Indeed, differences in sperm quality and processing, cycle timing, and overall differences in ovarian stimulation treatment have all been shown to affect IUI outcomes.

Wider implications of the findings: Our review demonstrates that infertile patients with proximal UTB diagnosed by HSG can expect good pregnancy outcomes after COH-IUI while patients with distal UTB have lower pregnancy odds and may benefit from laparoscopic assessment or IVF. This difference may reflect inherent diagnostic limitations of HSG or differences in underlying pathologies.

Trial registration number: Not applicable.

P-003 Bilateral versus unilateral cryptorchidism in non-obstructive azoospermia: outcomes of testicular sperm extraction and ICSI

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Study question: How successful is testicular sperm extraction (TESE) in non-obstructive azoospermia (NOA) in case of unilateral versus bilateral cryptorchidism?

Summary answer: NOA men with a history of unilateral cryptorchidism exhibited similar sperm retrieval rate but better clinical pregnancy rate than those who had bilateral cryptorchidism.

What is known already: Cryptorchidism is a very common genital disorder affecting almost 3% of male children and it represents one of the most frequent causes of NOA in adulthood. Although, it is well known that spermatogenesis is more impaired in bilateral cryptorchidism than in unilateral cryptorchidism, data available in the literature only refer to small cohorts or inhomogeneous population. To date, comparison of testicular sperm extraction and ICSI outcomes in men with a history of bilateral versus unilateral cryptorchidism is still lacking.

Study design, size, duration: This is a retrospective comparative study performed in the Centre of Reproductive Medicine and Andrology, University Hospital of Lille. Among the 1274 azoospermic men who underwent TESE procedure from 1995 to 2014, 314 had a history of cryptorchidism. Out of these patients, 35 had an additional obstructive cause and were therefore excluded. In the end, we identified and included a total of 249 patients with a history of cryptorchidism as only aetiopathogenetic factor for NOA.

Participants/materials, setting, methods: Patients were divided into two groups according to the history of cryptorchidism: bilateral (65.1%, 162/249) or unilateral (34.9%, 87/249). We compared hormonal profiles and sperm retrieval rates (SRR) between the two groups. Additionally, we studied ICSI outcomes with testicular frozen-thawed sperm.

Main results and the role of chance: This is the largest consecutive cryptorchid cohort reported to date. There was no difference in the andrological phenotype with similar FSH levels (21.7 IU/L vs. 18.8 IU/L; p = 0.17), inhibin B levels (35.5 pg/mL vs. 37 pg/mL; p = 0.15) and mean testicular volumes (7.7 mL vs. 7.9 mL, p = 0.71). Similar SRR were observed between the groups (62.3% vs. 59.8%; p = 0.7,8 respectively). Implantation rate was higher in the unilateral group (23.7% vs. 10.3%; p = 0.02). Cumulative pregnancy rates per embryo transfer and cumulative live birth rates were both significantly higher in the unilateral group (28.9% vs. 17.5%; p = 0.04 and 27.6% vs. 17.3%; p = 0.04, respectively).

Limitations, reasons for caution: This is a retrospective study, which inevitably creates a risk a bias towards the recording of the data. In addition, reproductive outcomes might be interpreted with caution regarding the assessment of the ovarian reserve, which has changed over time due to the evolution in serum AMH thresholds and ultrasound equipment.

Wider implications of the findings: We showed for the first time that ICSI outcomes were better in NOA patients with a history of unilateral versus bilateral cryptorchidism, despite similar SRR. This suggests that cryptorchidism could reflect a bilateral testicular impairment, even in unilateral cases, and should be considered when counseling patients before TESE attempts.

Trial registration number: not applicable.

P-004 Parameters associated with sperm quality prior to chemotherapy treatment in patients with lymphoma

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Study question: What parameters are associated with poor sperm quality in lymphoma patients prior to chemotherapy treatment?

Summary answer: The study shows a link between known prognostic factors of lymphoma (B symptoms, low hemoglobin and albumin levels) and sperm quality before chemotherapy.

What is known already: Chemotherapy treatment in patients with lymphoma may result in reduction in sperm quality. Therefore, patients are strongly advised to undergo sperm cryopreservation prior to the initiation of treatment. It is still unknown what factors are associated with increased semen analysis abnormalities prior to chemotherapy.

Study design, size, duration: Retrospective descriptive analytic study. One-hundred ninety one lymphoma patients attempting sperm cryopreservation between 1999 and 2016.

Participants/materials, setting, methods: One hundred and one Hodgkin lymphoma patients and ninety Non Hodgkin lymphoma patients were included in the study. Lymphoma prognostic factors (presence of B symptoms, albumin and hemoglobin levels, stage etc.) were compared between patients with normal and abnormal sperm (according to World Health Organization 2009 guidelines). Another parameter compared between the groups was the Prognostic Score Ratio (PSR), an index representing the number of negative lymphoma prognostic measures that exist in a given patient.

Main results and the role of chance: Among the prognostic factors of lymphomas, the following factors were found to be associated with reduced sperm quality: B symptoms (p = 0.021), albumin (p < 0.001) and hemoglobin (p < 0.001) levels. Logistic regression showed significant association of albumin and hemoglobin indexes with reduced sperm quality (p = 0.013, OR = 2.7 and

p = 0.015, OR = 13.5, respectively). However, in Hodgkin's lymphoma specifically, only hemoglobin was found to be statistically significant. Another measure that showed a significant relationship was the Prognostic Score Ratio (PSR) ($p < 0.001$).

Limitations, reasons for caution: The study included only patients who were eligible for sperm cryopreservation. Therefore, this study did not include very ill patients, who started chemotherapy immediately, or older patients who were not interested in sperm cryopreservation.

Wider implications of the findings: The prognostic factors mentioned above are related to a general inflammatory state and therefore might affect sperm production. A further investigation of inflammatory factors levels in plasma and seminal fluid is reasonable.

Trial registration number: Not applicable.

P-005 Effects of oral cysteamine on fertility in male mice

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Study question: Does oral cysteamine affect fertility in male mice?

Summary answer: Oral cysteamine does not affect fertility in male mice.

What is known already: Cystinosis is a rare autosomal recessive metabolic disease caused by mutations in the lysosomal membrane protein, cystinosin, which leads to intra-cellular accumulation of cystine crystals. The main clinical presentation is renal Fanconi syndrome, leading to end stage renal disease (ESRD) by the age of 10, if the patient is left untreated. Female cystinosis patients could get successful pregnancies. However, male patients suffer from unexplained infertility. The only available treatment for cystinosis is the oral cysteamine, which depletes the intra-cellular cystine crystals. However, the effects of oral cysteamine on male fertility was not investigated thoroughly yet.

Study design, size, duration: C57/BL6J male adult (2 months of age) wild type mice (n = 24) were randomized into 3 groups (n = 8 each); control group (fed with normal food), treatment group I (fed with 250 mg cysteamine/kg/day mixed with normal food), and treatment group II (fed with 500 mg cysteamine/kg/day mixed with normal food). After two months, mice were sacrificed. Body weights, testis weights, seminal vesicle weights, plasma samples, epididymal sperm, and testicular tissues were collected at sacrifice time.

Participants/materials, setting, methods: Plasma levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were evaluated, using the appropriate ELISA kit for each hormone. Testicular tissues were fixed in formalin, embedded in paraffin, and sectioned. The sections were stained by periodic acid-Schiff's (PAS) staining. Number of seminiferous tubules at stage VIII were quantified, relative to the total number of tubules. Results were compared between the control and treatment groups.

Main results and the role of chance: After two months of treatment, we found no statistically significant differences between groups (control, 250 mg, and 500 mg) regarding the total body weight (31.6 ± 1.9, 30.6 ± 2.7, and 29.6 ± 2.6 g respectively), body weight gain (5.9 ± 0.4, 5.0 ± 1.8, and 6.0 ± 1.8 g respectively), body weight gain normalized to body weight before treatment (23.4 ± 2.6, 19.6 ± 6.8, and 25.8 ± 8.0 % respectively), seminal vesicle weight normalized to total body weight (11.9 ± 1.2, 10.7 ± 1.9, and 10.9 ± 1.1 % respectively), testis weight normalized to total body weight (3.2 ± 0.2, 3.4 ± 0.3, and 3.3 ± 0.2 % respectively), and epididymal sperm count (2.5 ± 0.9, 3.6 ± 1.1, and 3.1 ± 1.2 million respectively), using one-way ANOVA test.

Moreover, there was no statistically significant difference between groups in plasma testosterone (4.4 ± 7.2, 3.4 ± 5.8, and 5.0 ± 7.3 ng/ml respectively) using Kruskal Wallis ANOVA on ranks test. In addition, after two months of treatment, plasma LH (4.6 ± 3.5, 4.4 ± 2.2, and 5.6 ± 3.3 ng/ml respectively), plasma FSH (2.5 ± 0.7, 2.8 ± 1.1, and 2.1 ± 0.5 ng/ml respectively) and number of tubules at stage VIII normalized to total number of tubules (13.9 ± 2.6, 12.5 ± 1.7, and 11.0 ± 2.1 % respectively) were similar.

Limitations, reasons for caution: The study is performed in wild type C57/BL6J mice, so the combined effect of cystinosis and cysteamine treatment was not investigated. Moreover, the effect of treatment is investigated after two months treatment, while the patients take cysteamine life-long. Hence, the results should be interpreted cautiously.

Wider implications of the findings: The results obtained in the study suggest that cysteamine treatment, the only available oral treatment for cystinosis, may have no negative impact on fertility.

Trial registration number: Not applicable.

P-006 A comparison of reproductive outcome using testicular sperm Vs. ejaculated sperm in ICSI for patients with abnormal DNA fragmentation

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Study question: Is testicular sperm better than ejaculated sperm in ICSI in terms of reproductive outcome in men with abnormal DNA fragmentation?

Summary answer: There is no significant difference in ICSI clinical outcome between using Testicular sperm or ejaculated sperm with abnormal DNA fragmentation patients.

What is known already: Abnormal levels of DNA fragmentation has negative impact on embryological parameters (fertilization, cleavage, blast).which lowers the level of pregnancy rate, ongoing pregnancy rate and increase miscarriage rate.

The topographic mapping of sperm DNA fragmentation in male genital tract represent that testicular sperm have lower DFI than ejaculated sperm which is better to use in ICSI.Using testicular sperm in ICSI can potentially solve the problem of abnormal DNA fragmentation and would improve the clinical outcome.

Study design, size, duration: Prospective cohort study included 111 couples have abnormal DNA fragmentation levels undergoing ICSI in GFC from June 2016 to July 2017.Patiens were randomized into two groups; first group was ICSI using testicular sperm and the second group was ICSI using ejaculated sperm.

Participants/materials, setting, methods: This study included 111 couples with abnormal DNA fragmentation and randomized into two groups. Sperm DNA fragmentation test was done by TUNEL assay using bench top flow cytometry and DFI cutoff value was (20%). Women were eligible to participate in the study if they were ≤37 years old and had ≥ 5 (MII)oocytes collected. Embryological data were recorded and results were compared using IMB SPSS Software Version 22 For Microsoft windows.

Main results and the role of chance: A total 111 cases with abnormal DFI underwent ICSI and divided into 53 cases injected using testicular sperm and 58 cases injected by ejaculated sperm with no significant difference in male or female age between the two groups. DFI level was (26.7%, 23.8%) in Testi-ICSI and Ejac-ICSI respectively with (P = 0.7). Fertilization rate was (62.98%, 77.16%) in Testi-ICSI and Ejac-ICSI respectively with (P = 0.1). Blastulation rate was (38.02%, 65.17%) with (P = 0.004) this significant value might be due to the larger number of D3 ET in Testi-ICSI group vs Ejac-ICSI group. Pregnancy rate was (50%, 51.02%) in Testi-ICSI and Ejac-ICSI respectively with (P = 0.9). Implantation rate was(41.4%, 26.7%) in Testi-ICSI and Ejac-ICSI respectively with (P = 0.1).Ongoing pregnancy was (48%, 42.8%) in Testi-ICSI and Ejac-ICSI respectively with (P = 0.5) and the miscarriage rate was (7.40%, 16%) in Testi-ICSI and Ejac-ICSI respectively with (P = 0.16).So there is no significant difference favoring Testi-ICSI over Ejac-ICSI in men with abnormal DNA fragmentation.

Limitations, reasons for caution: Ten patients vitrified all their embryos in both groups, and were excluded from the pregnancy rate, implantation rate and ongoing pregnancy rate. Also there are many factors might affect DFI such as infertility duration, use of medication, presence of varicocele, elevated ROS levels and other relevant male factors.

Wider implications of the findings: More studies are needed with larger number of cases for more statistically powered findings, to determine which is better for ICSI cycles with men who have abnormal DNA fragmentation, the usage of testicular sperm or ejaculated sperm.

Trial registration number: N/A.

P-007 Male accessory sexual glands secretion as a potential predictor of fertilization in conventional IVF

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Study question: Do male accessory sexual glands secretions have a predictive value on the fertilization outcome in conventional IVF?

Summary answer: Prostatic hyper-secretion may have a negative impact in the fertilization rate.

What is known already: The use of conventional in vitro insemination (CIVI) has been declining worldwide while intracytoplasmic sperm injection (ICSI) has dramatically increased. The main argument supporting ICSI overuse is to prevent fertilization failure. Basic seminal parameters are usually the criteria for choosing between CIVI and ICSI. Although an association between male accessory sexual glands secretion and fertilization has been shown in animal models, its evaluation has been sidelined from the basic exam of male fertility due to the absence of clinical studies.

Study design, size, duration: Eighty five patients younger than 40 yo, with more than 4 mature oocytes, with male partners with normal semen parameters, undergoing conventional IVF between November 2015 and October 2016 were included in this controlled, prospective, blinded, multicenter study. One-way analysis of variance or Kruskall-Wallis analysis was performed to detect any possible differences between parameter means. All the categorical variables were compared with a Chi-square test among groups.

Participants/materials, setting, methods: An aliquot of the IVF semen sample was centrifuged to keep in the supernatant a free-cell plasma fraction. Fructose seminal vesicle and citric acid prostate secretions were estimated by spectrophotometry. Fructose levels between 150-450 mg/dL and citric acid of 350-670 mg/dL were considered as normal while lower or higher values were considered as pathological. No differences in patients age, basic seminal parameters and number of recovered metaphase II oocytes were observed between groups.

Main results and the role of chance: No differences were observed in fertilization rates between patients with normal and hyper seminal vesicle secretion (0.79 ± 0.03 vs 0.80 ± 0.05) or normal vs hypo secretions (0.79 ± 0.03 vs 0.80 ± 0.07) were compared. Differences in fertilization rates were found between normal and hyper prostatic secretions (0.84 ± 0.03 vs 0.70 ± 0.04, $p < 0.01$) but not when normal vs hypo secretions (0.84 ± 0.03 vs 0.74 ± 0.04) were compared.

Limitations, reasons for caution: To determine the true extent of any clinical benefit of these preliminary results a randomized clinical trial will be necessary. Research is needed to understand the putative relationship between prostatic secretions and fertilization potential.

Wider implications of the findings: Our data suggest a negative correlation between a pathological prostatic hyper-secretion and sperm fertilization potential. We have described for the first time the use of a simple, inexpensive and objective method that could be useful to choose the most suitable type of in vitro insemination between CIVI and ICSI.

Trial registration number: N/A.

P-008 Technical standardization as a first step to maximize the prognostic value of ejaculate related parameters in a homologous intrauterine insemination programme

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Study question: Does standardization of the laboratory procedures help to evaluate the prognostic value of ejaculate related determinants in an intrauterine insemination (IUI) programme.

Summary answer: When technical parameters were held stable, time-interval between sample production and insemination significantly influenced positive hCG outcome, clinical pregnancies and live birth rate after IUI.

What is known already: IUI remains a relatively simple, non-invasive, cheap treatment option for a well selected population with a sufficient number of motile spermatozoa. There is a considerable amount of literature on several prognostic factors either related to patient characteristics, semen sample or the IUI procedure itself. But, results are controversial due to the lack of technical standardization and good-quality prospective cohort trials. Although minimum standards required were defined by WHO and ESHRE Special Interest Group Andrology, large inter-laboratory variations occur. For example, inseminating motile count varies between 0.8–5 million to maximize predictive performance (our own validation was ≥ 2 million).

Study design, size, duration: A prospective cohort study of 1795 homologous IUI cycles were included from April 2013 to March 2017. Cycles with cryopreserved semen or with two consecutive semen samples were excluded. Only complete semen samples prepared by density gradient centrifugation were included. The IUI procedure was divided into 3 phases to study the influence of the determinants: pre-analysis (abstinence time); analysis (macroscopic and microscopic examination and sperm preparation) and post-analysis (after sample preparation and before insemination).

Participants/materials, setting, methods: Five hundred and eighty one couples presenting at the Centre for Reproductive Medicine underwent 1447 IUI cycles. Semen samples were analyzed using WHO 2010 recommendations and prepared with a two-step discontinuous density gradient. Reprotoxicity for disposables and media was controlled. Staff members were trained in basic semen analysis and semen preparation and participated regularly in internal and external quality control. A soft IUI catheter was rinsed with medium and the inseminating volume was held constant.

Main results and the role of chance: IUI resulted in a positive hCG in 15.3% cycles with 14.5% clinical pregnancies and 12.6% live births per cycle. Pre-analysis phase: Only complete samples (93.4% of all samples) were considered for this analysis. Although not significantly different, abstinence time of 0-1 days reduced live births from 12.5 to 8.5% as compared to 2-7 days as recommended by WHO. Analysis phase: Analysis was initiated within 60 minutes (99.2% of all samples) after semen production. Semen viscosity (>3 cm) reduced live births from 13.0% to 8.6% but not significantly. Total motile sperm count and sperm morphology in the native semen did not influence clinical IUI outcome. After density gradient, inseminating motile count was ≥2 million (as previously validated). Post-analysis phase: Under standardized conditions, the time interval between sample production and effective IUI was significantly better if held <110 mins ($P = 0.01$; 17.1% versus 12.3% positive hCG; 16.2% versus 11.5% clinical pregnancies; 14.2% versus 9.5% live births, Receiver operating curve: area under the curve: 0.533; sensitivity 69.6 and specificity 42.3).

Limitations, reasons for caution: All participants in this study were already selected according to initial sperm parameters and reasons of subfertility. Clinical parameters such as female and male age, indication and lifestyle parameters were not taken into account.

Wider implications of the findings: This is the first monocentric study to standardize the technical aspects for IUI, paving the way to prospective multicenter approach. With technical determinants under control, attention can now be drawn to clinical variables influencing IUI outcome.

Trial registration number: not applicable.

P-009 Effect of varicocele repair on sperm DNA fragmentation: A systematic review

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Study question: Does the level of sperm DNA fragmentation decrease after varicocele repair in patients with male infertility?

Summary answer: Varicocele repair decreases sperm DNA fragmentation levels in infertile men with clinical varicocele.

What is known already: Varicocele, the leading cause of male infertility, can impair sperm quality and fertility via various oxidative stress (OS) mechanisms. Imbalance between excessive reactive oxygen species (ROS) production and antioxidant protection causes alterations in nuclear and mitochondrial sperm DNA, thus rendering the spermatozoa less fertile in a subset of varicocele men. Sperm DNA fragmentation (SDF) is known to be elevated in men with clinical varicocele in both abnormal and normal semen parameters by the current World Health Organization criteria. Surgical varicocele repair has been advocated as a means of reducing the oxidative-induced sperm DNA damage in men with varicocele-associated infertility.

Study design, size, duration: We conducted a systematic search using MEDLINE/PubMed, Scielo, and Google Scholar to identify all relevant studies published until December 2017. The search combined terms related to "sperm DNA fragmentation", "sperm DNA damage", "sperm chromatin integrity OR damage", "varicocele", "varicocelectomy OR varicocele repair", with the filters "human" and "English" language. For the advanced search, article types selected were: clinical study, comparative study, journal article, meta-analysis, observational study, randomized controlled trial (RCT), case series, and systematic review.

Participants/materials, setting, methods: Participants were men with varicocele subjected to varicocele repair in whom sperm DNA fragmentation had been measured before and after surgery regardless of assay type. The preoperative and postoperative levels of SDF in semen were the primary outcomes. The secondary outcomes were levels of other oxidative stress markers and pregnancy rates according to postoperative SDF levels.

Main results and the role of chance: We identified 21 studies (3 RCT, 7 prospective, 7 retrospective, 4 not specified) including 1,270 infertile men with varicocele subjected to surgery. Twelve studies included a control group and/or evaluated other OS markers. The majority of studies included men with clinical varicocele and abnormal semen parameters according to the WHO criteria. Despite the use of different SDF sperm assays, the mixed design, and variable sample size, all studies involving infertile men with palpable varicocele unequivocally reported a significant postoperative decrease in SDF rates in a follow-up period ranging from 3 to 12 months. A single report including men with subclinical varicoceles showed that SDF levels remained unchanged after surgery. Of the 4 studies out of 21 reports providing pregnancy outcomes, the overall postoperative SDF rates fell in men from couples who had a successful pregnancy than those who did not. Among studies assessing OS markers, the vast majority reported higher levels of seminal OS or sperm DNA decondensation in infertile men with clinical varicocele than healthy fertile counterparts without varicocele and men with subclinical varicocele. Although these studies (n = 8) unequivocally reported significant reductions in SDF after varicocelectomy, two studies have failed to demonstrate reduction in OS markers after varicocelectomy.

Limitations, reasons for caution: Not all risk factors such as participant age, use of over the counter medication, and smoking were consistently reported. These factors may influence the rates of SDF and varicocele repair outcomes. Another limitation refers to the quality of included studies, which also varied.

Wider implications of the findings: Current evidence based on careful review of published studies confirms the effectiveness of varicocelectomy as a means of both reducing oxidatively-induced sperm DNA damage and potentially improving fertility. Varicocele repair should be offered as part of treatment option for male partners of infertile couples presenting with palpable varicoceles.

Trial registration number: NA.

P-010 Effects and mechanisms of a heterozygous truncation mutation in BNC1 on male subfertility

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Study question: What is the effects and mechanisms of basonuclin1(BNC1) gene truncation mutation in male infertility.

Summary answer: BNC1 truncating mutation would lead to impaired spermatogenesis via expression dysregulation of several genes essential for spermatogenesis including Tex14, Klhl10, Spatc1, Hook1, Tssk6, and Tnp2.

What is known already: Basonuclin (BNC1) is expressed primarily in proliferative keratinocytes and gametogenic cells. However, its role in spermatogenesis is not clear. Previous study reported that BNC1 was present in spermatogonia, spermatocytes, and spermatids, but absent in Sertoli cells, and *Bnc1*-null male mice were sub-fertile, losing germ cells progressively with age. We recently discovered a novel heterozygous BNC1 truncating mutation, which led to male subfertility both in mice and human.Yet, the underlying molecular mechanism of BNC1 mutation on male subfertility remains unknown.

Study design, size, duration: Transgene mouse model study. Generate BNC1 truncation mutation mice. Investigate spermatogenesis of wild-type (+/+), heterozygous (+/tr), and homozygous (tr/tr) BNC1 truncation mutation mice. Assess expression profiling and direct binding (ChIP-seq) studies of wild-type and heterozygous BNC1 truncation mutation mice testis. Explore the underlying mechanisms of BNC1 on spermatogenesis.

Participants/materials, setting, methods: We generated BNC1 truncation mutation mice using homologous recombination. Based on the subfertility phenotype, expression profiling study was carried out. Key differentiated genes were confirmed by RNA extraction and real-time RT-PCR. Furthermore, ChIP-seq and ChIP-qPCR study with BNC1 in mouse testis was used to identify spermatogenesis specific gene promoters targeted by BNC1.

Main results and the role of chance: BNC1 truncation mutation male mouse model exhibited infertility or subfertility, significantly increased serum FSH and LH levels, lowered testerone level, and decreased testis size. Taken together, the data from the mouse model indicated BNC1 truncation mice had impaired spermatogenesis. Go-analysis, pathway-analysis, Gene-act-network, co-expression network of differentiated expression genes and ChIP-seq bindings were analyzed, and Tex14, Klhl10, Spatc1,Hook1, Tssk6, and Tnp2 were considered to be the possible genes involved in the mechanisms of BNC1 truncation mutation leading to impaired spermatogenesis.

Limitations, reasons for caution: Further studies performed with germ cells of different stages may help to explore the entire molecular mechanisms involved in the function of BNC1 in spermatogenesis.

Wider implications of the findings: Our findings described the first time the effects and mechanisms of BNC1 truncation mutation in spermatogenesis disorder, and provided unique evidences that BNC1 was an important transcription factor involved in spermatogenesis. The testis-specific role of BNC1 identifies this regulator as a potential target for male contraceptive intervention.

Trial registration number: No.

P-011 Efficacy of triple seminal lavage in patients with positive serology for HIV and HCV

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Study question: Evaluation of sperm recovery and viral load in samples from patients affected by HIV and HCV after triple washing and qPCR.

Summary answer: The qPCR for detection of viral load after triple seminal washing is essential for serodiscordant couples, although it is undetectable in the blood.

What is known already: Coombs et al., 1998 reported that in 27% of cases, the theory of compartmentalization occurs: there is a disagreement between blood viral load and semen. Reproductive and serodiscordant couples for HIV and HCV can perform in vitro fertilization without risk of transmission of the disease to descendants. However, seminal washing is recommended in situations where the man is a carrier of the virus.

Study design, size, duration: A cross sectional study where 125 seminal samples from HIV and HCV patients were evaluated from January 2012 to December 2017.

Participants/materials, setting, methods: A total of 125 seminal samples from HIV-positive patients were evaluated, 83 of them by HIV and 42 by HCV. The samples were submitted to triple washing using the techniques of Sperm-Wash, Density Discontinuous Gradient and Swim up for subsequent quantification of viral load by qPCR. Only samples from ejaculate were included. The efficacy and the seminal recovery rate were evaluated after the triple washing.

Main results and the role of chance: An overall recovery of 8% sperm after triple washing was found. Comparing the serologies, the seminal recovery of patients with HIV of 7.69% and 8.65% of HCV was observed. The qPCR was performed and the presence of the virus was found in 1.6% (2/125) of the samples after the triple washing. Both samples were from men infected with HIV virus with undetectable viral load in the blood. The clinical decision for these cases was not to proceed with oocyte fertilization.

Limitations, reasons for caution: More studies are needed with greater casuistry to confirm the data found in this study.

Wider implications of the findings: Although patients have undetectable viral load in the blood, this study demonstrated the need to perform the triple semen washing associated with quantification of the viral load by qPCR to prevent transmission of the virus to the descendants.

Trial registration number: None.

P-012 Male fertility under fire- the association between semen parameters and armed conflict related stress: a population-based study

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Study question: Whether the population exposure to the stress, peaking during two large military operations (MO), was associated with poor semen quality.

Summary answer: This ecological study showed that acute psychological stress can potentially impact sperm quality resulting in decreased sperm motility and reduced normal sperm forms.

What is known already: Psychological stress has been established as having a negative impact on infertility. However, research examining the association between psychological stress and semen quality has been inconsistent. Many studies were small and did not asses stress objectively or dealt with selected study populations.

Study design, size, duration: An ecological study. The data for 11,195 consecutive semen analyses of 6,819 couples between 2009-2017 were analyzed.

Participants/materials, setting, methods: In recent years the civilian population of Southern Israel experienced multiple alert sirens and rocket fire on a daily basis, creating a stressogenic environment. MO adjacent samples were defined as those obtained during the MO and the two subsequent months following cease-fire. Semen analyses were collected as part of basic fertility evaluation and conducted according to WHO criteria. Multivariate analysis was performed using Logistic Regression models.

Main results and the role of chance: Comparison of 659 semen analyses taken during and adjacent to MO to 10,536 semen samples obtained during the rest of the time period showed similarity in the mean age (31.8 ± 7.8 vs. 32.1 ± 8.1 years, respectively, $p = 0.246$), BMI (26.1 ± 4.7 vs. 26.1 ± 8.1, $p = 0.813$) and smoking (44.2% vs. 43.5%, $p = 0.320$). In the MO adjacent group, mean total motility was lower (47.4%±22.9% vs. 49.5%±22.0%, $p = 0.019$), as well as mean progressive motility (39.7%±21.5% vs. 43.3%±21.5%, $p < 0.001$) and mean normal sperm forms (3.4%±1.9% vs. 3.7%±2.3%, $p = 0.010$).

The prevalence of low progressive motility was higher in the MO group (37.5% vs. 30.8%, $p < 0.001$). In multivariate analysis MO adjacency adjusted for age, BMI and smoking was found to be associated with lower progressive motility (adjusted O.R = 1.47 C.I 95% 1.16-1.61).

Limitations, reasons for caution: This is an ecological study assigning stress based on the exposure of the total population. Also, we did not take into consideration the time before the operations when missiles were already fired and the stress may have been increasing.

Wider implications of the findings: We detected a negative association between psychological stress and semen quality. This finding is of public health importance, as stress is common and might be a modifiable factor. In a clinical setting, psychological stress should be considered upon the interpretation of semen analysis data. Future studies should address causality.

Trial registration number: not applicable.

P-013 The impact of male smoking on intracytoplasmic sperm injection outcomes in In vitro fertilization procedures

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Study question: How does male cigarette smoking influence in vitro fertilization outcomes?

Summary answer: The results suggest that male smoking negatively influences the IVF outcome resulting in reduced ongoing pregnancy and delivery rate.

What is known already: Cigarette smoke comprises toxic, mutagenic chemicals and carcinogens such as cotinine, cadmium, nicotine metabolites, which were found in the seminal plasma at concentrations proportional to those in the serum, suggesting that they can cross the blood-testis barrier providing genotoxic environment for germ cells. An evidence based review suggests that oxidative stress and the causing genetic and epigenetic changes that result from smoking may correlate directly with reduced sperm parameters and reduced sperm function.

Study design, size, duration: This prospective study encompassed the period from 2012 to 2013 with 702 performed cycles from which 329 (46.9%) men were non-smokers and 373 (53.1%) were smokers The exclusion criteria for women were poor responders (basal FSH greater than 10mIU/ml, less than 5 pre-antral follicles) and patients above 39 years of age. The male exclusion criteria were azozoospermia, severe oligozoospermia, donation, chromosome alteration, genitourinary infections and operations, chemotherapy treatment and diabetes mellitus.

Participants/materials, setting, methods: To minimize the bias, only the first IVF cycle was analyzed. Binary logistic regression analysis was used to study the effect of male smoking on clinical pregnancy and delivery after controlling for potential confounding variables, (maternal age, maternal smoking status and asthenozoospermia). Significant differences were considered all values of $p < 0.05$

Main results and the role of chance: Our study indicates that male smoking does not have a significant influence on the clinical pregnancy rate (OR = 1.21, 95%CI 0.89-1.25). After the adjustment for asthenozoospermia (OR = 1.22,CI 0.91-1.65) and female smoking (OR = 1.21 CI 0.89-1.62), male smoking was again confirmed to be non-significant predictor for reaching clinical pregnancy. These findings support the observations of ICSI as preferred fertilization method. The results also demonstrated that male smoking compromised the quality of the ongoing pregnancy. Significant number of early miscarriages was observed among group of smoker men (18.3% as opposed to 5.6% $p < 0.05$ among smokers and non-smokers respectively) resulting in lower ongoing pregnancy rate in this group of patients (45.9% vs 35.9% $p < 0.05$). We found that male smoking generally is

significant factor for increasing the chances for not achieving delivery for 1.53 times (CI 1,13-2,07). Women older than 35 years in group of paternal smoker had 2.27 times (CI 1,39-4,56) higher risk for not achieving delivery. It is likely that if the oocyte's repair capacities are inadequate (low oocyte quality/increased age), a low rate of embryonic development resulting in a high rate of early pregnancy loss.

Limitations, reasons for caution: Our study should be further investigated. The data on smoking and male fertility reinforce the preferred preventive approach of discouraging smoking and eliminating exposure to tobacco smoke in particular while trying to conceive. Antioxidant supplementation may then be taken together to improve the patient's health outcomes.

Wider implications of the findings: It is also very important to comment that all smokers are not infertile, which suggests that genetic variations or polymorphisms in DNA repair, apoptosis and xenobiotic metabolism genes among smokers may increase susceptibility to infertility.

Trial registration number: N/A.

P-014 Is "real time" conventional TESE (cTESE) an obsolete sperm retrieval technique in patients with non obstructive azoospermia (NOA)?

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Study question: How successful is cTESE as a sperm retrieval technique in patients with NOA when performed in a systematic fashion?

Summary answer: Our results show that "real time" cTESE is a highly efficient sperm retrieval technique in patients with NOA.

What is known already: Recent reports in the literature suggest that there is a tendency to replace cTESE with mTESE in NOA patients in many centers around the world. mTESE was introduced as a surgical approach in patients with very small testicles with the notion of preserving an already diminished testicular parenchyma but it has not proven to be superior in most cases of NOA. In order to perform mTESE an operating microscope is required and a highly trained microsurgeon needs to be always available at the time of egg retrieval, if done simultaneously.

Study design, size, duration: Retrospective clinical study, January 1996 to December 2016: all patients who underwent cTESE were included in the study. All patients were surgically approached in the same fashion by the same team. In some instances the procedure was carried out at the time of egg retrieval and in other cases for diagnostic purposes and sperm cryopreservation. The diagnosis was obtained post operatively in most patients.

Participants/materials, setting, methods: A total of 635 patients underwent cTESE under local anestesia with IV sedation. A scrotal incision was performed on one side with up to four testicular incisions at random until sperm were observed. Only if no sperm were present in any of the chosen areas, was the contralateral testis approached in the same fashion. A small specimen was separated for histopathological diagnosis and the patient discharged four hours later.

Main results and the role of chance: Out of 635 patients who underwent cTESE we were able to identify 388 patients with the histological diagnosis of NOA who had their first testicular biopsy along with sperm retrieval attempt. In 30 cases the procedure was a repeat, in 20 cases the patients had been unable to produce a fresh sample the day of oocyte retrieval, in 10 cases the procedure was undertaken due to absolute asthenospermia/necrospermia or severe oligospermia, in 28 cases the patients had a single testis, in 100 cases the diagnosis was obstructive azoospermia (OA), and in 59 cases we were unable to retrieve their histological reports. All these cases were excluded from our study. In the 388 patients identified with the confirmed diagnosis of NOA we found sperm for ICSI or cryopreservation in 234 (234/388; 60%). In 167 cases a unilateral procedure was sufficient (167/388; 43%), in 33 cases sperm were only found in the contralateral testis (33/388; 8%), and in 35 cases (35/388; 9%) a bilateral procedure was needed in order to obtain enough sperm for ICSI or cryopreservation since only scarce sperm could be harvested from each testicle. In patients with a single testis the sperm retrieval rate was 28.5% (8/28).

Limitations, reasons for caution: This is a retropective study. Only one sperm analysis with centrifugation was performed preoperatively to rule out cryptozoospermia.

Wider implications of the findings: Our extensive experience indicates that "real time" cTESE provides optimal sperm retrieval rates when performed in a systematic fashion. The procedure is simpler and less invasive than mTESE, carries less post operative complications and there is no need for an operating microscope and a trained microsurgeon.

Trial registration number: not applicable.

P-015 Correlation between spermatic dna fragmentation and sperm motility in infertile subjects

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Study question: Why the pathophysiology of the testicular damage associated with varicocele remains unclear, sperm DNA damage has been identified as a potential explanation for this cause of male infertility?

Summary answer: Current study determines the extent of sperm nuclear DNA damage in patients with varicocele, and to examine its relationship with parameters of seminal motility.

What is known already: Varicocele is one of the most common adrological pathologies in the general population and particularly common in infertile men. The varicocele negatively influences spermatic function is well documented although the exact mechanisms are still not understood. In fact, infertility may be associated to a variety of spermatogenetic conditions ranging from normozoospermia to moderate oligoasthenoteratozoospermia (OAT), to azoospermia. Several authors have suggested that patients with varicocele have a significantly higher DNA fragmentation index. Studies show that varicocele samples contain a higher proportion of spermatozoa with abnormal DNA and immature chromatin than those from fertile men as well as infertile without varicocele.

Study design, size, duration: One hundred and fifty subjects analyzed in this study ranged in age between 20-50 with the median age of 35 and attending to Andrology Service for infertility diagnosis and other andrological problems. For each patient included in the study, the spermatozoon population was determined. On the basis of the usual seminologic criteria, patients considered in this phase of the study were characterized by isolated asthenospermia, in according to WHO 2010

Participants/materials, setting, methods: Semen samples from 60 patients with clinical varicocele and 90 infertile men without varicocele were examined. Varicocele sperm samples were classified as normal or pathological according to the 2010 World Health Organization guidelines. Sperm DNA damage was evaluated using the Halosperm kit, an improved Sperm Chromatin Dispersion (SCD) test. All data were calculated as average with standard deviation on experiments which were repeated and analized statistically using the statistical program SPSS.

Main results and the role of chance: DNA fragmentation index (DFI: percentage of sperm with denatured nuclei) values was significantly higher in patients with varicocele, either with normal or abnormal (DFI 25.8 ± 3.2 vs 17.4 ± 2.8- $P < 0,01$) semen profiles. Therefore, most significant data of our study came from the analysis of correlation between the DFI values calculated and progressive a + b motility values expressed in % and calculated in patients with varicocele associated with the condition of isolated asthenozoospermia.

Statistical analysis of the data found a semi-empirical correlation of 0,9982 between the index of percentage fragmentation (DFI) and percentage sperm motility according to the: Eq.1: DFI = 49,48 exp (-0,022 motility)

Limitations, reasons for caution: no limitations took place in the study.

Wider implications of the findings: Results of our study, on the higher frequency of DNA fragmentation presented in the sperm cells of infertile patients with varicocele compared to patients suffering from other typologies of

infertility and the fertile controls, supporting the hypothesis which has been proposed by other authors.

Trial registration number: not applicable.

P-016 Development of sperm searching system using artificial intelligence in assisted reproductive technology

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Study question: This study aims to improve the accuracy of normal sperm selection and to reduce burden on embryologists by introducing AI incorporating their skills and experiences to support detection and selection.

Summary answer: Efficient sperm detection and selection, and improving ART treatment outcome will be expected by development of this AI system.

What is known already: The number of ART cycles in Japan has been increasing year by year. Detection and selection of morphologically normal and motile spermatozoa are carried out by the embryologists out of the cell suspension of prepared semen or testicular tissue. However, the treatment outcome is greatly dependent on the individual technique and experience, and it takes a lot of time and labors. It is difficult for all embryologists to detect and select them with equivalent reproducibility. AI excels in universalizing the elements such as technology and experience.

Study design, size, duration: We conducted a preliminary experiment as a collaborative research between our center and the faculty of engineering of Yokohama National University from 2017. In the training data, 8020 positive examples (spermatozoa) and 25522 negative examples (non-spermatozoa) were labeled and AI was made to identify as spermatozoa.

Participants/materials, setting, methods: Embryologists performed labeling target cells in selecting spermatozoa during ICSI and then recorded on HDD and transported to research facilities. Thereafter, we aim to establish the system in following 4 steps: (1)detection and indexing of spermatozoa, (2)extraction of morphology and motility characteristics of spermatozoa, and accurate reproduction of embryologists' point of view in combination with multidimensional feature quantities, (3)assessment of spermatozoa by ensemble learning, (4)collection and evaluation of data by the urological specialists and embryologists.

Main results and the role of chance: Although the linear discrimination plane was learned from the histogram for both the learning data and the test data by the histograms of oriented gradient (HOG) feature extraction method, and the recall rate 0.99 was obtained, the false positive rate> 0.50 in the test data.

Limitations, reasons for caution: Limitation is that this system is still in the development stage.We are now in the stage of confirmation of sperm detection and grading capability with outlier detection on non-sperm HOG by nonlinear discrimination, and it is not yet applied to clinical application.

Wider implications of the findings: Using AI is a new attempt for searching spermatozoa not reported in the past, and establishing this system make it possible to search sperm with high accuracy.

Trial registration number: not applicable.

P-017 Ultrasonographic (US) visualization of the scrotal vas deferens

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Study question: Can the normal scrotal vas deferens be visualized by ultrasonography (US)?

Summary answer: US can visualize the normal scrotal vas deferens and may provide further insights into pathological conditions of the proximal seminal tract.

What is known already: Obstructive azoospermia results from obstruction of the seminal tract between the rete testis and the ejaculatory duct. Determination of the location of the obstruction is extremely important for selecting an appropriate management. Due to its firmness, the vas deferens can be easily palpated through the scrotum. However, subtle changes in the vas deferens including luminal dilation are difficult to identify by physical examination. Although transrectal US clearly reveals the caudal junction of the vas deferens and seminal vesicles, US visualization of the scrotal vas deferens is considered to be difficult due to the complex structure of the spermatic cord.

Study design, size, duration: This prospective observational study involved 463 infertile men who visited our andrology outpatient clinic from April to December 2017. We excluded from the study patients who had any clinical signs of seminal tract abnormality.

Participants/materials, setting, methods: Because unilateral orchiectomy had been performed in two patients, a total of 924 vas deferens in 463 patients aged 25 to 69 years were examined. After physical examination of the scrotum, we evaluated the testis, rete testis, epididymis and scrotal vas deferens by US using a 14-MHz linear array transducer.

Main results and the role of chance: Of the 924 normal vas deferens, only one with very severe varicocele was not visualized by US. The ultrasonographic features of the normal vas deferens are as follows. Above the proximal tortuous part connected to the tail of the epididymis, the vas runs linearly up to the external inguinal ring. The thick muscular wall of the vas appears hypoechoic, and the lumen is shown as "double-line echoes" at the center of the tube. By color Doppler US, while the vas itself lacks blood flow signals, pulsatile signals of the artery of the vas deferens are evident beside the vas.

Limitations, reasons for caution: US does not allow visualization of the vas deferens from the inguinal canal to just before the ampulla.

Wider implications of the findings: Our experience provides further information for diagnosing anatomical abnormalities of the proximal seminal tract in certain conditions, including vasal obstruction after inguinal hernia repair.

Trial registration number: Not required.

P-018 Ameliorative effects of Naringenin in altered testicular ultrastructural and antioxidant activity following use of antiretroviral therapy in Sprague Dawley rats, a randomised trial

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Study question: What is the role of naringenin a bioflavonoid on antiretroviral therapy induced testicular ultrastructural changes and altered fertility potentials in Sprague Dawley rats?

Summary answer: Antiretroviral therapy induced testicular ultrastructural changes associated with altered antioxidant activity, impaired semen parameters and decline in fertility index which was partly restored by Naringenin.

What is known already: Even though infection with Human Immunodeficiency Virus (HIV) depresses the health status of men, it does not eliminate the desire for future fertility yet testicular toxicity has been associated with Highly Active Antiretroviral Therapy (HAART). HAART induced histopathological changes in the testes has been linked to increased activity of reactive oxygen species that ultimately overwhelms the tissue's total antioxidant status. The seminiferous tubules has been shown to be particularly sensitive to the effects of HAART thereby rendering patients oligospermic and even azoospermic. Various flavonoids have been shown to mitigate against free radical/oxidative stress induced tissue damage.

Study design, size, duration: A randomized, controlled animal trial involving 30 male and 18 non- mated female Sprague Dawley rats. Animals were housed in standard cages under controlled environmental conditions (25oC and a 12-h light/dark cycle). The study lasted for a period of 10 weeks.

Participants/materials, setting, methods: Thirty male SD rats weighing 200–220 g, were randomly assigned into groups; viz **DW**: Distilled water, **H**: HAART, **N40**: Naringenin, 40 mg/kg, **N80**: Naringenin, 80 mg/kg, **HN40**: HAART+Naringenin, 40 mg/kg and HN80: HAART+Naringenin, 80 mg/kg. After treatment, copulation was allowed to take place. The number of

pregnancies and litters per group were noted. Semen was analysed. Oxidative enzyme activities were assayed using enzyme linked immunoassay and testicular ultrastructural changes were noted via electron microscopy.

Main results and the role of chance: Sperm count was significantly different between the groups. There was a significantly lower count in group H compared to DW [p = 0.01] and N40 [p = 0.032]. There were significantly lower progressive sperms in group H when compared to DW [p < 0.0001], N40 [P = 0.001], N80 [p = 0.00], HN40 [p = 0.00] and HN80 (HAART+Naringenin 80 mg/kg) [p = 0.001]. Group N40 [P = 0.001] and HN40 [P = 0.002] also displayed significantly lower progressive motility when compared to DW.

The testicular glutathione peroxidase levels was higher in the N40 and N80 groups when compared to H group. Lower levels were also observed in groups DW, HN40 and HN80. The N80 group showed significantly higher level of testicular catalase activity than groups DW (p = 0.024) and H (p = 0.003)

Group H displayed thickening and irregularity in the outline of the basement membrane. Normal cellular progression in the germinal epithelium was altered. Spermatids appeared in the basal compartment. There was widening of the internuclear space between Sertoli cells. Degenerative changes appeared in the nuclear membrane, abnormally formed spermatid heads and disorganised axonemes were observed the in the midpiece.

The fertility index was higher in the DW group than in the H and N40 groups. The number of pubs per group were also higher.

Limitations, reasons for caution: A larger sample size would have been more convincing especially in the aspect of fertility index. The study considered the effect of a FDC of HAART, it is therefore difficult to associate the effects observed to a single drug in the combination.

Wider implications of the findings: The study suggests that HAART has deleterious effects on testicular function and male fertility. This agrees with earlier findings. Naringenin, a bioflavonoid may be a useful adjuvant therapy in protecting against testicular toxicity.

Trial registration number: The Animal Research Ethical Committee (AREC), UKZN, South Africa approved this research with a reference number AREC/046/016D.

P-019 CD147 deficiency is associated with impaired acrosome reaction in patients with asthenozoospermia

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Study question: What is the etiological factor underlying the impaired acrosome reaction in asthenozoospermia infertile patient?

Summary answer: Our study showed that CD147 deficiency is associated with impaired acrosome reaction in patients with asthenozoospermia.

What is known already: Asthenozoospermia, a condition which sperm exhibit motility lower than the normal threshold, accounts for approximately 18% of all male subfertility and infertility cases. A previous study has shown that deficient human β-defensin 1, a small antimicrobial peptide released into the epididymis and female reproductive tract, underlies poor sperm motility observed in asthenozoospermia patients. Intriguingly, sperm from asthenozoospermia patients has been shown to have a significantly lowered rate of ionophore-induced acrosome reaction, suggesting that the infertility outcome of asthenozoospermia patients could be attributed to multifaceted factors. However, the molecular mechanisms underlying defects in acrosome reaction leading to infertility remain incompletely understood.

Study design, size, duration: Sperm from fertile men (n = 36) and asthenozoospermia patients (n = 27) were included in the study. The samples were evaluated for the protein level of CD147 on sperm and used as an in vitro model to study the effect of soluble CD147 on sperm functions and its underlying molecular mechanism.

Participants/materials, setting, methods: The level of CD147 on sperm was assessed by immunofluorescence staining and semi-quantitative Western blot. Sperm samples were treated with recombinant CD147 proteins or conditioned media containing soluble CD147. Sperm functions were evaluated by computer-assisted sperm analysis (CASA), fluorescein-Pisum sativum agglutinin (FITC-PSA) assay, hyaluronan binding assay and oocyte penetration assay. Calcium imaging was used to examine the mechanism underlying the effect of CD147 on sperm functions.

Main results and the role of chance: The localization of CD147 was shifted from the mid-piece to the head region upon capacitation. The expression levels of CD147 were lower in sperm from asthenozoospermia infertile patients that exhibited defects in both sperm motility and acrosome reaction. The reproductive female tract-derived soluble CD147 interacted with sperm-bound CD147. The dimerization of CD147 induced a 2-fold increase in intracellular calcium level and a 3-fold increase in the percentage of acrosome reaction in normal capacitated sperm. Soluble CD147 treatment restored the acrosome reaction from 10% to 37% and enhanced the fertility potential of sperm from asthenozoospermia patients.

Limitations, reasons for caution: The gain- and loss-of-function in human sperm were only achieved by neutralizing antibody against CD147 and recombinant CD147. A more reliable genetic overexpression or knockout approach was not possible in human sperm.

Wider implications of the findings: CD147 deficiency may underlie the acrosome reaction defects in male infertile patients. CD147 is a potential diagnostic marker for male infertility and soluble CD147 is a feasible approach to enhance the fertility outcome of assisted reproductive technologies.

Trial registration number: Not applicable.

P-020 Sperm vitality and DNA fragmentation index (DFI) are good predictors of progressive sperm motility in oligozooasthenospermic men treated with metabolic and essential nutrients

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Study question: The goal of this study was to examine correlation between sperm vitality and DFI with progressive sperm motility after 6 months therapy of oligoasthenospermic men.

Summary answer: The increase of the vitality of spermatozoa and decrease DFI have very good predictive and diagnostic characteristics of progressive sperm motility after 6 months therapy.

What is known already: Sperm vitality is a reflection of the proportion of live, membrane-intact spermatozoa determined by either dye exclusion or osmoregulatory capacity under hypo-osmotic conditions. Sperm DNA damage has been associated with adverse reproductive outcomes and has been increasingly utilised in the management of male infertility in the era of IVF and ICSI. L-carnitine is essential for the normal mitochondrial oxidation of fatty acids, protects cell membrane and DNA against damage induced by free oxygen radicals. Both L-C and acetyl-L-carnitine (ALC) also have important roles in the post-gonadal maturation of spermatozoa.

Study design, size, duration: The study was randomized, double blind, placebo controlled (DBPC) and examined the effect of test formulation, Proxeed Plus, containing L-C 2 g and ALC 1 g, as well as antioxidants, vitamins and minerals, in men with idiopathic oligo-asthenozoospermia (age group18-50 years). The protocol was 2 months wash-out and 6 months treatment (T-2, T0, T + 3, T + 6), with test formulation (125 patients) or placebo (50 patients).

Participants/materials, setting, methods: The subjects with oligoasthenozoospermia and history of infertile marriage more than one year, were randomized to receive treatment or placebo in a double blind protocol. Analysis of ejaculate was done according to WHO 5th guideline. Progressive sperm motility (A + B grade of rapid) was done manually. DFI was evaluated by Halosperm kit

(Halotech DNA, S.L) and sperm vitality was done by the one-step eosin-nigrosin technique.

Main results and the role of chance: The results of treated group: DFI (%) was T0 = 38,50 (32,00-48,75), T3 = 35,50 (25,50-44,00) and T6 = 31,00 (25,00-41,00); sperm vitality (%) was T0 = 0.52 (0.43-0.60), T3 = 0.57 (0.46-0.64) and T6 = 0.56 (0.56-0.65; the progressive sperm motility: T0 = 28.00% (12.00 ± 38.00), T3 = 30.00%(12.00 ± 39.00) and T6 = 31.00%(20.00 ± 41.00). All showed significance of $p < 0.001$ by Friedman test. The increase of spermatozoa vitality has the best predictive and diagnostic characteristics. Men who have increased this parameter by 1% have 1,119 times more likely to have a progressive motility of spermatozoa greater than 20% after 6 months of therapy. If the spermatozoa vitality after six months of therapy increases by 5.9% and more (cut off value), the probability that sperm motility will be greater than 10% or 20% is 100% (PPV = 100%). If DFI drops by more than 3% (cut-off), after 6 months of therapy, it can be expected, with moderate accuracy, that men have sperm motility greater than 10% (AUC = 0.793; $p < 0.001$). DFI reduction (odds ratios = 1.106 with 95% confidence intervals) independently increases the likelihood that sperm motility is > 10%. Placebo group: no significant difference in sperm motility, vitality and DFI, between T0 and T6.

Limitations, reasons for caution: No technical limitations.

Wider implications of the findings: This analysis showed that the increase of the vitality of spermatozoa has the best predictive and diagnostic characteristics of progressive sperm motility after 6 months therapy. Further, the percentage of change in DFI after therapy can be used with moderate accuracy in the detection of men with better sperm motility.

Trial registration number: TXT-001-B

P-021 Does replacement of the sperm donor increase the chance of achieving a pregnancy?

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Study question: to assess whether replacing a sperm donor affects the chances of achieving pregnancy via intrauterine insemination treatments.

Summary answer: A sperm donor replacement may increase the chances to conceive in women who failed to achieve a pregnancy within a few AID treatments.

What is known already: The primary recipients of donor sperm are single women, lesbian couples and heterosexual couples suffering from male infertility. Artificial insemination by sperm donor (AID) is a widely used treatment to achieve pregnancy in those cases. There is a common belief among some of the recipients that replacement of the donor will improve their chances to conceive in cases of repeated failures.

Study design, size, duration: Study population included 312 women who conceived using artificial insemination by sperm donor.

Participants/materials, setting, methods: Group A consisted of 242 women were treated with one donor only and group B of 70 women were treated with one donor for a few cycles and with a different donor for additional cycles. Statistical analysis was conducted using a multivariate model, with consideration of various factors such as age, hormonal treatment, number of inseminations, etc.

Main results and the role of chance: The age of the participants in groups A and B was similar with mean±SD of 37.02 ± 3.58 and 37.26 ± 3.38, respectively. Baseline FSH levels on day 3 of the cycle was similar in groups A and B. There was a significant difference between the number of AID cycles till pregnancy achievement with a single donor (group A) and changed donor (group B, $p < 0.001$) with mean number of 3.78 ± 1.90 and 6.07 ± 2.95, respectively. Further subdivision of groups A and B according to the type of fertility treatment showed significant difference between the number of AID cycles of the first and the second donors of group B ($p < 0.001$).

Limitations, reasons for caution: A retrospective study of women who achieved pregnancies.

Wider implications of the findings: Our results suggest that among women who failed to achieve a pregnancy within a few AID treatments, a sperm donor replacement may increase their chances to conceive regardless of ovulation protocol. Further study is needed to provide explanation for this phenomenon.

Trial registration number: 0726-15-TLV.

P-022 Impact of cigarette smoking on sperm DNA methylation patterns and gene expression

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Study question: Are there any implicating consequences of cigarette smoking on sperm DNA methylation level and gene expression?

Summary answer: Cigarette smoking leads to an alteration in the sperm DNA methylation patterns of *PTPRN2* and *TYRO3* genes. Besides, an influence on gene expression level.

What is known already: Various factors affect the sperm DNA methylation patterns including, but not limited, to other environmental, social, racial and biological factors. The effect of cigarette smoking on DNA methylation and consequently gene expression is still considered a contradictory issue.

Study design, size, duration: This study is a prospective cohort study. A total of 108 human semen samples were collected during the period of September 2016 to July 2017. Fifty-nine samples were obtained from fertile heavy smokers, who smoked ≥ 20 cigarettes per day (as a case), and forty-nine from fertile non-smokers males (as control).

Participants/materials, setting, methods: All participants came from the same region without demographic differences such as race. They were all in reproductive age and normal body mass index. Thirty samples (15 Cases and 15 controls) were subjected to Infinium 450 K BeadChip arrays to identify variations in sperm DNA methylation between case and control groups. Furthermore, the local deep bisulfite sequencing was applied to 108 samples to validate CpGs, followed by qPCR to assess the gene expression levels.

Main results and the role of chance: The results of 450 K BeadChip arrays revealed a significant difference in 7 CpGs of the DNA methylation level between case and control groups. Five of them were overlapped with the annotated SNPs. Therefore, they were excluded from this study. The remaining CpGs(cg23841288, and cg19169023) were directly linked to the *PTPRN2*, and *TYRO3* genes and underwent further validation using deep bisulfite sequencing. According to the results of validation, significant differences in the methylation patterns at more than one CpGs and related to *PTPRN2* and *TYRO3* genes amplicon in cases compared to control groups were shown. Whereas, nine of twelve CpG sites in the *PTPRN2* gene-related amplicon ($p \leq 0.006$, $p \leq 0.004$, $p \leq 0.01$, $p \leq 0.01$, $p \leq 0.01$, $p \leq 0.01$, $p \leq 0.03$, $p \leq 0.006$ and $p \leq 0.01$ respectively) and three CpGs of four tested CpGs in the *TYRO3* gene ($p \leq 0.008$, $p \leq 0.005$, p and $p \leq 0.02$ respectively) were found. Furthermore, the present study demonstrates significant differences in the gene expression levels of the *PTPRN2* and *TYRO3* genes in the cases compared to controls ($P \leq 0.01$, and $P \leq 0.04$ respectively), with fold change (0.6 and 1.2 respectively).

Limitations, reasons for caution: The sample size was a constraint towards getting a strong outcome. Therefore, more samples are required to demonstrate the impact of this variation on sperm DNA methylation patterns and the effect of genes expression on human male fertility.

Wider implications of the findings: The study results were a concurrent confirmation towards the drawbacks of cigarette smoking. It should be noted that significant correlations between the variation in the sperm DNA methylation patterns and semen parameters were found. This was furtherly elucidated through the positive results of gene expression of the related genes.

Trial registration number: NO.

P-023 Assessment of sperm apoptosis by flow cytometry technique using 7-Amino-Actinomycin D staining

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Study question: Does the analysis of sperm vitality and apoptosis by the flow cytometry technique offer an interesting sperm biomarker to predict male fertility potential?

Summary answer: Flow cytometry allows identifying the apoptotic spermatozoa not individualized by classic microscopic evaluation.

What is known already: Conventional sperm analysis is a subjective method and shows an average prediction of male infertility. New processes can indicate a more accurate prediction for the state and function of the sperm. Therefore, flow cytometry has become an important technique in sperm evaluation and is increasingly used both for routine assessment and for research in human reproduction.

Study design, size, duration: This basic research study was performed on semen samples collected from 52 patients attending our center for couple infertility investigation.

Participants/materials, setting, methods: Semen analysis was carried out according to WHO 2010 guidelines. Sperm vitality was evaluated respectively by the stain exclusion method (Eosin-Nigrosin) and the flow cytometry technique using 7AAD (7-Amino-Actinomycin D) a DNA specific marker witch label dead spermatozoa. Sperm mitochondrial membrane potential was also analyzed by the flow cytometry technique using JC1 staining.

Main results and the role of chance: Sperm vitality values assessed by Eosin-Nigrosin method were significantly higher than those noted by cytometry analysis (74.5% vs 62.3%, $p < 0.001$). The apoptotic sperm levels were significantly correlated with sperm motility ($r=-0.283$, $p = 0.04$), sperm mitochondrial membrane potential ($r = -0.395$, $p = 0.005$) and leukocyte concentrations ($r=+0.434$, $p = 0.01$).

Limitations, reasons for caution: The present study was performed in relatively small number of samples. Further studies with larger sample size are needed.

Wider implications of the findings: Active oxygen species produced by leukocytes cause significant damage to sperm membranes with severe alterations of mitochondrial functions inducing sperm apoptosis. Sperm mitochondrial membrane potential combined with sperm apoptosis level could be interesting parameters for predicting natural conception and might be used prior to IVF/ART program.

Trial registration number: No trial registration number.

P-024 The impact of seminal hyperviscosity on sperm parameters and male fertility

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Study question: Is there an effect of increased seminal viscosity on sperm parameters and does it contribute in male reproduction disorders?

Summary answer: Semen hyperviscosity significantly affects sperm parameters mainly motility and negatively decreases male fertility rates.

What is known already: Semen hyperviscosity (SHV) is a condition that can impair the physical and chemical characteristics of seminal fluid, which leads to an adverse impact on sperm functions. SHV also leads to certain technical difficulties in the handling of samples, such as when using different methods of semen preparation for *in vitro* fertilization (IVF) programs. Some procedures used to treat SHV may damage sperm structure and should be revised.

Study design, size, duration: This retrospective study was performed on semen samples collected over period of 8 years from 2585 patients attending our center for couple's infertility.

Participants/materials, setting, methods: Semen analysis was caried out according to WHO guidelines. Sperm viscosity was estimated by gently aspirating it into a wide bore pipette, allowing semen to drop by gravity and observing the lenght of any thread, getting 3 groups: G1 (normal viscosity; $n = 2139$): sperm leaves the pipette in seperate drops; G2 (moderate hyperviscosity; $n = 56$): sperm drop from a thread more than 2 cm long; G3 (severe hyperviscosity; $n = 390$): sperm flows very difficult or stays blocked.

Main results and the role of chance: The observed prevalence of SHV was 17.2% (446/ 2585). The mean values of sperm parameters including: volume, numeration, motility, vitality and normal morphology in G1 group were significantly higher than those in G2 and G3 ($p = 0.02$, $p = 0.01$, $p < 0.001$, $p < 0.001$, $p < 0.001$; respectively). Asthenospermia associated to SHV would be related to liquefaction process disturbance secondary to semen kallikreins variability and the abscence of semenogelins matrix hydrolysis. Hypofunction of the prostate or seminal vesicles result in the developmet of SHV.

Limitations, reasons for caution: A potential limitation for this study is its retrospective design. Moreover, semen hyperviscosity does not seem to be induced by a single pathogenic factor, but rather by several (biochemical, enzymatic, and genetic) factors that act in synergy. These factors should be studied further.

Wider implications of the findings: Biochemical changes on SHV could impair male fertility linked to a reduce of seminal antioxydant capacity, sperm membrane lipid peroxidation and sperm nuclear DNA damage. Otherwise, the mechanism in which the depletion of antioxidants in the seminal plasma of patients occurs has not been fully elucidated.

Trial registration number: not applicable.

P-025 Genome wide DNA methylation analysis of ROS-mediated sperm chromatin integrity damage suffering from idiopathic male infertility

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Study question: The objective of this study was to assess genome-wide sperm DNA methylation alteration between idiopathic male infertility with ROS-mediated DNA damage and normal fertile males.

Summary answer: The results show that there are significant differences in sperm DNA methylation levels between sperm chromatin integrity damage patients and fertile males.

What is known already: It is now clear that ROS-mediated sperm chromatin integrity damage is critical in male infertility. What is not clear is whether ROS could induce epigenetic alterations that further contribute to male infertility.

Study design, size, duration: A genome-wide DNA methylation study was performed using sperm samples of men who were idiopathic infertility with sever sperm chromatin integrity damage. Human semen samples were collected during the period between July 2017 to September 2017.

Participants/materials, setting, methods: Firstly, Infinium 850 K Methylation BeadChip arrays were used to determine whether sperm DNA methylation alterations between 4 infertility males with high sperm DNA damage compared to 4 proven fertile males. Then the top five sites with highest difference in methylation levels were underwent to further analysis using deep bisulfite sequencing in a large samples (100 controls and 100 cases).

Main results and the role of chance: We observed significantly different DNA methylation profiles for the sperm chromatin integrity damage patients and fertile controls. The DNA methylation patterns showed that in the cases, 225 of the 445 CpG sites were hypermethylated and 220 hypomethylatedwere (relative to the fertile controls). Furthermore, we identified genes that may provide insight into the mechanism of ROS-mediated idiopathic male infertility. The top 5 CpGs were found to be directly linked to the genes ZFAT, SOX6, SCARNA17, RP11-5P22.1 and MIR300. Currently, these results are under validation through local deep bisulfite sequencing and are planned to be tested on a larger sample cohort. Gene Ontology analysis indicated the genes are associated with some molecular function and biological process, such as phosphatase binding, DNA binding, nucleic acid binding transcription factor activity, cell differentiation regulation of metabolic process and RNA polymerase II transcription factor activity. KEGG pathway analysis suggested that the DMRs were distributed among pathways of ErbB signaling, Gap junction, GnRH signaling and pathways in cancer, which will lay the theoretical foundation to further excavating the functional genes of ROS-related DNA damage.

Limitations, reasons for caution: The DNA methylation patterns in sperm chromatin integrity damage patients should be confirmed with an mRNA expression analysis and correlated with immunohistochemical data on protein

expression and localization. More studies are needed to elucidate the mechanisms relating to these alterations and to identify their significance and consequences on male infertility.

Wider implications of the findings: The comprehension of mechanisms leading to epigenetic modifications associated with ROS may help better understand the male infertility, as well as aid in the development of potential biomarkers for better male infertility diagnostics and novel therapeutic strategies.

Trial registration number: No.

P-026 Evaluating the predictability of oxidation–reduction potential in male factor infertility in conjunction with semen analysis: A multicenter study

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Study question: Identify if ORP measure could reliably predict semen samples that meet normal reference range of WHO criteria from those that fail to meet across fertility centers.

Summary answer: Of the 1644 samples analyzed, the ORP measure provided the greatest predictability when distinguishing abnormal from normal semen quality among patients undergoing infertility evaluation.

What is known already: Discrete measures of free radicals, antioxidant activity, and oxidative damage suggest an ambiguous relationship between the redox system and male fertility. Oxidation-reduction potential (ORP) measures the balance between all oxidants and antioxidants providing a comprehensive status of the redox system. In previous studies, ORP has been tested in semen samples using the MiOXSYS System as an alternative method for measuring oxidative stress and distinguishing normal controls from male factor infertility patients; thus ORP levels may be used to clarify the relationship between the redox system and semen parameters associated with male infertility.

Study design, size, duration: This prospective study was carried out jointly by nine participating fertility centers on 1644 subjects. The study was approved by the institutional ethics committee and subjects were consented prior to participation. Subjects were grouped into those that had all normal semen parameters (concentration, total concentration, total motility, progressive motility, and morphology) according to WHO 2010 guidelines and those who failed to meet one or more criteria.

Participants/materials, setting, methods: Exclusion criteria included azoospermia, presence of sexually transmitted disease or chronic diseases, use of prescription, OTC medications or antioxidants. Samples were collected and semen parameters assessed using the WHO 2010 guidelines. ORP was measured (mV) using the MiOXSYS system and normalized to concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration >0.999 × 10^6 sperm/mL were included.

Main results and the role of chance: In the current study, using an ORP test in conjunction with the semen analysis measures resulted in the detection of abnormal semen quality with a 98.1% sensitivity, 40.6% specificity, 94.7% positive predictive value, and 66.6% negative predictive value. ORP provides a unique and statistically significant contribution in classifying semen quality into abnormal and normal status. A logistic regression performed on all measures (six semen analysis parameters and ORP measure) revealed the predictability of identifying abnormal / normal semen quality within the samples (Table 1). Measures were categorized according to overall contribution and significance. ORP ranked the highest (beta 2.88, p = 0.01) in terms of predicting abnormal / normal semen quality, followed by progressive motility (beta 2.29, p = 0.001), and total motility (beta .494, p = 0.005). Utilizing ORP and semen analysis combined, the overall performance characteristics (Table 2) were 98.1 sensitivity, 40.6 specificity, 93.3% positive predictive value, and 66.7% negative predictive value.

Table 1

	BETA	S.E.	DF	Sig.
(ORP)	2.880	1.152	1	0.012**
(SpermTotal)	−0.150	0.202	1	0.458
(PRMot)	−2.297	0.211	1	0.001***
(TotalMot)	0.494	0.177	1	0.005***
(NormlMorph)	−0.115	0.097	1	0.233
(SpermConc)	−0.356	0.186	1	0.055
(Volume)	0.149	0.162	1	0.358

Limitations, reasons for caution: A number of healthy controls with proven fertility was limited in comparison to the male infertility group. While semen parameters are an important part of the assessment of the infertile male, the gold standard is the reproductive outcome. Pregnancy outcomes were not prospectively measured in the infertile group.

Wider implications of the findings: Abnormal ORP levels will be especially useful in pinpointing the altered functional status of the sperm in patients with idiopathic male infertility and thereby directing those men toward accurate therapeutic management.

Trial registration number: (not applicable) WIRB PRO NUM: 20170761

P-027 Ultrasonographic (US) analysis of seminiferous tubules in patients with complete deletion of the AZFc region

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Study question: Can US findings regarding seminiferous tubules predict sperm production in patients with complete deletion of the AZFc region?

Summary answer: Thick seminiferous tubules on US images imply sperm production in patients with complete deletion of the AZFc region.

What is known already: Microdeletion of the AZF region located on the long arm of chromosome Y is the most frequent genetic cause of spermatogenic failure. Complete deletion of the AZFc region causes a variable clinical phenotype ranging from azoospermia to severe oligozoospermia.

We have previously demonstrated that seminiferous tubules over 200 μm in diameter can be visualized by US, and thick seminiferous tubules (over 300 μm in diameter) suggest a high rate of successful sperm retrieval in patients with non-obstructive azoospermia.

Seminiferous tubules in patients with complete deletion of the AZFc region have not yet been analyzed by US.

Study design, size, duration: This retrospective study included 802 consecutive patients with non-obstructive azoospermia or severe oligozoospermia who underwent an evaluation for Y chromosome microdeletions at our andrology clinic from June 2014 to October 2017.

Participants/materials, setting, methods: All of the study patients underwent a physical examination, endocrine assessment, karyotyping, Y chromosome microdeletion analysis and US evaluation of the testis, which involved measurement of the testicular volume and the diameter of seminiferous tubules.

US was performed using a 10- or 14-MHz linear array transducer. To optimize US images for visualization of the seminiferous tubules, gain and contrast were appropriately adjusted with graphics software.

Main results and the role of chance: Complete deletion of the AZFc region was identified in 49 patients. After we excluded 2 patients with chromosomal abnormalities and 1 patient with orchitis, 46 patients were included in this study. Of these patients, 31 had sperm in the ejaculate, whereas 15 were azoospermic. Microdissection TESE (micro-TESE) was performed in 6 of the 15 patients with azoospermia, and testicular sperm were retrieved in 2 of these 6 patients.

Thick seminal tubules, defined as more than 300 μm in diameter on US images, were visualized in all patients with sperm in the ejaculate and in both patients with testicular sperm. In contrast, thick seminal tubules were not observed in any of the 4 patients in whom sperm was not retrieved by micro-TESE.

Almost 90% (33/37) of patients with complete deletion of the AZFc region had sperm in the ejaculate or testis. Thick seminiferous tubules on US images in patients with complete deletion of the AZFc region indicate sperm production. Furthermore, a lack of thick seminiferous tubules on US images may suggest a poor outcome of micro-TESE in patients with complete deletion of the AZFc region.

Limitations, reasons for caution: Only 6 of 15 azoospermic patients with complete deletion of the AZFc region underwent micro-TESE, and thick seminiferous tubules were not observed in US in any of the 9 patients who did not choose micro-TESE. More data will be needed before we can draw definitive conclusions concerning this small category.

Wider implications of the findings: US analysis of seminiferous tubules may be helpful for estimating the potential for sperm production in patients with various types of genetic disorders.

Trial registration number: Not applicable.

P-028 Adverse effects of Reactive Oxygen Species in semen to the development of ICSI cleavage embryo

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Study question: Does Reactive Oxygen Species (ROS) in semen have adverse effects to the embryo development in ICSI?

Summary answer: The ROS level in semen was significantly higher in non-successfully developed cleavage embryo group compared to successful developed cleavage embryo group.

What is known already: Reactive Oxygen Species(ROS) in semen is created from immature spermatozoa and seminal leukocytes. High ROS level results in lipid peroxidation, DNA damage and induction of apoptosis, which has been reported to have negative efffect to sperm concentration, motility and male fertile capacity.

Study design, size, duration: This is a retrospective study between March 2013 and December 2016. 887 embryos from 141 cycles were analyzed, which were divided into to successfully developed group and non-successfully developed group and the level of ROS was compered between two groups.

Participants/materials, setting, methods: 77 infertile couples who perfomed ICSI at our center were enrolled. 887 embryos from 141 cycles were analyzed.The ROS level were measured by monolight 3010® using whole semen, which were compared 1) between fertilized group and non fertilized group, 2) between good cleavage embryo group and non-developed embryo group. 3) The cut-off ROS level was calculated for the prediction of good cleavage embryo development.

Main results and the role of chance: 1) The mean ROS level were 50842 (±178426) relative light unit(RLU) and 50118 (±157274) RLU, respectively. No significant difference was observed between the two groups($p = 0.952$). 2) The ROS level was significantly higher in non-developed embryo group compared to good cleavage embryo group(81450 ± 128610 RLU vs 35645 ± 128610 RLU, $p = 0.026$). 3)The cut off value was 6601 RLU calculated from receiver operating characteristic curve (Area under the curve = 0.539), and the embryos were diveded into high ROS group and low ROS group using this cut-off value. Good cleavage embryo rate was significantly lower in high ROS group compared to low ROS group(51.5% vs 63.8%, $p = 0.004$).

Limitations, reasons for caution: This is a retrospective and a single-institute study, prospective and multi center analysis is needed for further investigation. Anti-oxidant capacity in semen against the ROS were not evaluated in this study.

Wider implications of the findings: ROS in semen have adverse effect under the environment of embryo development in ICSI, especially for the good cleavage embryo development than for the fertilization. It may help unveiling the mechanism of cleavage embryo development.

Trial registration number: not applicable.

P-029 Pregnancy rate and infertility in patients with varicocele and/or oligoasthenoteratozoospermia: evaluation of antioxidant supplementation effect on sperm parameters

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Study question: Are functional substances able to treat male infertility and which is the role of varicocele and oligoasthenoteratozoospermia?

Summary answer: The use of functional substances is an efficacious strategy to handle male infertility. All sperm parameters and pregnancy rate significantly increased in treated subjects.

What is known already: Varicocele has adverse effects on spermatogenesis and to date is considered as the first cause of male infertility. Many factors negatively affecting semen quality act through decreasing energy availability by mitochondrial dysfunction and sperm are also vulnerable to reactive oxygen species because their accumulation leads to membrane damage, instability and functional alterations. However, for normal sperm cell function, a delicate redox balance of reduction and oxidation is required. Thus, a therapeutic strategy would need to use supplements to increase sperm energy metabolism, minimize free radical damage and improve the cellular processes connected with the formation and maturation of sperm.

Study design, size, duration: To evaluate, utilizing a randomized double-blind placebo controlled trial, the effect of supplementation with selected naturally compounds on pregnancy rate and sperm quality. The effect was evaluated in subjects with oligo or asthenoteratozoospermia, as well as with or without varicocele.

Participants/materials, setting, methods: With a block randomization 104 patients were enrolled: 52 had grade I-III varicocele and 52 were not affected. Patients were further divided in two groups consisting of the supplementation arm and the placebo arm. The supplementation formulation consisted of L-carnitine, fumarate, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, zinc, folic acid and vitamin B12. Spermogram evaluation was done at the beginning of treatment and after 6 months, at the end.

Main results and the role of chance: Adverse events occurred only in the treatment group: 4 patients had nausea and 3 vertigo or headache. Twelve pregnancies occurred during follow-up time: 10 in supplementation group (9 non-varicocele and 1 varicocele) and 2 in placebo group (1 non-varicocele and 1 varicocele). One spontaneous abortion was reported in placebo arm. Mean changes of number of sperm ($10^6 \times$ mL) after treatment were 1.7 in the placebo group and 9.8 in the supplemented group ($p = 0.0186$). Mean changes of sperm concentration ($10^6 \times$ mL) after treatment were 13.0 in the placebo group and 46.9 in the supplemented group ($p = 0.0117$). Mean changes of progressive motility of sperm (%) were 1.7 in the placebo group and 5.9 in the supplement group ($p = 0.0088$). Mean changes of total motility of sperm (%) were 1.6 in the placebo

group and 7.3 in the supplement group (p = 0.0120). Analyzing typical and atypical morphology there was, respectively, a difference of -6.1 and 5.9 in the placebo group while -6.7 and 3.6 in the supplement group.

Limitations, reasons for caution: We did not compare the effect of this treatment with surgical treatment of varicocele and we did not evaluate DNA fragmentation and level of ROS. Furthermore, latest evidences report that evaluating OS can be a diagnostic tool in predicting the best responders to supplementation.

Wider implications of the findings: Oxidative stress is a cause of male infertility with significant negative effect on semen parameters and varicocele is an additional cause of poor sperm quality. The use of functional substances is an efficacious strategy to handle male infertility. All sperm parameters and pregnancy rate increased in treated subjects.

Trial registration number: PXP-001A

P-030 Novel measures of sperm DNA damage increase its usefulness to diagnose male infertility and predict live births following both IVF and ICSI

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Study question: Predictive power of novel Comet parameters that quantify damage levels in the semen sample and utility in prediction of ART live birth rates.

Summary answer: The proportion of sperm with low or high levels of DNA damage provides discriminatory information for male infertility diagnosis and treatment outcomes.

What is known already: The Comet assay measures the DNA damage in individual sperm enabling the degree of heterogeneity of the whole sperm population to be assessed.

Study design, size, duration: Retrospective study of 381 men of couples attending a Lister Fertility Clinic for IVF or ICSI treatment were recruited during 2015-2017

Participants/materials, setting, methods: 381 couples attending for IVF or ICSI. Sperm DNA damage quantified using the average Comet Score (ACS) and two novel parameters: the low and the high Comet Score. Sperm from 166 men with idiopathic infertility were compared with those from 76 sperm donors, using ROC analysis. 79 IVF and 229 ICSI cycles were included to determine thresholds for each parameter using ROC analysis. Thresholds were used to compare live birth rates (LBRs) following ART.

Main results and the role of chance: 80% of sperm from fertile men had less than 33% DNA damage. ACS, HCS and LCS were all highly predictive of male infertility (ROC > 0.9, p < 0.0001). IVF LBRs declined sharply once sperm DNA damage exceeded all threshold levels with HCS showing the sharpest decline. ICSI LBRs were also impacted by sperm DNA damage with highest LBRs in men whose sperm DNA approached the fertile range. Trends in IVF and ICSI differed in that IVF LBRs decreased as damage increased whereas in ICSI, LBRs decreased but then remained stable.

Limitations, reasons for caution: A prospective study choosing IVF or ICSI based on these thresholds should be performed to confirm this data.

Wider implications of the findings: The proportion of sperm with low or high levels of DNA damage provides discriminatory information for male infertility diagnosis and chance of success by IVF compared to ICSI. Implementing these new test thresholds will enable better selection of ART treatment pathways.

Trial registration number: None.

P-031 High concentrations of interleukin-18 induce Leydig cell apoptosis and steroidogenesis disorder

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Study question: Do high concentrations of interleukin (IL)-18 influence a drop in steroidogenesis by increasing apoptosis of Leydig cells?

Summary answer: High IL-18 concentrations increased apoptosis-related proteins (cleaved caspase [CC]-8 and -3) and death receptor pathway-related mRNAs levels but reduced streroidogenic acute regulatory (StAR) protein levels.

What is known already: IL-18, which is produced by germ cells, Leydig cells and resident macrophages, is an indispensable cytokine in the maintenance of testis homeostasis. IL-18 is known to be an inflammasome-mediated cytokine, and IL-18 levels are increased by inflammatory stimulation such as lipopolysaccharide (LPS) in mouse testis. We showed that endogenous IL-18 induced testicular germ cell apoptosis during endotoxin-mediated inflammation when the plasma IL-18 levels were very high (Inoue T. *et al. Reproduction.* 2015;150:105-114).

Study design, size, duration: LPS stimulation: Mouse Leydig cell line TM3 cells and mouse macrophage cell line RAW264.7 cells were stimulated with 200 or 1,000 ng/mL LPS and were sampled at 0, 1, 6, 12, 24 and 48 hours. Recombinant IL-18 (rIL-18) stimulation: TM3 cells were stimulated with 0.1, 1, 10 and 100 ng/mL rIL-18 and were sampled at 12 h. Vehicle: TM3 or RAW264.7 cells were incubated in the medium only.

Participants/materials, setting, methods: Cells were cultured on 6-well microplate (4×10^5 cells) with DMEM-F12 medium for 48 h at 37°C, 5% CO_2. CC-3, CC-8, IL-18 and StAR protein levels were measured by western blotting. The expression levels of *Tnf-α, Il-6, Il-18, Fas, Fas ligand (FasL), Tnfr1* and *Fadd* mRNAs were analysed using reverse transcriptional real-time PCR. Statistical analysis was performed using Student's t-test or Welch's t-test and Tukey-Kramer's post-hoc test (statistically significant: $p < 0.05$).

Main results and the role of chance: LPS significantly increased *Tnf-α* and *Il-6* expressions at 1 h ($p < 0.01$) and *Fas* and *Tnfr1* expressions at 6 h ($p < 0.01$). LPS also increased CC-8 and CC-3 expressions at 6 and 12 h, respectively, and these levels were maintained until 48 h. However, *FasL* and *Fadd* mRNA expressions did not differ significantly between the LPS and control groups. LPS stimulation did not increase IL-18 protein and mRNA expressions in TM3 cells. The expression of StAR was reduced at 12 h and maintained until 48 h after LPS stimulation. These results suggest that LPS-induced Leydig cell apoptosis may lead to steroidogenesis reduction. We hypothesised that IL-18 level increased in testes after inflammation was derived from immune cells. *Il-18* expression in RAW264.7 was significantly increased at 6 h ($p < 0.01$) and maintained at a high level until 48 h after LPS stimulation. High-concentrated (10 or 100 ng/mL) rIL-18 increased CC-3, CC-8, *Tnf-α, Fas* and *Fadd* expressions. Contrariwise, high-concentrated rIL-18 reduced the expression of StAR protein. There was no significant difference between control and rIL-18 stimulation on *FasL* and *Tnfr1* mRNA expressions. These results suggest the overproduction of IL-18 induced Leydig cell apoptosis and reduced steroidogenesis during acute inflammation.

Limitations, reasons for caution: The limitation of this study is that Leydig cell-macrophage interactions during inflammatory condition could not be evaluated. Therefore, even though our results may not completely explain the relationship between Leydig cell apoptosis/steroidogenesis and immune cell-derived overproduction of IL-18, they should help to elucidate the underlying cell-cell interactions.

Wider implications of the findings: Our results suggest that the overproduction of IL-18 may induce Leydig cell apoptosis, which may lead to the reduction of steroidogenesis. Monitoring IL-18 levels and controlling the overexpression of IL-18 could be a new therapeutic target to prevent Leydig cell apoptosis and the dysfunction of steroidogenesis during acute inflammation.

Trial registration number: Not applicable.

P-032 Magnetic-activated cell sorting as a complement to swim-up improves efficiency of spermatozoa separation

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Study question: Is it beneficial to use swim-up before or after magnetic-activated cell sorting (MACS) for effective spermatozoa selection for IVF?

Summary answer: Our results showed that the combination of swim-up and magnetic-activated cell sorting brings better results in sperm selection when the first step is the swim-up.

What is known already: Spermatozoa selection is one of the most important steps in IVF methods. Magnetic-Activated Cell Sorting (MACS) is a selection method which reduces apoptotic spermatozoa in sample and improves IVF outcome. Many reports indicate that MACS is a beneficial technique compared to classical sperm selection techniques. However, the possible positive effects of this method in clinical application are still debatable. Although a positive effect of swim-up and MACS on spermatozoa selection was presented, further studies are needed to investigate the benefits of using the combination of swim-up and MACS on separation of spermatozoa.

Study design, size, duration: A prospective study was carried out to investigate the effects of spermatozoa separation procedure on concentration, motility and DNA integrity after using swim-up and MACS methods and their combinations. A total of 26 normozoospermic patients were included in this study from January to December 2017. A sample from each volunteer was divided into 5 subgroups according to separation procedure as follows: 1) native 2) swim-up 3) MACS 4) MACS/swim-up 5) swim-up/MACS.

Participants/materials, setting, methods: Semen samples from the volunteers were separated by swim-up, MACS or combination of these methods according to the manufacturer's instructions (Miltenyi Biotec). The analysis of sperm concentration and motility was performed both in raw and subgrouped semen samples according to the WHO manual. Sperm DNA fragmentation was assessed using sperm chromatin dispersion test (Halosperm G). Proportion of spermatozoa with fragmented DNA was expressed as DNA Fragmentation Index (DFI). P value <0.05 was considered statistically significant.

Main results and the role of chance: Compared to spermatozoa concentration in the native sperm samples (average 81.4 mil/ml), this parameter significantly decreased in all the other groups. A statistically significant decrease was observed in swim-up (23.2 mil/ml), MACS (29.6 mil/ml) swim-up/MACS (16.8 mil/ml) and also MACS/swim-up (23.4 mil/ml). Total sperm motility was significantly higher in all the groups where swim-up method was applied (92.7 %), swim-up/MACS (84.5 %) or MACS/swim-up (88.1 %) in comparison with native ejaculate (52.7 %) and MACS method alone (37.8 %). In the case of sperm chromatin integrity, no significant differences were observed among native (DFI 30.52 %), MACS (DFI 28.65 %) and MACS/swim-up (DFI 22.7 %). However, swim-up/MACS significantly reduced the proportion of spermatozoa with fragmented DNA (DFI 13.8 %) as well as swim-up alone (DFI 18.2 %). Indeed, the combination of swim-up/MACS significantly decreased the proportion of spermatozoa with fragmented DNA in comparison with native ejaculate and also with MACS method alone. Based on our results we recommend to use swim-up followed by MACS method. This approach brings better results in sperm selection – lower proportion of spermatozoa with fragmented DNA and also better gain in terms of total sperm count usable for next IVF or ICSI methods.

Limitations, reasons for caution: The limitation is the number of semen samples included and analysed in this study which slightly reduced the power of the statistical analysis.

Wider implications of the findings: Combination of the swim-up method followed by MACS is the most effective. MACS have its limitations regarding sperm concentration and volume for loading. Indeed, loading of raw semen to column may reduce the filtering function of MACS, since dead/apoptotic sperm cells bound to the MACS column compete with motile/non-apoptotic spermatozoa.

Trial registration number: not applicable.

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P-033 The impact of semen infections over sperm parameters in primary infertile men - results of a real-life, cross-sectional study

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Study question: To assess the prevalence of urogenital infections and their impact on sperm and hormonal parameters in Caucasian-European men presenting for primary couple infertility.

Summary answer: Semen infections are highly prevalent in infertile Caucasian-European men. Non-obstructive azoospermia (NOA) is more frequently found in patients with a positive semen culture.

What is known already: Among the possibly reversible factors associated with male infertility and abnormal semen parameters, acute and chronic infections of the male urogenital tract are of great relevance as they account for 15-20% of all cases. Overall, cellular reactions against the pathogenic agents and activation of immunity cells present in the seminal fluid can lead to defects of spermatozoa function, obstruction of the seminal tract and even deterioration of spermatogenesis. However, the debate about the cause-effect relationship between genitourinary infections and male infertility is far from over and the available guidelines do not give clear indications yet.

Study design, size, duration: Real life, cross-sectional study based on a cohort of 2464 consecutive white-European men assessed at a single academic centre for couple's infertility (non-interracial infertile couples only) between September 2006 and September 2016.

Participants/materials, setting, methods: Demographic, clinical and laboratory data of the entire cohort were analyzed. Health-significant comorbidities were scored with Charlson Comorbidity Index (CCI; categorized 0 vs. ≥1). Semen analysis was based on WHO 2010. Only patients assessed with semen culture tests were included in the analysis. Hypogonadism was defined as a total testosterone level <3.0 ng/ml. Descriptive statistics and logistic regression models tested the association between sperm infection and clinical, seminal, and hormonal characteristics in the entire cohort.

Main results and the role of chance: Overall, 1662 patients (67.5%) underwent sperm cultures. Of them, semen cultures were positive in 271 patients (16.3%). Most commonly identified bacteria were Ureaplasma spp. and Enterobacteriaceae spp. (32.9% and 14.8%, respectively). A concomitant infection with 2+ agents was reported in 15.1% cases. Patients with a positive semen culture had higher mean (SD) BMI [26.1 (4.0) vs. 25.6 (3.3)], lower ejaculate volume [2.99 (1.6) vs. 3.35 (1.8) ml], lower inhibin B levels [98.1 (74.8) vs. 115.9 (83.3) pg/ml], were more frequently active smokers compared to those without semen infections (all $p \leq 0.02$). No significant correlations were found between the different infectious agents and sperm parameters. Azoospermia (NOA + OA) was more frequently reported in patients with positive vs. negative semen cultures (27.7% vs. 17.0%, $X^2 = 16.29$; $p < 0.001$); semen infections were more frequently found in NOA patients ($X^2 = 17.84$; $p < 0.001$) but not in OA men ($p = 0.8$). At multivariate analysis, a positive semen culture achieved independent predictor status for azoospermia (NOA + OA; OR 2.50, $p < 0.001$) and for NOA (OR 2.59, $p < 0.001$), after accounting for

CCI status, genetic abnormalities, history of cryptorchidism and hypogonadal status.

Limitations, reasons for caution: No comparison with a same-race, age-matched cohort of fertile individuals. Lack of data regarding potential molecular alterations in spermatogenesis, which may be important to understand the possible impact of infections on semen parameters.

Wider implications of the findings: Infections of the urogenital tract are thought to be potentially modifiable causes of male infertility, so that a detailed and extensive investigation on them may be advisable to prevent long, potentially unnecessary infertility treatments.

Trial registration number: N/N.

P-034 The effect of autologous platelet-rich plasma on human sperm parameters: in vitro experimental study

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Study question: Study the effect of autologous PRP (platelet-rich plasma) on sperm parameters in the presence and absence of oxidative stress.

Summary answer: Autologous PRP improves the quality of the sperm, more so in the presence of oxidative stress.

What is known already: Around 65 to 80% of infertile men suffer primary testicular defects in spermatogenesis, detected by abnormal semen analysis. In addition to standard parameters, specialized tests, such as oxidative stress measurement and sperm DNA fragmentation test, can help in the diagnosis and prognosis of infertility. The role of oxidative stress in male infertility is well established and all sperm parameters are highly affected by its damage. Recently, a review of growth factors, cytokines, and antioxidants present in PRP and their effect on sperm parameters have lead to a theoretical conclusion that PRP might be a novel therapeutic option for male infertility.

Study design, size, duration: Semen samples of 30 healthy men in their fourth decade attending a fertility clinic, between November 2016 and December 2017, were enrolled in this cohort study. After obtaining a written informed consent from all patients, a questionnaire about age, previous diseases and habits was filled, and biological samples (blood and sperm) were obtained.

Participants/materials, setting, methods: After establishing H2O2 suitable concentration (10 mM) for oxidative stress induction, semen samples were prepared and cultured in the presence or absence of H2O2. Following preparation of autologous PRP, semen samples were left untreated or treated with increasing concentrations of PRP (2%, 5% and 10%). After 24 hours, sperm motility, morphology, vacuolization, viability, DNA fragmentation, and oxidative stress assessment were evaluated using light microscopy, Spermoscan kit, eosin-nigrosine staining, halosperm kit and nitroblue tetrazolium test, respectively.

Main results and the role of chance: This study demonstrated the harmful effect of oxidative stress on sperm parameters, showing a significant increase in the percentage of vacuolization ($p = 0.047$) and sperm DNA fragmentation ($p = 0.030$), and a significant decrease in the percentage of progressive ($p = 0.026$) and total motility ($p = 0.012$) in the H2O2 treated group compared to non-stressed sperm. As expected ROS positive sperm cells were extremely higher in the H2O2 treated samples. When different concentrations of PRP were added to all specimens, an improvement of the studied parameters were noted mainly with PRP 2%, leading to the conclusion that a concentration of 2% is the best to achieve a positive effect on sperm parameters. As expected the percentage of dead sperm and the cell morphology didn't differ between the group treated with PRP and the untreated group ($p > 0.05$). However, DNA fragmentation, and ROS positive cells were significantly decreased when PRP was added to both stressed ($p < 0.0001$) and non-stressed samples ($p = 0.025$ and $p = 0.008$ respectively), and immotile sperm and vacuolization were also decreased when PRP was added but only for the stressed specimens ($p < 0.0001$). Finally, the same beneficial effect of PRP was noted with increased percentage of progressive and total motility in the stressed group only ($p < 0.0001$).

Limitations, reasons for caution: Due to financial limitations, we could not be able to include infertile patients in the present study. The correlation of our findings with sperm parameters in patients with fertility problems would give better understanding of the involvement of oxidative stress in male infertility.

Wider implications of the findings: Findings from the present study contribute to the understanding of sperm physiology and would have relevance in the diagnosis and/or treatment of infertile patients.

Trial registration number: Not applicable.

P-035 Effect of paternal age on semen parameters and the live birth rate of in-vitro fertilization treatment

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Study question: To determine the effect of paternal age on semen parameters and the live birth rate of first IVF cycles.

Summary answer: Paternal age is negatively correlated with some semen parameters but it could not predict the likelihood of a live birth in the fresh IVF cycle.

What is known already: Two systematic reviews looking at the effect of paternal age on assisted reproduction outcomes showed conflicting results on both semen parameters and IVF outcomes. The conclusion drawn was that except for volume and possibly motility, other sperm characteristics such as concentration and morphology did not alter with age and advancing paternal age was not associated with adverse IVF outcomes.

Study design, size, duration: This is a retrospective analysis of all first IVF cycles carried out between 2004 and 2014 at the Centre of Assisted Reproduction and Embryology, The University of Hong Kong - Queen Mary Hospital. Only those using ejaculated sperms were included for analysis to avoid any potential bias. Those requiring preimplantation genetic diagnosis and using donor sperms or surgically retrieved sperms were excluded from the study.

Participants/materials, setting, methods: Women received ovarian stimulation using either the long gonadotrophin releasing hormone agonist or antagonist protocol. Embryo transfer was performed two or five days after the oocyte retrieval. Up to three embryos were transferred before 2006 and a maximum of two embryos were transferred after 2006. Luteal phase was supported with vaginal progesterone pessaries or intramuscular injection of hCG. Data of all first IVF cycles was retrieved from the Assisted Reproduction Clinical Database of the Centre.

Main results and the role of chance: A total of 3549 first IVF cycles fulfilling the inclusion criteria were analysed. In the raw semen, paternal age is negatively correlated with semen volume, progressive motility, total motility and total motile count with normal morphology (TNMC) ($p < 0.005$). It is positively correlated with sperm concentration ($p < 0.001$) but does not correlate with sperm count, normal morphology and total motile count (TMC). Paternal age does not correlate significantly with fertilization rate, both in the conventional insemination group, ICSI group and overall ($p = 0.786$, 0.977 and 0.810 respectively). Paternal age positively correlates with maternal age (Spearman's correlation coefficient 0.487, $p < 0.001$). Paternal age is significantly lower in those who attained pregnancy, ongoing pregnancy and live birth compared with those who did not ($p < 0.001$). Logistic regression showed that maternal age, total numbers of oocytes retrieved and number of embryos transferred, but not paternal age, were the significant factors which independently predicted the likelihood of live birth in the first IVF cycles after controlling for the others ($p < 0.001$).

Limitations, reasons for caution: We do not have the information on congenital abnormalities of the live born and the long term data of the babies born from IVF/ICSI. The present study examined patients who have undergone IVF treatment and the results may not be applicable to natural conception.

Wider implications of the findings: Infertile couples can be counselled that although there is a decline in some semen parameters with paternal age, the live birth rate of IVF depends on the maternal age, the number of oocytes retrieved and the number of embryos transferred but not the paternal age.

Trial registration number: Not applicable.

P-036 Animal enriched serum contains vitamin E and omega-3 fatty acids; a new suggestion to improve frozen-thawed human sperm quality

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Study question: This study was performed to evaluate the effects of ram enriched serum contains vitamin E and omega-3 fatty acids on human sperm cryopreservation.

Summary answer: Low concentration of enriched serum contained vitamin E and fish oil + vitamin E in commercially freezing medium improved frozen-thawed human sperm parameters.

What is known already: Cryopreservation media has crucial role in protection of sperm against cryo-injuries during freezing-thawing. Therefore, developing this media can improve the fertility potential of sperm during this process. It seems that using nutrients in freezing media is leading to improve thawed sperm parameters. Although effectiveness roles of antioxidants and omega 3 fatty acids for improvement of sperm quality after freezing are reported, the potential of dietary enriched serum contains vitamin E is currently unclear. Therefore, the purpose of this study was to evaluate the addition of enrich serum contains these nutrients in commercially freezing media for cryopreservation of human sperm.

Study design, size, duration: To produce enriched serum, 16 rams (n = 4) were fed diets as follows: Control (CTR), vitamin E (VITE; 200 IU/ram/day), fish oil (FO; 40 g/ram/day) and fish oil + vitamin E (FO+VITE). In the second phase, semen samples were collected from 25 normospermic men and then were divided into six equal experimental groups for cryopreservation in freezing media (SpermFreez™, Fertipro) containing enriched 5% ram serum as mentioned above.

Participants/materials, setting, methods: Fetal Bovine Serum (5% FBS) and commercially freezing media without serum (Control) were used as other experimental groups. To evaluate the effects of enriched serum on the quality of frozen-thawed sperm, several parameters such as motion characteristics (CASA), viability (Eosin-nigrosin), DNA fragmentation (SCSA) and total ROS (Chemiluminescence assay) were recorded. Data were analyzed using SPSS and statistical differences among various groups were determined by ANOVA and Tuckey's post hoc test.

Main results and the role of chance: The highest significant ($p < 0.05$) percentage of sperm motility and viability were observed in groups containing VITE (49.78 ± 1.96 and 44 ± 8.03) and FO+VITE (51.28 ± 2.71 and 48 ± 7.74) compared to control group (38.66 ± 1.89 and 37 ± 8.47), respectively. Moreover, FBS produced the lowest significant ($p < 0.05$) percentage of motility and viability (33.66 ± 1.88 and 30 ± 7.33) compared to control group, respectively. Interestingly, adding serum contains FO+VITE to freezing media improved curvilinear velocity (VCL) than other groups (53 vs. 31, 36, 37, 44, 48 µm/s for FO +VITE, FBS, FO, control, CTR, VITE, respectively). ROS concentrations were not significantly ($p > 0.05$) affected by the serum supplementation. Flow cytometry parameters in this study confirmed our results related to motility and viability. Straight linear velocity (VSL) as well as Average path velocity (VAP) were changed by serum inclusion. This improvement is mostly related to characteristic of antioxidant activity of vitamin E and omega-3 fatty acids. This combination has several characteristic that may improve the quality of sperm such as membrane flexibility and improve signal transduction during cryopreservation.

Limitations, reasons for caution: The limitations of the present study included the limited volume of sample test for more investigations and functional tests. We cannot test the fertility potential of frozen-thawed sperm in current study.

Wider implications of the findings: Our study has clinical relevance and may result in considerable improvement from a wide range of cryopreservation procedures and assisted reproductive techniques (ART) which warrants further studies. Furthermore, this attitude may improve the aspects of sperm freezing protocols in several species.

Trial registration number: Not applicable.

P-037 Male's advanced age negatively impacts embryo development on short-term insemination IVF cycles with younger female patients

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Study question: Do the couple ages affect short-term insemination conventional in vitro fertilization (c-IVF) results?

Summary answer: Male's advanced age on short-term insemination c-IVF has a negative influence on fertilization and embryo development.

What is known already: Female age has been shown in multiple studies to be a predictor of poor reproductive success with assisted reproductive technology (ART). In addition, the influence of male age is suggested in undergoing ART.

Study design, size, duration: This retrospective cohort study included a total of 3249 c-IVF cycles of infertile couples between January, 2012 and October, 2017 at Yanaihara Women's clinic.

Participants/materials, setting, methods: Male and female patients were divied into two age groups (31-37 years, and 38-44 years), and a male age on IVF outcomes for short-term insemination was studied. The protocol for all the cycles was insemination for 3 hours, PB check(two polar body, 2PB) 4-6 houers later, and fertilization check(two pronuclei, 2PN) 20 hours after insemination. We compared the rates of fertilization, embryo development, and clinical pregnancy on day 5 after ET among different age groups.

Main results and the role of chance: In the 31-37 years age female group between the same age male partner group and older age male partner group (38-44 years), the second polar body release rates (80.6% vs 77.5, $p < 0.01$), fertilization rates (74.0% vs 70.5% $p < 0.05$), Day 3 good embryo rates (56.2% vs 52.0%, $p < 0.01$) and blastocyst formation rates (57.8% vs 49.2%, $p < 0.01$) werer compared, and all the above outcomes were significantly lower in the older male partner group. However, there were showed no significant differences on day 5 good blastocyst rates (53.5% vs 51.6%) and clinical pregnancy rates (52.9% vs 52.9%) after embryo transfers. Women 38-44 years groups showed no statistically significant differences in the rates among different male age partner groups ($p > 0.05$).

Limitations, reasons for caution: Male age affects short-term insemination IVF outcomes, but female factors outperforms male factor in cases with older patients.

Wider implications of the findings: Male age affects c-IVF outcomes, and it is necessary to think about treatment options with the male age as well as female age in consideration.

Trial registration number: None.

P-038 The effect of paternal trans fatty acids diet on sperm quality and expression profile of PPAR genes in the testis of rat offspring

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Study question: Does the paternal trans fatty acids diet influence on sperm quality and expression profile of *PPAR* genes in testis tissue of rat offspring?

Summary answer: Paternal intake of trans fatty acids caused to significant decrease in sperm parameters and expression profile of *PPARβ* and *PPARγ* genes in male offspring rats.

What is known already: High fat diets especially trans fatty acids can affect on the expression of a wide variety of metabolic factors including peroxisome proliferator-activated receptor, *PPAR*. *PPARs* are nuclear receptors that their normal expression levels in testis tissues of adults are associated with their normal spermatogenesis. Recently, it has been shown by investigators that suboptimal diet during male gametogenesis can influence the metabolic status of the offspring.

Study design, size, duration: This study is included 20 adult male Wistar rats (5 in each groups) that their fathers were fed for 60 days in the following four diet groups: control diet (C) - control diet with trans fatty acid (CT) - a diet containing vitamin E (E) and a diet containing vitamin E and trans fatty acid (ET).

Participants/materials, setting, methods: Epididymal tissue was taken from male offspring rats and sperm parameters were analyzed by using CASA computer analysis. On the other hands, total RNA was extracted from testis tissues and after cDNA synthesis the mRNA expression of *PPARα*, *PPARβ* and *PPARγ* genes were measured by using quantitative PCR. Data were statistically analyzed using one-way Anova.

Main results and the role of chance: The results of this study showed statistical significant differences between concentration, progressive and total motility of sperm parameters among groups. Duncan's test showed that sperm concentration in group with vitamin E intake was significantly higher than other groups ($P < 0.05$). Also, the mean of progressive and total motility of sperms in groups C and E were significantly higher than two other groups. The expression of *PPARβ* gene in two groups (E and C) was significantly higher than other groups (the CT and ET). Also the expression of *PPARγ* gene in group E was significantly higher than other groups. Eventually, the expression of *PPARγ* gene in group C was significantly higher than ET group.

Limitations, reasons for caution: The difference in nutrition of fathers before dietary treatment and also the low sample size of offspring in each group can be a limited factor in this study.

Wider implications of the findings: This study showed paternal trans fatty acid consumption can have negative effects on normal spermatogenesis in male offspring, resulting to decrease in the quality and quantity of sperms. Additionally, contrary to our expectations, vitamin E supplement could not neutralize enough the negative effect of trans fatty acids in this regard.

Trial registration number: NA.

P-039 Changes in ultrasonographic (US) images of the seminiferous tubules during gonadotropin replacement therapy in men with hypogonadotropic hypogonadism

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Study question: Are changes in US images of seminiferous tubules during gonadotropin replacement therapy related to the progression of spermatogenesis in men with hypogonadotropic hypogonadism?

Summary answer: The density of thick seminiferous tubules on US images is related to the extent of spermatogenesis induced by gonadotropin therapy in men with hypogonadotropic hypogonadism.

What is known already: Gonadotropin replacement therapy using hCG and rFSH is effective for inducing spermatogenesis in patients with hypogonadotropic hypogonadism. However, the progression of spermatogenesis during hCG +rFSH treatment in these patients has not been well elucidated. We previously demonstrated that thick seminiferous tubules on US images suggest a high rate of successful sperm retrieval in patients with non-obstructive azoospermia (ESHRE2016 P-014). US analysis of seminiferous tubules may provide valuable information for determining the extent of spermatogenesis in patients with hypogonadotropic hypogonadism who are being treated with gonadotropins.

Study design, size, duration: This prospective observational study involved 7 azoospermic patients with male hypogonadotropic hypogonadism who underwent hCG+rFSH therapy at our outpatient clinic from May 2008 to November 2017.

Participants/materials, setting, methods: We studied 7 men with hypogonadotropic hypogonadism, aged 34 to 49 years. Of these patients, 6 had idiopathic hypogonadotropic hypogonadism, including 2 patients with Kallmann syndrome and 1 had iatrogenic hypogonadotropic hypogonadism after brain surgery. Semen analysis and US evaluation of the testis were performed before and during gonadotropin therapy. To optimize US images for visualization of the seminiferous tubules, gain and contrast were appropriately adjusted with graphics software.

Main results and the role of chance: Gonadotropin replacement therapy induced spermatogenesis in 5 of the 7 patients. The first appearance of sperm in the ejaculate was achieved after a median duration of treatment of 3 months. Although testicular volume in these patients increased from 2.5 to 4.0 mL, there was no further increase in testicular volume 3 months after the initiation of treatment. Thick seminiferous tubules, defined as more than 300 μm in diameter on the US image, and the first sperm in the ejaculate were detected at almost the same time. Furthermore, an increase in sperm output was associated with an increase in the density of thick seminiferous tubules.

In contrast, two patients remained azoospermic 10 and 24 months after the initiation of treatment, respectively. Neither an increase in testicular volume nor thick seminiferous tubules on US were observed in these patients.

An increase in testicular volume only suggests the induction of spermatogenesis by gonadotropin therapy in patients with hypogonadotropic hypogonadism, while the extent of spermatogenesis can be assessed by the change in the density of thick seminal tubules on US images.

Our results suggest that the appearance of thick seminiferous tubules on US images may predict a good future response to gonadotropin therapy in hypogonadotropic hypogonadism.

Limitations, reasons for caution: In patients with intrascrotal calcification including severe testicular microlithiasis, testicular parenchyma cannot be assessed by US.

Wider implications of the findings: The status of spermatogenesis in patients whose semen is not available for testing due to an ejaculatory disorder may be estimated by the US analysis of seminiferous tubules.

Trial registration number: Not applicable.

P-040 Dishevelled-associated activator of morphogenesis 1 or DAAM1: expression and localization in human testicular tissue and spermatozoa

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Study question: Is the protein Dishevelled-associated activator of morphogenesis 1 (DAAM1) present in human testicular tissue and spermatozoa and, if so, where is it located.

Summary answer: DAAM1 is present in both human testicular tissue and spermatozoa localized in the germ cells and Sertoli cells and in the tail in mature spermatozoa.

What is known already: DAAM1 is a protein involved in the nucleation of actin filaments and in cytoskeletal organization; however, to date it has not been described in the human male reproductive tract. In a recent study, DAAM1 has been shown to be expressed in the post-natal development of rat testis and spermatozoa. The level of expression of DAAM1 in the rat decreases during development and changes its localization; from the cells near the basement membrane towards the lumen of the tubule. In the spermatozoa it is found in the tail.

Study design, size, duration: Human testicular tissue was obtained from 15 males, with an average age of 37 years, following Testicular Sperm Extraction (T.E.S.E.), while human sperm samples were collected from 20 patients undergoing regular sperm analysis, at 'Centro di Fecondazione Assistita (CFA)" in

Naples. Only samples with concentration higher than 20 mil/ml, and normal motility and morphology according to WHO 2010 were used.

Participants/materials, setting, methods: Part of the tissue was frozen and stored in liquid nitrogen until protein extraction. The proteins were separated by SDS-PAGE and identified by Western Blot. The remaining tissue was fixed in formalin, embedded in paraffin and stained with haematoxylin and eosin to check the quality of the tissue and identify the cell types. Subsequently immunofluorescence was performed both on the tissue and spermatozoa.

Main results and the role of chance: Western blot analysis confirmed the presence of the protein in human testicular tissues. The tissues samples showed different levels of DAAM1 expression (O.D. DAAM1/Actin ratio ranges from 0,9 – to 2,3). We have also shown the presence of this protein in all sperm samples studied (O.D. DAAM1/Actin ratio ranges from 0,07 – to 1). In testicular tissue the protein signal was localized in the cytoplasm of Sertoli cells, gonocytes, spermatogonia and spermatids. In spermatozoa, the protein was mainly detectable inside the flagellum.

Limitations, reasons for caution: Human testicular tissues were obtained from males following surgical intervention (Testicular Sperm Extraction, TESE) at 'Centro di Fecondazione Assistita" of Naples; these men enrolled in the study were affected by azoospermia. A limit of the study was the absence of material and experimentation on healthy individuals.

Wider implications of the findings: This is the first report to show the presence of DAAM1 in human testicular tissues and spermatozoa. Further studies are needed to elucidate the role of DAAM1 in human spermatogenesis and male fertility.

Trial registration number: This is basic science.

P-041 Diagnostic value of sperm DNA fragmentation for predicting IVF fertilization failure in males with mild or medium asthenospermia

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Study question: Is sperm DNA fragmentation (SDF) valuable for predicting IVF fertilization failure in males with mild or medium asthenospermia?

Summary answer: SDF greater than 31.25% could be associated with higher probability of IVF fertilization failure in males with mild or medium asthenospermia.

What is known already: Low levels of natural fertility are detected in couples with a high percentage of SDF. But studies on effects of SDF on IVF/ICSI outcomes report controversial results.

Study design, size, duration: Retrospective clinical study.

Participants/materials, setting, methods: Data from a cohort of 116 couples who underwent their first IVF cycle between January 2016 and December 2017 at an University-affiliated teaching hospital under criteria as follows: males with progressive sperm rate between 10% and 32%, woman age < 38 years, number of oocyte retrieved ≥5. Women diagnosed with PCOS were excluded. The SDF was detected by chromatin dispersion test.

Main results and the role of chance: The area under the receiver operating characteristic curve was 0.772 ($p = 0.003$). A cutoff value for SDF to predict the IVF fertilization failure is 31.25%. When the SDF exceeded 31.25%, the fertilization rate, number of embryo and number of good embryo were statistically decreased, but the cleavage rate, good embryo rate and clinical pregnancy rate were not affected.

Limitations, reasons for caution: A weakness of our study is that SDF was measured on neat semen samples 1 to 2 months before the initiation of IVF instead of on sperm collected the day of oocyte retrieval.

Wider implications of the findings: High rate of SDF may be used as a clinical indication for ICSI in couples with mild or medium asthenospermia.

Trial registration number: not applicable.

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P-042 The assessment of clinical intracytoplamic sperm injection (ICSI) outcome using immotile testicular spermatozoa in non-obstructive azoospermia (NOA)

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Study question: What is the fertilization rate, embryonic development, and clinical outcome for TESE-ICSI using immotile testicular spermatozoa even after pentoxifyllin administration among NOA couples?

Summary answer: Better fertilization and embryonic development were achieved and clinical pregnancy rate per embryo transfer (ET) by motile spermatozoa was significantly higher than by immotile spermatozoa.

What is known already: The testicular spermatozoa are often immotile immediately after testicular sperm extraction (TESE) and especially after thawing of frozen testicular samples and spermatozoa showing reliable signs of vitality are preferred for ICSI because the use of motile sperm significantly increases the efficacy of the ICSI procedure. To select TESE-retrieved viable spermatozoa for ICSI, pentoxifylline administration or hypo-osmotic swelling test (HOST) were often used, however, the testicular sperm from microdissection (micro) TESE and frozen-thawed tissue are often immotile and we have often observed the NOA patients whose testicular spermatozoa are immotile only even after pentoxifylline administration.

Study design, size, duration: We performed a retrospective study based on a reproduction center in Japan and evaluated 966 NOA patinets including after orchidopexy, Klinefelter syndrome, cryptozoospermia, mumps orchitis, azoospermia factor (AZF) microdeletion and unexplained NOA and 369 couples had 646 TESE-ICSI cycles were performed between September 2013 and December 2017. TESE-ICSI was performed with only motile spermatozoa (268 patients 482 cycles, Mo+), motile/immotile spermatozoa (58 patients 98 cycles, Mo+/-), and immotile spermatozoa only (43 patients 46 cycles, Mo-).

Participants/materials, setting, methods: Sperm preparation medium with pentoxifylline added (1 mg/mL) was applied to a droplet of testicular cell suspension in the ratio 1:1 when we could not detect motile spermatozoa. When immotile spermatozoa was injected even after pentoxifyllin administration, oocytes were incubated with calcium ionophore (A23187) for 15 minutes. Two pronuclei (2PN), blastocyst development, good-quality blastocyst, and clinical pregnancy rates (CPR) were compared. Statistical analysis was performed using unpaired t-tests and chi-squared tests.

Main results and the role of chance: Wives age was not different between groups. The sperm retrieval rate with micro TESE was 36.5% (353/ 966), in which motile spermatozoa were retrieved in 79.6% (281/353). Hormonal and any clinical data did not predict before TESE whether motile spermatozoa could be retrieved. 2PN and blastocyst rates of oocytes injected by motile spermatozoa (57.8% and 50.4%, respectively) were significantly higher than by immotile spermatozoa (41.1% and 15.4%, respectively) ($P < 0.001$). Good-blastocyst rates of these oocytes were not observed significant differences (42.7% and 47.4%, respectively). CPR per embryo transfer in embryo by motile spermatozoa was significantly higher than by immotile spermatozoa (35.2% (227/ 644) and 10.6% (7/66), respectively). In all 11 pregnancies of Mo+/- group, all embryo which lead to pregnant were injected by only motile spermatozoa. Another 5 patients with immotile spermatozoa injected into oocyte could achieve pregnancy of Mo+/- group (double embryo transfer using motile and immotile sperm; four from AZFc microdeletion and one from unexplained NOA. Seven patients with immotile spermatozoa injected into oocyte achieved pregnancy of Mo- group; four had delivered healthy babies from 2 unexplained NOAs, Klinefelter syndrome, and post orchidopexy and the other three were ongoing pregnancy from unexplained NOA.

Limitations, reasons for caution: There was a lack of standardization with respect to the frozen-thaw and fresh cycles. The safety and screening for congenital malformations among these children has not been fully investigated. No study compared the outcome of ICSI with initially immotile and induced motile testicular sperm.

Wider implications of the findings: The patients with NOA no longer should be considered sterile without performing micro TESE-ICSI. Sperm motility is the most important to deliver a baby in TESE-ICSI. However, embryologists should not give up when only immotile spermatozoa was injected even after pentoxifyllin administration.

Trial registration number: Not applicable.

P-043 Successful clinical outcome of ICSI cycles with sperm of HIV- positive men after antiretroviral treatment

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Study question: Does the clinical outcome of ICSI with extensively processed semen of HIV-positive male patients reach acceptable values?

Summary answer: A high clinical pregnancy rate is obtained after ICSI with extensively processed sperm of HIV- positive man who underwent antiretroviral treatment.

What is known already: HIV men undergo antiretroviral therapy before undergoing ICSI treatment. However, in ejaculated semen, the HIV virus may still be present in the cell-free fraction or bound to lymphocytes. Therefore, the preparation of semen for assisted reproduction still requires special attention in order to reduce the viral load in the final sperm fraction. Special devices for the preparation of sperm samples from HIV-positive men are available in order to improve safety. Different combinations of sperm preparation techniques can be applied.

Study design, size, duration: A single-centre retrospective analysis was performed on ICSI cycles of HIV-serodiscordant couples with HIV^+-male partners, referred to our unit between August 2009 and December 2017. The male patients were under antiretroviral therapy and had a blood viral load <40 copies/ml. The semen samples were tested before and after extensive preparation for the presence of HIV-virus using COBAS-AmpliPrep/COBAS-TaqMan-HIV-1 quantitative test. The final sperm fraction was frozen and released for later ICSI if no HIV viruses detected.

Participants/materials, setting, methods: Twenty-five couples underwent 45 fresh ICSI cycles and 32 frozen embryo cycles. Based on sperm origin and quality, different semen preparation protocols were performed: density-gradient-centrifugation (DGC) combined with swim-up for good-quality semen, DGC only for fair to low-quality semen, and sperm wash for very low-quality semen or for testicular sperm. The pro-insert Kit (Nidacon) was used during DGC procedure. The end-point was clinical pregnancy with fetal heart beat (CP) per patient.

Main results and the role of chance: From the 45 fresh ICSI cycles, 5 cycles had no embryo transfer (ET) due to abnormal fertilization (n = 3) or insufficient embryo quality (n = 2). In 13 cycles, all embryos were frozen for different reasons, while fresh ET was performed in 27 cycles (27/45; 60.0%). In total, 90 embryos were frozen. Regarding the frozen cycles, 43 embryos were thawed in 32 cycles, all resulting in ET. The CP rate per ET was 33.3% (9/27) in cycles with fresh ET and 25% (8/32) in frozen cycles. The total number of CPs obtained by the 25 couples who started an ICSI cycle was 17 (68.0%). In total, we report 9 deliveries in the fresh cycles, and 3 deliveries, 3 ongoing pregnancies and 2 miscarriages in the frozen cycles. In addition, 42 embryos are still frozen, and have the potential to increase the cumulative delivery rate. Twenty-one of these embryos belong to 8 patients who did not get pregnant so far. It can be concluded that the outcome of ICSI cycles in the present patient population is re-assuring.

Limitations, reasons for caution: The present study is limited by its observational retrospective nature and by the small number of patients included. The analysis is restricted to sero-discordant couples with HIV-positive men. A similar analysis will be performed for HIV-positive women only, and for HIV sero-concordant couples.

Wider implications of the findings: Our data show that despite the antiretroviral treatment and extensive processing of the semen in cases of HIV-positive men, the outcome of ICSI treatment in sero-discordant couples is successful. Moreover, the use of semen fractions with undetectable HIV virus in ICSI cycles is reassuring for the patients and clinical staff.

Trial registration number: none.

P-044 Impact of the sperm DNA fragmentation on clinical outcome in treatments with oocyte donation

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Study question: Which is the impact of sperm DNA fragmentation in male patient on clinical outcomes oocyte donation programs?

Summary answer: The sperm DNA fragmentation of male patient has no impact on clinical outcome in oocyte donation programs.

What is known already: It has been observed that the high sperm DNA fragmentation (DNA fragmentation index >30%) in normozoospermic males could be a cause of infertility. Different studies show a correlation between high levels of sperm DNA fragmentation and the decrease of the pregnancy rate in homologous treatments. On the other hand, it is known that oocytes from healthy young women such as donors have the ability to repair sperm DNA.

Study design, size, duration: The present retrospective study was performed on 173 couples undergoing cycles of ICSI with cryopreserved donated oocytes between June 2016 and December 2017.

Participants/materials, setting, methods: The mean age was 42.5 years for female recipients and 44.5 years for male patients. The donated metaphase II oocytes were obtained and cryopreserved from donors with a mean age of age 24.1 years. The sperm DNA fragmentation was evaluated by TUNEL test and fluorescence microscopy few weeks before ICSI. The percentage of sperm DNA fragmentation was calculated on fresh sample and sperm preparation after density gradient treatment.

Main results and the role of chance: The DNA fragmentation of the spermatozoa resulted to be 14.8%±13.1% (mean±SD) for the fresh sample, and 7.7%±10.02% (media±SD) after sperm preparation.

No correlation was observed between the two samples and the age of the patient (in fresh r = 0.13; selected spermatozoa r = 0.03), between the two samples of spermatozoa and the result of bHCG test (in fresh r = 0.00; selected spermatozoa: r = 0.05), and the two samples of spermatozoa and clinical pregnancy rate (in fresh r = 0.06; selected spermatozoa: r = 0.03).

Limitations, reasons for caution: Limitations might be represented by the limited number of cases with fragmentation over 30% and by the fact that DNA fragmentation has been evaluated few weeks before ICSI.

Wider implications of the findings: Cryopreserved oocytes from young donors have the ability to repair the sperm DNA. In these cases the valuation of sperm DNA fragmentation, in the diagnostic phase, couldbe avoided.

Trial registration number: No one.

P-045 Prevalence of HPV infection in potential sperm donors and in men from infertile couples in the czech republic

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Study question: What is the prevalence of hrHPV infection in potential sperm donors (SD) and men from infertile couples (IM)? Does the presence of hrHPV influence the semen parameters?

Summary answer: HrHPV prevalence in SD was 28.9% compared to 35.1% in IM. Men with hrHPV+semen sample had lower sperm volume, sperm concentration, and total sperm count.

What is known already: HPV infection could play a role in human fertility like other sexually transmitted diseases (STDs). Unlike other STDs, HPV is not tested obligatorily for gamete donors or infertile couples, although it might affect fertility and pregnancy outcome.

Study design, size, duration: In this laboratory-based study semen samples and penile swabs of potential sperm donors (n = 97) and men from infertile couples (n = 328) were collected between July 2013 and November 2016. All participants filled a questionnaire focused on their health status and sexual behavior.

Participants/materials, setting, methods: Semen samples were analyzed for the presence of 14 hrHPV genotypes by cobas 4800 HPV system (Roche) than genotyped using PapilloCheck HPV-Screening system (Greiner Bio-One) which detects 18 hrHPVs. Penile swabs were analyzed only by PapilloCheck HPV-Screening system. The association between hrHPV positivity, sexual behavior, and semen parameters was assessed using statistical software R.

Main results and the role of chance: The hrHPV prevalence in SD (n = 97) was 28.9% compared to 35.1% in IM (n = 328, $P = 0.312$). Penile swabs were more frequently hrHPV+ than semen samples in both IM (32.3% vs. 11.9%, $P < 0.001$) as well as in SD (26.8% vs. 6.2%, $P = 0.006$).

Men with hrHPV+ semen sample had lower semen volume (median volume 2.5 ml vs. 3 ml, $P = 0.009$), sperm concentration (median concentration 16×10^6/ml vs. 31×10^6/ml, $P = 0.009$) and total sperm count (median count 46×10^6 vs. 82×10^6, $P = 0.009$). No association between penile hrHPV positivity and semen parameters was found.

HPV16 was the most frequent hrHPV genotype in penile swabs in both SD (6.19% [6/97]) and IM (5.49% [18/328]). The most frequently occurring genotype co-infection was HPV42 in SD (5.15% [5/97]) and HPV44/55 in IM (3.35% [11/328]). In semen samples the single-genotype infection was HPV66 in SD (2.06% [2/97]) and HPV53 in IM (2.13% [7/328]).

Men who tested hrHPV+ in penile swabs from both groups had more sexual partners than those who tested hrHPV- (in SD median number of 6 vs. 3 [hrHPV + vs. hrHPV-] with $P = 0.008$; in IM, median number of 6 vs. 4 with $P = 0.003$).

Limitations, reasons for caution: Only penile swabs and semen samples have been analyzed in this study. No data about female partners, number of pregnancies and abortion rates were evaluated.

Wider implications of the findings: HPV positivity of the semen during a time of donation or use may be of interest for facilities dealing with the treatment of infertility because of the risk of transfer and interference with the further development of the pregnancy.

Trial registration number: not applicable.

P-046 Diagnostic evaluation of static oxidation-reduction potential with MiOXSYS System (Male Infertility Oxidative System) in normospermic versus teratozoospermic patients: a retrospective study

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Study question: Does evaluation of static oxidation-reduction potential (sORP) with MiOXSYS System relate with WHO 2010 (World Health Organization) sperm parameters and DNA fragmentation in infertile patients?

Summary answer: There is association between sORP and sperm parameters: sORP levels are higher in patients with lower concentration, motility and morphology and with higher DNA fragmentation.

What is known already: Spermatozoa have a physiological equilibrium between ROS (Reactive Oxygen Species) and anti-oxidants. This equilibrium assures ordinary functions such as chromatin compaction, lipid membrane modification and sperm-oocyte fusion. When ROS are overproduced this equilibrium is lost, generating oxidative stress (OS), possible cause of lipid peroxidation, apoptosis and DNA damages or fragmentation. Seminal plasma contains anti-oxidant factors (enzymatic and non-enzymatic) to prevent post-ejaculation OS, however many studies demonstrate that in infertile men anti-oxidant levels are low and ROS are high; these concentrations may be influenced by both exogenous and endogenous factors: smoking, alcohol assumption, infection and inflammation of male uro-genital tract.

Study design, size, duration: Semen from 84 infertile men were evaluated, according to WHO 2010, during June and July 2017. On their first visit at Reproductive Medicine Unit, patients underwent semen analysis and sORP evaluation and divided in two groups according to semen morphology. Group A (N = 45) included patients with morphology ^{3} 4 and group B (N = 39) patients with morphology <4. Men with ejaculatory dysfunction, varicocele, sexually transmitted diseases and exposed to radiation or chemotherapeutic agents were excluded.

Participants/materials, setting, methods: Semen samples were produced after 72-120 hours of sexual abstinence and analyzed after complete liquefaction, evaluating semen parameters and DNA fragmentation with TUNEL (Terminal deoxynucleotidyl transferase UTP-driven Nick End Labeling) test. MiOXSYS was used to evaluate sORP (expressed in millivolt/sperm concentration 10^6/mL), a 'snapshot' of the current balance of the redox system. A higher sORP level indicates an imbalance in the activity of all available oxidants relative to all available antioxidants in ejaculates.

Main results and the role of chance: Mean male age was 42.1 ± 6.3 in group A and 38.2 ± 6.5 in group B ($p < 0.05$). Semen volume was 3.2 ± 2.3 mL in group A and 3.0 ± 2.0 in group B (NS). Sperm concentration was 47.2 ± 18.0 mil/mL in group A and 24.7 ± 19.0 mil/mL in group B ($p < 0.05$). Sperm motility (progressive plus not progressive) was 66.7 ± 7.4 and 44.3 ± 20.0 in group A and B respectively ($p < 0.05$). Morphology was 4.2 ± 0.5 in group A and 2.1 ± 0.7 in group B (NS). Round cells concentration was 2.3 ± 2.0 and 2.7 ± 2.0 mil/mL in group A and B respectively (NS). DNA fragmentation measure with TUNEL test resulted to be 7.5 ± 6.4 in group A and 11.0 ± 8.1 in group B ($p < 0.05$). sORP level was 1.6 ± 2.5 and 15.5 ± 34.3 in group A and B respectively ($p < 0.05$). Statistical analysis was made using Student t-test. Correlation between sORP level and sperm concentration, motility and DNA fragmentation resulted statistically significant, while there isn't any statistically significant correlation with morphology, semen volume and round cells concentration. A small concentration of white blood cells in semen, such as polymorphonuclear granulocytes and lymphocytes, should contribute to ROS production, but our study didn't highlight this correlation and maybe further studies should be performed to confirm this parameter.

Limitations, reasons for caution: Our study included only infertile patients, but including a group of men with proven fertility may improve the cutoff values for sORP and increase the sensitivity of the assay. Another possible limit is sORP levels are normalized for sperm concentration, leading to an over estimation in men with severe oligozoospermia.

Wider implications of the findings: Currently available assays for OS measure only discrete quantity of oxidants (ROS by chemiluminescence assay), antioxidants (total antioxidant capacity [TAC] assay) or post-hoc damage (MDA assay) and are tedious, time consuming and expensive. MiOXSYS System is innovative in measuring ORP level in semen for its easiness and readiness.

Trial registration number: Not applicable.

P-047 Sperm preparation for Intra-Cytoplasmic Sperm Injection (ICSI) in cattle

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Study question: Main aim is to develop improved sperm pre-treatment procedures required to trigger acrosome reaction (AR) and sperm DNA decondensation for bovine ICSI.

Summary answer: Lyshophosphatidylcholine (LPC)/Heparin (Hep) combined with L-glutathione (GSH)/Hep increases the proportion of 2 pronuclei (PN) after bovine ICSI compare to LPC/Hep or GSH/Hep alone.

What is known already: Bovine ICSI can overcome deficiencies of poor sperm viability and motility, typically associated with frozen-thawed sexed semen. Unlike human IVP, technical challenges exist with bovine ICSI, leading to low fertilization rates and poor embryo development. This is may be due to suboptimal sperm preparation (capacitation, AR), delay in sperm chromatin decondensation and/or deficient egg activation.

Study design, size, duration: Percoll-gradient separated semen from a single frozen-thawed sire were split into three treatments (T): T1, exposure to Hep (80 μg/ml, 15 min), involved in capacitation followed by LPC (15 min, 100 μg/ml) which induces AR; T2, co-incubation in GSH (15 mM) and Hep (80 μM) for 20 hours (h) required to facilitate the sperm DNA decondensation; T3, combination of both treatments. Then, pre-treated spermatozoa were microinjected into bovine mature oocytes and activated chemically.

Participants/materials, setting, methods: Sperm viability and AR were determined simultaneously by a lectin fluorescence staining, counting at least 200 spermatozoa per replicate in four repeated experiments. Decondensed sperm heads were evaluated under a bright field microscope in three replicates.

Microinjected oocytes (n = 175) were exposed to a novel activation protocol optimized previously. Calcium ionophore-A23187 (5 µg/ml for 5 min) followed by strontium chloride (20 mM) and cyclohexamide (10 µg/ml) for 6 h. Pronuclear formation (16h-post-activation) and blastocyst quality were assessed by fluorescent probes.

Main results and the role of chance: We found that sperm pre-treated with LPC/Hep under the working conditions described before led to 0.81 ± 0.021 of AR spermatozoa over the total sperm population. When, sperm were exposed to GSH/Hep 0.53 ± 0.054 showed decondensed heads. After the application of both treatments, slightly lower proportion (0.44 ± 0.008) of spermatozoa had decondensed sperm heads. However, the majority were AR (0.97 ± 0.013).

PN assessment 16 hpa following ICSI indicated that LPC/Hep + GSH/Hep increased (P = 0.012) the proportion of 2PN zygotes relative to LPC/Hep or GSH/Hep (0.31 ± 0.058, 0.042 ± 0.026, 0.16 ± 0.055, respectively). Finally, microinjected oocytes using pre-treated sperm with the optimal protocol (LPC/Hep + GSH/Hep) were cultured until the blastocyst stage (192 hpa). We observed similar proportions of cleaved (48 hpa) and blastocysts between the treatment group (0.67 ± 0.059 and 0.09 ± 0.056) and non-treated sperm (0.61 ± 0.062 and 0), and IVF control (0.81 ± 0.038 and 0.27 ± 0.073). Total cell number between treatment and controls was similar but LPC + GSH embryos were less competent than those derived by IVF.

Limitations, reasons for caution: Aggressive sperm treatment (LPC/Hep + GSH/Hep) reduced sperm concentration, restricting sperm counting during the evaluation process.

Wider implications of the findings: With further development bovine ICSI has the potential to improve success with sex-sorted semen in bovine in vitro embryo production. However, the use of sex-sorted semen leads to poor fertilisation rates compared to non-sorted semen from the same sire as a result of the sorting process.

Trial registration number: Not applicable.

P-048 The effects of incubation at two different oxygen concentrations on oxidation-reduction potential of bull semen of various breeders

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Study question: Do higher oxygen concentrations in incubation conditions have a more negative impact on the oxidation reduction potential (ORP) of thawed bull semen?

Summary answer: This preliminary analysis revealed a nonsignificant difference in ORP levels of spermatozoa incubated at two different oxygen concentrations.

What is known already: High concentrations of reactive oxygen species have been reported to exert negative effects on sperm quality including increased DNA fragmentation. Lowering oxygen levels in embryo culture was also reported to enhance blastocyst development rates. However, there is no consensus between different IVF laboratories on the incubation conditions of sperm cells before use for IVF or ICSI.

Study design, size, duration: Thawed bull semen samples were divided into five aliquots including one for ORP measurement at zero timepoint, two for the incubation conditions at 39°C, 5% CO_2 and 5% O_2 and two for the conditions at 39°C, 5% CO_2 and 20% O_2. ORP measurements were done at 0, 4-6 and 18-22 hours. ORP values were normalized and represented as mV/10^6 spermatozoa.

Participants/materials, setting, methods: Seven cryopreserved bull semen samples of different breeders were used in the study. Two straws from each sample were thawed and washed by density gradient centrifugation (Sperm Grad TM, Vitrolife, Sweden). Total number of spermatozoa was counted for each and the sample was then diluted with commercial media for bovine IVF (BO IVF, IVF Bioscience, UK).

Main results and the role of chance: Compared to starting levels, an increase was observed in ORP values under both oxygen levels at both time intervals (66.1 vs 70.05 and 80.7 mV/10^6 (6 h, 5% and 20% O_2, respectively); 66.1 vs 87.8 and 90.8 mV/10^6 (22 h, 5% and 20% O_2, respectively) ($p > 0.05$ for both).

When differences between both oxygen concentrations in time were evaluated, a slight increase was found in 20% compared to 5% at both times, but the differences were not statistically significant ($p > 0.05$).

Limitations, reasons for caution: The main limitation of our study was bull variability. Since ORP values were found to be higher in the semen of worst quality, it might be better to use semen of comparable quality to make a generalized interpretation.

Wider implications of the findings: Our findings showed that especially within 4-6 hours of incubation, higher oxygen level seemed to increase ORP levels slightly more than lower oxygen concentration, but the difference did not reach significance. However, this finding may be important to focus on best culture conditions for human spermatozoa before IVF and ICSI.

Trial registration number: None.

P-049 Impact of spermatozoa origin and quality on fertilization, pregnancy, miscarriage and live births: a comparative study of azoospermic, cryptozoospermic, normozoospermic patients and donors as control

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Study question: This study aims to compare reproductive outcomes after ICSI in a large patient series considering spermatozoa from non-ejaculated origin and ejaculates of different quality baseline.

Summary answer: Reproductive outcomes, in terms of pregnancy, miscarriage and live births, after using non-ejaculated spermatozoa are similar or even better compared with ejaculated spermatozoa.

What is known already: Azoospermic patients represent 5% to 15% of infertile males, so they are scarce but not rare. Therefore, it is important to research in depth clinical outcomes and new treatments to improve management of azoospermic patients. Nowadays, the use non-ejaculated spermatozoa in ICSI when severe male factor is present is still a concern. It is widely discussed whether different degrees of DNA fragmentation or maturation status of spermatozoa from non-ejaculate sources impair ICSI outcomes, affecting to fertilization and embryo development. Published studies regarding this issue often consider limited sample size or they do not include donor and/or ejaculates of different qualities.

Study design, size, duration: Retrospective cohort study held in HUiP La Fe (Spain) from April 2010 to April 2017. In total, 2638 ICSI cycles followed by double fresh embryotransferences of cleavaged embryos were included. Study groups considered, established according to semen origin and diagnostic. Post-vasectomy controls and patients currently reporting active sexual transmitted diseases were excluded. Normal fertilization rate, embryo quality, biochemical pregnancy, miscarriage rate and live births were reproductive variables considered to be compared among study groups.

Participants/materials, setting, methods: Study groups comprised testicular/epididymal spermatozoa (T:183 cycles), fresh cryptozoospermic (C:510 cycles) and normozoospermic (N:1563 cycles) ejaculates and donor sperm (D:382 cycles) as control group. ANCOVA and Chi-square tests were performed to establish the impact of spermatozoa origin and quality on reproductive variables. Moreover, spermatozoa origin and quality were studied together with female age by logistic regressions and ordinal logistic regressions to demonstrate the impact of both effects on the reproductive outcome. P-values<0.05 were considered significant.

Main results and the role of chance: No differences regarding number of oocytes retrieved or MII oocytes were reported but it was observed, as

expected, differences in sperm count and progressive motility in fresh and prepared sperm among the groups. Fertilization rate (FR) of testicular/epididymal spermatozoa (66.49 ± 1.58) was the lowest ($p < 0.01$) in comparison with cryptozoospermic patients (71.05 ± 0.94), normozoospermic males (77.89 ± 0.53) and donors (77.67 ± 1.23). However, testicular/epididymal spermatozoa presented the highest pregnancy rate (PR) (T: 56.29%; C:37.2%; N:36.10; D:39.84; $p = 0.029$). In addition, testicular/epididymal spermatozoa presented the lowest miscarriage rate (MR) although differences did not reach statistical significance (T: 12.94%; C:18.82%; N:19.96; D:18.37; $p = 0.493$). No differences were found neither in the quality of embryos transferred (QET), live birth rate (LBR) nor percentage of singletons/multiplets deliveries. Outcomes regarding the impact of female age considered together with spermatozoa origin confirmed that (QET), MR and LBR are influenced by maternal age ($p = 0.01$, $p < 0.01$ and $p = 0.04$) but not by the spermatozoa's origin ($p > 0.05$ in all cases). It is also revealed that FR was only influenced by spermatozoa origin ($p < 0.01$), not by female age ($p = 0.147$). Furthermore, PR is not only strongly influenced by female age ($p < 0.01$) but also it is still observed that spermatozoa origin tends to have an impact on achieving pregnancy ($p = 0.08$).

Limitations, reasons for caution: Even though only transferences of embryos at the same development stage were considered to prevent biased conclusions, further studies could consider a cohort of transferences in other embryo development stages as blastocysts. Also the current study does not distinguish about the cause of azoospermia (obstructive or no obstructive).

Wider implications of the findings: Outcomes derived from this large series study are a valuable aid to azoospermic patient counseling. It demonstrates that the use of non-ejaculated spermatozoa has similar or better likelihood of success that the use of donor sperm, offering a treatment option for those azoospermic patients who desire biological offspring.

Trial registration number: This study is not a clinical trial.

P-050 Myoinositol is a good freezing supplement to improve human semen parameters and DNA fragmentation

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Study question: What is the effect of myoinositol as a supplement on sperm parameters, DNA fragmentation, total antioxidant capacity, the level of malondialdehyde and reactive oxygen species in human sperm freezing process?

Summary answer: Myoinosito improves sperm motility, morphology, Total antioxidant capacity and decreases DNA fragmentation and the level of malondealdehyde (MDA).

What is known already: sperm freezing has detrimental effects on sperm parameters, like motility, morphology, ROS level and sperm DNA. Myoinositol belongs to the vitamin B complex group and has many phisiological function. It plays a role in the regulation of calcium intracellular concentration, reproductive disorders and improve the mitochondrial function.

Study design, size, duration: 40 Fresh semen samples were obtained from normospermic men. All the samples after primary analysis, were immediately freezed in two groups, group 1: myoinositol (2 mg per ml) + freezing medium (Fertipro), group 2: freezing medium. After one month, all samples were thawed and assessed.

Participants/materials, setting, methods: semen parameters in two groups were analyzed by CASA for motility and morphology and live ratio. Also, the level of total ROS, TAC, DNA fragmentation and MDA were evaluated by DCFH(by Fluorimetry), ELISA kit, TUNEL assay by flow cytometry and ELISA respectively.

Main results and the role of chance: Our data clearly showed that total motility, morphology and live ratio were higher in myoinositol group. Also, DNA fragmentation is notably lower in myoinositol than control(20.72 vs 15.28, $p = 0.001$). TAC is higher ($p < 0.0001$) and MDA is lower ($p = 0.007$) significantly in myoinositol than normal.The level of ROS was decreased in myoinositol group but not significant ($p = 0.1$).

Limitations, reasons for caution: It is better to use this supplement on freezing process of oligoastenotratospermic (OAT) patients.

Wider implications of the findings: It seems that the effect of myoinositol on mitochondria and other parts of the sperm, improve semen parameters after thawing. So, myoinositol protect normal sperm from detrimental effect of freezing. This study showed the effective role of myoinositol for the first time in human sperm parameters during freezing process.

Trial registration number: NA.

P-051 Improving effectiveness of a human sperm cryopreservation program for Assisted Reproduction by testing the effect of different cryoprotectants and freezing protocols on the post-thawing outcomes

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Study question: Evaluate thawing outcomes of two different commercial cryoprotectants, testing its mode of use in combination with each different freezing protocol, in a sperm cryopreservation program.

Summary answer: Liquid nitrogen vapors freezing achieves more viable spermatozoa than slow freezing. Moreover, the effectiveness of a cryoprotectant can be increased by varying its addition method.

What is known already: Sperm freezing is the only method implemented nowadays in clinical practice to preserve male fertility. In this scenario, it is paramount to achieve the best semen quality after thawing since sample's availability is limited. Cryoprotectants are needed during the freezing process. However, at high concentrations, they are toxic. To minimize its toxicity, addition and removal methods are important. The most common extender of cryoprotectants is egg yolk. However, it derives from animal origin. Hence, it could be a potential source of infectious animal diseases. Therefore, more research is needed to find new animal-free extenders that contribute to standardize sperm freezing.

Study design, size, duration: Prospective cohort study held in Hospital La Fe (Spain) from June to November 2017. In total, 29 normozoospermic males were considered. Sperm count (SC), progressive (PM) and total sperm motility (TM) were evaluated using a CASA system. Aliquots from the same sample were used to test each freezing protocol performed (liquid nitrogen vapors, LN and slow freezing, SF) using both cryoprotectants (Test Yolk Buffer, TYB and a commercial cryoprotectant with animal-free extenders, AFE).

Participants/materials, setting, methods: Firstly, 13 samples were mixed with AFE following provider's recommendations (ratio cryoprotectant:sample 2:1). In last 16 samples, another addition procedure, consisting on diminishing the ratio to 1:1, was tested for AFE. In both cases TYB was mixed volume to volume. Each mix was loaded in two 0.5 ml straws, one used for SF, and the other used for LN. T-tests of dependent and independent samples were performed to compare cryopreservation outcomes. P-values<0.05 were considered significant.

Main results and the role of chance: LN leads to a higher number of viable spermatozoa than SF when TYB is used (15.14 ± 4.37 vs 9.24 ± 3.39, p-value = 0.066) while it is equally effective as SF when AFE is used (1.48 ± 0.82 vs 2.39 ± 3.77, p-value = 0.211). Considering the first sample set, use of AFE is associated with a decreased total motile sperm count (TMSC) when compared with TYB in both freezing protocols (LN: 1.48 ± 0.82 vs 15.14 ± 4.37, p-value = 0.003; SF: 2.39 ± 3.77 vs 9.24 ± 3.39, p-value = 0.016), worsening cryopreservation outcomes. However, a tendency of increased TMSC is observed in LN freezing when the mix ratio of AFE is diminished in the second sample set compared with the ratio used for AFE in the first sample set (3.49 ± 1.92 vs 1.48 ± 0.82, p-value = 0.084). Moreover, another comparison considering the

second sample set in which the mix ratio had been changed was performed. Interestingly, no significant differences were found when comparing the use of AFE and TYB in LN freezing (p-value = 0.294).

Limitations, reasons for caution: The current study prevents biased conclusions since it uses aliquots from the same sample to test freezing protocols and both cryoprotectants. However, in further studies the sample size should be increased and other patients apart from normozoospermic males should be included to validate the results.

Wider implications of the findings: LN freezing leads to sperm cryopreservation program's optimization due to its equal effectiveness and reduced operation costs. Moreover, if AFE is going to be used as animal-free extender cryoprotectant, diminishing the volume of AFE is recommended since outcomes are improved, so similar results as those associated to TYB are reached.

Trial registration number: This study is not a clinical trial.

P-052 Human Sperm Aminopeptidase N is related to embryo development and viability during ART

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Study question: to investigate the association between the levels of aminopeptidase N (APN) present in human spermatozoa with embryo development and viability.

Summary answer: Semen samples with less number of Aminopeptidase N molecules per spermatozoon are related to higher-quality early-embryos, more evolved blastocysts and blastocyst viability during IVF procedures.

What is known already: 20%-30% of men with normal seminal-parameters have impaired fertility ability and inability to achieve pregnancy, suggesting that male infertility can be caused by different deficiencies not yet described. One of the major predictors of ART success is embryo quality, and abnormal embryos have been linked to poor sperm quality. Sperm molecular features, such as proteins, are involved in fertilization and embryo development. Aminopeptidase N can be a relevant biomarker due to its high concentration in spermatozoa and its role in human sperm motility, mussel acrosome reaction and several seminal pathologies. However, its role in embryo development is not completely understood.

Study design, size, duration: This prospective cohorts study was conducted February 2014 to July 2015. Normal and/or pathologic semen samples of couples undergoing oocytes donation cycles, at Clínica IVI Bilbao, were used to determine the APN levels in human spermatozoa and to analyze the association between the APN and human embryo development and viability.

Participants/materials, setting, methods: A total of 81 human semen samples and 611 embryos were examined. Sperm samples and embryo quality were examined following WHO and "Asociación Española para el Estudio de la Biología de la Reproducción" (ASEBIR) guidelines, respectively. APN levels were measured by semi-quantitative and quantitative flow cytometry assays, in the same processed sperm sample used for the *in vitro* techniques. Statistics: Spearman´s rank correlation, Kruskal-Wallis and Mann-Whitney U-test.

Main results and the role of chance: Sperm samples with higher percentages of APN-positive spermatozoa and lower levels of this enzyme per spermatozoon correlate to sperm samples with better motility. In regard to embryo quality, embryos with higher implantation potential came from semen samples with less APN molecules per spermatozoon in early human embryos, at day 2 and 3 of development. Similarly, in the later phase of *in vitro* development, blastocyst with higher implantation potential, such as fully expanded and hatching blastocyst, also came from semen samples with less APN molecules per spermatozoon. Furthermore, concerning embryo viability at day 5 and 6 of development, we observed similar results, viable human blastocysts came from semen samples with less number of APN molecules per spermatozoa than non viable blastocysts.

Limitations, reasons for caution: Duration of the study.

Wider implications of the findings: APN levels can provide very valuable information for semen sample quality and embryo development in humans. In conclusion, the sperm APN could be a potential sperm biomarker to orient embryologist for embryo selection in IVF procedures in order to obtain a future embryos with higher implantation potential.

Trial registration number: CEISH/61/2011.

P-053 The impact of sperm chromatin condensation and ploidy on IVF/ICSI outcome

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Study question: To explore whether sperm chromatin condensation and ploidy affect the *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) outcome.

Summary answer: Our data show that sperm nuclear chromatin condensation and ploidy constitute critical parameters for a successful IVF/ICSI outcome.

What is known already: Fifteen per cent of the couples worldwide face infertility problems, while half of them are affected by male factor infertility. The development of medically assisted reproduction technology has allowed infertile couples to achieve a pregnancy. Sperm morphology, concentration and motility are taken into account for the selection of the appropriate assisted reproduction procedure. However, noteworthy percentages of IVF/ICSI cycles remain unsuccessful, suggesting the need of additional parameters for the evaluation of sperm quality, such as sperm nuclear chromatin condensation and ploidy. Sperm flow cytometry (SFC) has been suggested as a reliable method for the evaluation of these parameters.

Study design, size, duration: The study population was consisted of 280 couples that referred to the Ioannina University Medical School IVF Unit for IVF or ICSI due to male factor infertility. Men with karyotype abnormalities, Y microdeletions, hypogonadotropic hypogonadism and seminal tract disorders were excluded from the study. Furthermore, women suffering from endometriosis, polycystic ovary syndrome, ovulatory disorders and reproductive organ malformations were also excluded so as to analyze the real impact of male factor infertility on IVF/ICSI outcome.

Participants/materials, setting, methods: Semen analysis was performed according to World Health Organization guidelines. SFC after Acridine Orange and Propidium Iodide stainings was used to study the chromatin condensation and the ploidy of human spermatozoa, respectively. For the ovarian stimulation, a standard GnRH-antagonist protocol was used. IVF was performed in 130 couples and ICSI in 150 couples. The fertilization rate, the embryo quality and the clinical pregnancy rate per cycle begun were the IVF/ICSI outcome measures.

Main results and the role of chance: SFC analysis revealed that 70 ± 20.3% of the spermatozoa in the IVF group and 61.3 ± 19.9% of the spermatozoa in the ICSI group had fully condensed chromatin (FCC). Furthermore, 16.3 ± 12.2% of the spermatozoa in the IVF group and 18.5 ± 16.7% of the spermatozoa in the ICSI group were aneuploid.

In the IVF group, couples with FCC≥70% presented higher fertilization ($p = 0.006$) and clinical pregnancy ($p = 0.019$) rates compared to couples with FCC < 70%. Furthermore, they were characterized by higher grade A ($p = 0.008$) and lower grade C ($p = 0.004$) embryo rates. As concerns the ICSI group, couples with FCC≥61.3% were characterized by higher fertilization ($p = 0.01$) and clinical pregnancy ($p = 0.008$) rates compared to couples with FCC < 61.3%. Additionally, they presented higher grade A ($p = 0.009$) and lower grade C ($p = 0.03$) embryo rates.

In the IVF group, couples with aneuploidy rate<16.3% presented higher fertilization ($p = 0.008$) and clinical pregnancy ($p = 0.017$) rates compared to couples with aneuploidy rate≥16.3%. Moreover, they were characterized by higher grade A ($p = 0.01$) and lower grade C ($p = 0.005$) embryo rates. Regarding the ICSI group, couples with aneuploidy rate<18.5% showed higher fertilization ($p = 0.01$) and clinical pregnancy ($p = 0.02$) rates compared to couples with aneuploidy rate≥18.5%. Finally, they were characterized by higher grade A ($p = 0.01$) and lower grade C ($p = 0.03$) embryo rates.

Limitations, reasons for caution: Our findings were based on 280 couples undergoing IVF or ICSI due to male factor infertility. Larger population and multicenter studies are needed to verify our preliminary results.

Wider implications of the findings: SFC analysis could provide a better screening of the male partner's clinical condition leading to a more appropriate treatment. The combination of SFC with sperm selection methods could help in the fractionation/isolation of spermatozoa with normal genetic constitution, fully condensed chromatin and elevated pregnancy potential post-ICSI from defective semen samples.

Trial registration number: N/A.

P-054 Metabolomics profiles of the seminal plasma in samples collected following long (4-5 days) and short (2 hours) sexual abstinence periods

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Study question: Is there a within-subject variation in Seminal plasma metabolomics profiles following long (4-5 days) and short (2 hours) sexual abstinence periods?

Summary answer: Short ejaculation abstinence was associated with lower amounts of all detected seminal plasma metabolites, except for pyruvate and taurine.

What is known already: The metabolome is a dynamic environment and can be influenced by genetic or environmental cues. Metabolomic profiling of seminal plasma has been suggested as a possible approach for a fast and non-invasive diagnosis in the evaluation of male infertility, but the metabolomics profiles in normozoospermic men have not been thoroughly investigated. The different sperm motility characteristics in samples collected following long and short abstinence periods have been previously documented but the possible change of seminal metabolomic profiles following different abstinence periods has not been studied.

Study design, size, duration: Repeated measures study based on seminal plasma from 31 normozoospermic men who delivered a semen sample after 4-7 days of abstinence, followed by a second sample 2 hours later. The study was conducted during 2015–2017.

Participants/materials, setting, methods: Participants included normozoospermic male partners of couples attending IVF treatment at the Fertility Unit of Aalborg University Hospital (Aalborg, Denmark). All semen samples were assessed for concentration, total counts and motility characteristics by using the SCA® (Microptic S.L., Barcelona, Spain) computer aided sperm analysis system. Metabolite profiles of the seminal plasma were determined using untargeted Nuclear Magnetic Resonance Spectroscopy by a Bruker AVIII-600 NMR spectrometer (Bruker Biospin, Germany and Switzerland).

Main results and the role of chance: 30 metabolites were identified in the ejaculates. Among these, the concentration of pyruvate was higher in samples collected after a two hours abstinence (vs. 4-7 days), and the concentrations of fructose, acetate, choline, methanol, N-acetylglucosamine, O-acetylcarnitine, uridine and sn-glycero-3-phosphocoline were lower. There was no difference for the remaining 21 metabolites. Sperm concentration was also lower in samples obtained after the short abstinence period. Consequently, the absolute amounts of pyruvate and taurine per spermatozoa were higher in samples obtained after the short abstinence ($P < 0.001$ and $P < 0.05$ respectively).

Limitations, reasons for caution: The possible physiological explanations for the findings cannot be determined by the study, and furthermore we do not know if the metabolomics patterns we detected are different from those of men with oligozoospermia. The effect of sperm metabolism after ejaculation and during liquefaction could not be determined in this study.

Wider implications of the findings: The concomitant increase in percentages of motile spermatozoa and pyruvate and taurine per spermatozoa might indicate that these two metabolites are beneficial for sperm motility, which should be further investigated in future studies.

Trial registration number: -

P-055 Varicocelectomy in patients with elevated serum FSH: correlation between changes in semen parameters and serum FSH

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Study question: Can varicocele repair improve semen quality and decrease serum FSH levels in infertile patients with elevated serum FSH and varicocele?

Summary answer: Even in patients with elevated serum FSH, varicocele repair can significantly improve semen parameters with a substantial decrease in serum FSH.

What is known already: Varicocele is present in approximately 35% of infertile men and in 81% of those with secondary infertility. Most previous studies have demonstrated that varicocele repair is associated with a significant improvement in semen parameters as well as spontaneous pregnancy rates. Furthermore, the benefits of varicocelectomy have been reported even among patients with non-obstructive azoospermia or hypogonadism. However, the role of varicocelectomy in patients with severe spermatogenic failure and hormonal abnormalities, including those with an elevated serum FSH level, is still under debate.

Study design, size, duration: We retrospectively reviewed the records of 2,368 consecutive patients who underwent varicocelectomy for subfertility at our clinics from July 2003 to September 2017.

Participants/materials, setting, methods: Multiple semen analyses were performed before and after varicocelectomy. The total motile sperm count per ejaculate was calculated as semen volume x sperm concentration x percent motility divided by 100. We defined a greater than 50% increase in the total motile sperm count per ejaculate at three months after surgery as a significant improvement.

Microsurgical subinguinal varicocelectomy was performed under local anesthesia.

Main results and the role of chance: The serum FSH level was greater than 10 mIU/mL in 282 patients. After we excluded 43 patients with azoospermia, 5 patients with prior endocrine therapy and 27 patients who were lost to follow-up, 207 patients were included in this study. After surgery, 135 of these patients (65.2%) experienced a significant improvement of semen parameters.

Preoperative serum FSH levels were not significantly different between the improved patients (14.0 ± 4.3 mIU/mL) and not-improved patients (15.6 ± 5.9 mIU/mL). Serum FSH was measured in 34 improved patients and 15 not-improved patients after surgery. The decrease in the serum FSH level in the improved patients (1.8 ± 2.6 mIU/mL) was significantly greater than that in the not-improved patients (-0.6 ± 3.2 mIU/mL).

These data indicate that varicocelectomy in patients with elevated serum FSH levels and varicocele improves semen quality associated with a decrease in the serum FSH level.

Limitations, reasons for caution: Because we measured serum FSH only in selected patients after surgery, further studies with more patients will be needed to avoid being misled by changes in serum FSH levels induced by varicocele repair.

Wider implications of the findings: In patients with non-obstructive azoospermia, changes in the serum FSH level after varicocele repair may predict the outcome of microdissection TESE.

Trial registration number: Not applicable.

P-056 Delayed and impaired development in early mouse embryo was induced by sperm DNA damage

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Study question: Does sperm DNA damage affect to developmental ability and developmental events such as pronuclear formation, DNA replication, and cleavage?

Summary answer: DNA damage in spermatozoa induced slowing DNA replication at the pronuclear stage and delayed time points of early developmental events.

What is known already: Some studies have indicated that DNA damage in mammalian spermatozoa could induce some developmental defects such as low fertilization rates, poor embryo quality, and developmental arrest. Further, it is suggested that spontaneous miscarriage and childhood disease may be related to sperm DNA damage. However, the mechanisms of these defects on embryos with damaged sperm DNA have not yet been cleared.

Study design, size, duration: Spermatozoa obtained from C57BL/6J mouse were exposed to 1 mM H_2O_2 for 30 min to induce DNA damage (oxidatively damaged spermatozoa: ODS), and embryos were produced by ICSI with ODS and normal spermatozoa (control) (n = 70-80 embryos/group). The timing of developmental events (pronuclear formation, DNA replication, syngamy, and cleavage) and the developmental ability were compared between ODS and control groups.

Participants/materials, setting, methods: Time-lapse monitoring has been used to examine the developmental events of each embryo. After 4.5-6.0 hr from ICSI, DNA replication in pronuclei was estimated by using the DNA replication assay kit with EdU. Further, DNA damages in male pronucleus and nuclei in developing embryos were detected by immunohistochemical staining with gamma-H2AX antibody.

Main results and the role of chance: There was no significantly difference in the rate of male and female pronuclear formation between ODS and control groups. However, DNA replication assay at 5.5 hr after ICSI revealed that the rate of EdU-positive pronuclei in ODS embryos was significantly lower than that of control (58.8% vs 81.3%, $p < 0.05$). The time-lapse analysis revealed that the average time points of syngamy, 1st cleavage, and 3rd cleavage of ODS embryos were delayed remarkably compared with control (16.9 hr vs 14.7 hr, 20.1 hr vs 17.0 hr, 43.5 hr vs 40.1 hr, respectively, $p < 0.01$). By immunohistchemical staining, male pronucleus and nuclei in embryos with ODS were positive for gamma-H2AX, and extranuclear DNA fragments were frequently observed in ODS embryos. In addition, the blastocyst rate of ODS embryos was significantly lower than control (15.4% vs 76.7%, $p < 0.05$).

Limitations, reasons for caution: This study was conducted using a mouse model with artificially induced DNA damage by exposing to H_2O_2. This finding does not directly represent human reproductive medicine.

Wider implications of the findings: This study shows clearly that sperm DNA damage induced the delay of developmental events and impaired the developmental ability. Also, our results suggest that damage on sperm DNA persists during early development. Therefore, this study may provide novel insights for understanding the relationship between sperm DNA damage and embryonic development.

Trial registration number: Not applicable.

P-057 Effect of varicocelectomy on oxidation reduction potential in varicocele associated male infertility

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Study question: Does varicocelectomy improve Oxidation Reduction Potential (ORP) as an independent measure of oxidative stress (OS) in infertile men?

Summary answer: Elevated oxidative stress is related with poor semen quality in men with varicocele. Varicocele surgery reduces the ORP levels and improves sperm count.

What is known already: Patients with varicocele tend to have poor sperm quality and are at higher risk of being infertile. Although the pathophysiology of infertility in males with varicocele has been extensively studied, the underlying mechanism remains unclear. Many reports have shown that oxidative stress is one of the underlying mechanisms of poor semen quality in infertile men with varicocele. ORP has been validated as a diagnostic marker of poor semen quality and oxidative stress in men with infertility.

Study design, size, duration: Prospective, case control study of 43 infertile patients with clinical grade 2-3 varicocele attending a male infertility clinic during January to November 2017.

Participants/materials, setting, methods: All patients underwent microsurgical subinguinal varicocelectomy. Full medical history and clinical examination was collected. Semen samples were done using WHO Fifth edition criteria and ORP levels were measured by MiOXSYS analyzer before surgery and 3 months post-varicocelectomy. The results were compared by Wilcoxon rank sum test and Spearman's correlation test and a P value < 0.05 was considered significant.

Main results and the role of chance: Table 1 compares results of semen parameters and ORP values before and 3 months after the surgery (n = 43). All semen parameters (concentration, total motility and normal sperm form) showed an improvement post- surgery but it was not statistically significant. However, the ORP level was significantly reduced after the surgery (10.4 ± 3.3 vs. 4.6 ± 1.1, p value <0.001). Table 2 shows the correlation between semen parameters and ORP before and after surgery. All semen parameters (concentration, total motility and normal form) were negatively correlated with ORP pre-operatively. However, only sperm count maintained this significant negative correlation post operatively.

Table I

	Before Surgery	After Surgery	P value
Sperm Count	25.2 +/− 3.4	29.9 +/− 3.1	0.029
Total Motility	44.8 +/− 3.1	46.1 +/− 2.3	0.645
Progressive Motility	9.3 +/− 1.6	10.3 +/− 1.6	0.64
Normal forms	5.5 +/− 1.6	3.6 +/− 0.4	0.893
sORP	10.4 +/− 3.3	4.6 +/− 1.1	<0.001
Wilcoxon Rank test			

Table II

	Before Surgery	After Surgery
Sperm Count	−0.871**	−0.827**
Total motility	−0.359**	−0.288
Progressive motility	−0.461**	−0.155
Normal forms	−0.526**	−0.332*
Spearman's correlation		

*p < 0.05
**p < 0.01

Limitations, reasons for caution: The small sample size of this study may be a limitation. However, we report on a pilot study describing the effect of varicocelectomy on oxidative stress measures using the ORP system.

Wider implications of the findings: ORP can be used as a prognostic factor for counselling patients before varicocelectomy.
Trial registration number: Non.

P-058 Oxidation reduction potential is correlated to spermatogenic testicular function in infertile men

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Study question: Is oxidative stress a clinical indicator for testicular function in infertile men?
Summary answer: Oxidative stress measured by oxidation reduction potential (ORP) can be considered a clinical indicator of spermatogenic and not endocrine function of the testis.
What is known already: Oxidative stress plays a major role in the pathogenesis of male infertility. ORP is a new marker of oxidative stress that has been shown to correlate inversely with important semen parameters in infertile men. ORP levels can be used to discriminate normal fertile men from infertile males. Besides semen parameters, testicular size and FSH are considered as prognostic markers of spermatogenesis.
Study design, size, duration: Cross sectional, retrospective study on 660 patients attending a male infertility clinic during January to March 2017.
Participants/materials, setting, methods: Medical records of recruited patients were reviewed. The data extracted included medical history, clinical examination, semen analysis (WHO 5th edition), ORP (using MiOXSYS system), hormonal assay including FSH, LH, prolactin, testosterone and estradiol as well as testicular size assessment by scrotal ultrasound. After checking for normal distribution of the data by the Chi-squared test, Spearman rank test was used to detect correlations between different parameters. Statistical significance was defined as $p < 0.05$.
Main results and the role of chance: ORP showed negative correlation with sperm count ($r = -0.793$, $P < 0.0001$), motile sperm count ($r = -0.579$; $P < 0.0001$), progressive motility ($r = -0.431$; $P < 0.0001$) and normal sperm morphology ($r = -0.458$; $P < 0.0001$). ORP also correlated with sperm DNA fragmentation ($r = 0.264$, $P < 0.0001$) (Table 1).

ORP levels showed significant correlation with the testicular size ($r = -0.386$; $P < 0.0001$), serum FSH ($r = 0.273$; $P < 0.0001$) and serum LH concentrations ($r = 0.182$; $P = 0.0002$), but not with testosterone, estradiol and prolactin.
Limitations, reasons for caution: The main limitation is the lack of fertile controls in this study.
Wider implications of the findings: ORP can be used as a marker of spermatogenesis in infertile men. Further studies should be carried out to demonstrate the effect of treating OS on spermatogenesis. Since varicocele patients show seminal oxidative stress, smaller testes and higher FSH values, ORP could possibly be used as adjunct indicator of varicocele.
Trial registration number: None.

P-059 Integrity of human sperm DNA assessed by the neutral comet assay and its relationship to oxidative stress and poor spermatic morphology

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Study question: The current study aimed to determine the extent of sperm nuclear DNA damage in patients with isolated teratozoospermia and to examine its relationship with oxidative stress.
Summary answer: Our results may explain the complex biological relationship between teratozoospermia, oxidative stress, and DNA damage.
What is known already: The physiopathology of the morphological abnormalities of the spermatozoa has not been completely understood. In the present study, we found an impaired seminal antioxidant status and an increased seminal level of both Lipid peroxidation and Iron can affect sperm nuclear integrity resulting in DNA Breaks and can be associated with poor sperm morphology.
Study design, size, duration: Semen samples from 60 patients with isolated teratozoospermia and 30 normozoospermic donors were examined. DNA damage was evaluated by Comet assay. Seminal antioxidant activities (Superoxide dismutase; Glutathione peroxidase; Catalase), Iron and malondialdehyde concentrations were measured spectrophotometrically.
Participants/materials, setting, methods: Methods and endpoints used:

patients: 60 patients with isolated teratozoospermia and 30 normozoospermic donors,

Methods: Comet assay, spectrophotometer, chemicals reagents.
Main results and the role of chance: Sperm DNA damage; malondialdehyde and Iron levels were more elevated in study groups than control groups. Nevertheless, the antioxidant enzymes activity was significantly lower in the group of patients with teratozoospermia compared to the controls. Sperm DNA damage was positively correlated to malondialdehyde and seminal Iron level, While reduced seminal antioxidant status was negatively associated with sperm DNA Breaks. Interestingly, we noted that sperm DNA damage; lipid peroxidation, Iron level, and impaired antioxidant status were negatively correlated to normal sperm morphology.
Limitations, reasons for caution: only in vitro study.
Wider implications of the findings: This work was supported by funds allocated to the Research Unit of Histology and Genetic UR12ES10 by the Ministère Tunisien de l'Enseingement Supérieur et de la Recherché Scientifique.
Trial registration number: not applicable.

P-060 Alterations in disease activity and fertility potential in men with rheumatoid arthritis by yoga based lifestyle intervention

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Study question: Can a simple lifestyle modification program including yoga bring about positive alterations in fertility potential and reduction in disease activity in men with Rheumatoid arthritis?
Summary answer: Yoga based lifestyle intervention may not only reduce disease severity, minimize usage of drugs with minimum side effects especially on sperm.
What is known already: The complex mechanism of Rheumatoid arthritis with infertility in men involves interactions between endocrine, immune, and reproductive systems. Association of autoimmunity with dysregulated androgen (hypogonadism) levels may cause transient infertility in men. Furthermore, usage of disease-modifying antirheumatic drugs (DMARDs) like cyclophosphamide, methotrexate, sulphasalazine etc. may result into decreased quantity and quality of sperm, reduced fertility potential and ultimately permanent infertility. These drugs can cross blood-testis-barrier and induce changes in sperm impairing spermatogenesis. Complementary and alternative medicine like yoga reduces seminal oxidative stress and its consequences like DNA fragmentation in sperm nuclear/mitochondrial genome.
Study design, size, duration: Seventy five males with RA were enrolled in this 12-week prospective, open-label, single-arm exploratory study, designed to explore the impact of yoga based lifestyle intervention (YBLI) on disease activity and fertility potential in men with Rheumatoid arthritis.
Participants/materials, setting, methods: The participants were evaluated for pre (day 0) and post (12th week) levels of C reactive protein (CRP), IL-6, IL-17A and soluble HLA-G for systemic inflammation. Sperm parameters as per WHO 2010 guidelines and reactive oxygen species (ROS), DNA fragmentation index (DFI), 8-hydroxy-2'-deoxy guanosine (8-OHdG) were estimated. Parameters of disease activity i.e. disease activity score (DAS28-ESR) and pain

acuity i.e. visual analogue scale (VAS) and disability quotient i.e. health assessment questionnaire (HAQ) were assessed.

Main results and the role of chance: The mean levels of ROS ($p < 0.05$***), DFI ($p < 0.05$**) and 8-OH2dG ($p < 0.05$**) levels were significantly reduced after 12 weeks of yoga intervention. We observed reduction in mean levels of CRP ($p < 0.05$***), IL-6 ($p < 0.05$**), IL-17A ($p < 0.05$**) and soluble HLA-G ($p < 0.05$**) at 12 weeks compared to base line level (day 0). There was reduction seen in DAS28-ESR ($p < 0.05$***), VAS ($p < 0.05$***) and HAQ ($p < 0.05$***) after 12 weeks with respect to the base line levels (day 0).

Limitations, reasons for caution: Compliance of patients for Yoga based lifestyle intervention was poor, hence we enrolled large number of patients to achieve the desirable sample size.

Wider implications of the findings: Adoption of yoga based lifestyle intervention as an integral part of our lifestyle may hold the key to reduce the disease activity, minimize dosage of DMARDs and its associated consequences like disabilities associated with physical, mental and reproductive health.

Trial registration number: Not applicable.

P-061 Reproductive potentials of testicular versus ejaculated sperm for intracytoplasmic sperm injection (ICSI) using sibling oocytes for men with high sperm dna fragmentation

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Study question: In couples with high sperm DNA fragmentation (SDF), is reproductive potential improved with intracytoplasmic sperm injection (ICSI) using testicular sperm (Testi-ICSI) compared to ejaculated sperm (Ejac-ICSI)?

Summary answer: ICSI with testicular sperm in couples with high SDF seemed not to have a beneficial effect on reproductive potential compared to using ejaculated sperm.

What is known already: High SDF may have an adverse impact in reproductive outcomes, reducing the fertilization rate, poor embryo development and high miscarriages. Although testicular sperm have higher aneuploidy rates, testicular sperm have lower levels of SDF than ejaculated ones. Therefore, some studies have been reporting better reproductive outcomes with ICSI using testicular sperm for men with high levels of SDF. However, these studies were done on women-based randomization, not on oocyte-based randomization.

Study design, size, duration: In this prospective pilot study, performed from November 2016 to November 2017, 29 women (32.2 ± 4.4 years old) whose male-partners (38.6 ± 4.5 years old) had high sperm DNA fragmentation (>30% DNA fragmentation index (DFI)) and unsuccessful antioxidant treatment. This study was performed to compare reproductive potential between Testi-ICSI (n = 158) and Ejac-ICSI (n = 169) in same patients with persistently high SDF despite prior antioxidant treatment.

Participants/materials, setting, methods: Sperm DNA damage was examined by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using flow cytometric analysis. All male-partners in this study had high sperm DNA fragmentation (> 30% DNA fragmentation index (DFI). Male partners were produced ejaculate sperm and underwent testicular sperm aspiration (TESA). Approximately half of mature eggs obtained per ICSI cycle were Testi-ICSI and half with Ejac-ICSI. Fertilization, good-quality blastocyst development and reproductive outcomes were analyzed between two groups.

Main results and the role of chance: Average seminal DFI of patients was 45.2 ± 12.7%. A total of 327 mature oocytes were injected; 158 with Testi-ICSI and 169 with Ejac-ICSI. Fertilization rate per oocyte was 65.7 ± 20.5 % and 78.1 ± 20.7 % in Testi-ICSI and Ejac-ICSI, respectively ($P < 0.01$). Good-quality blastocyst formation rates (> 4BB) per embryo were 60.2 ± 35.9 % and 65.5 ± 32.4 % in Testi-ICSI and Ejac-ICSI, respectively (NS). Seventeen cycles out of 29 ICSI cycles had fresh embryo transfer (ET), twelve cycles cancelled because of high risk of developing OHSS. In Testi-ICSI, 11 blastocysts were transferred in 7 ET cycles resulting in 3 clinical pregnancy rate (CPR) of 42.9% and an implantation rate (IR) of 27.3% (N = 3). It resulted in one live birth, one ongoing and one miscarriage. In Ejac-ICSI, 10 ET cycles were performed transferring 17 blastocysts obtaining the CPR and IR of 50 % (5/10) and 35.3% (6/17). One of the clinical pregnancies of this group resulted in an abortion. The other 4 pregnancies had live births.

Limitations, reasons for caution: Sample size is still too low and it should be enlarged; furthermore a high number of mature oocytes available for the injection per patient is needed. Finally, frozen embryos have still to be transferred before drawing final conclusions.

Wider implications of the findings: Due to variable factors between patients, study on sibling oocytes might be the actual valid method to see usefulness of Testi-ICSI. Our study implies that more studies are necessary before changing clinical practice for the couples with high SDF because of potential known/unknown risks of using testicular sperm.

Trial registration number: Not applicable.

P-062 Effect of paternal high values of sperm DNA fragmentation on the outcomes of PGT-A programs in couples with different age groups of female patients

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Study question: Does the maternal age influence the outcomes of the PGT-A programs with high levels of partner's DNA fragmentation?

Summary answer: A high level of DNA fragmentation does not affect the frequency of clinical pregnancy in women under 30 years of age in the PGT-A programs.

What is known already: High sperm DNA fragmentation in couples undergoing assisted reproductive technologies is associated with lower live birth rate.

Sperm DNA damage has a close inverse relationship with live-birth rates after IVF.

Study design, size, duration: Retrospective, experimental study of 126 couples, undergoing IVF treatment with the evaluation of functional semen parameters (concentration, motility, morphology), and sperm DNA fragmentation from January 2016 to January 2017

Primary endpoints:
The fertilization rates groups.
The rates of blastocyst formation in groups.
The rates of clinical pregnancy in groups.

Participants/materials, setting, methods: Sperm DNA fragmentation was evaluated using the TUNEL assay (the normal level of DNA fragmentation is < 15%).

ICSI+PGT-A program, fertilization and embryo culture according to the manufacturers recommendations of William A. COOK (Australia).

Array CGH (Agilent, USA) was used for 24-chromosome embryonic genome analysis.

The rates of fertilization, rates of blastocyst formation, and rates of implantation were evaluated.

Main results and the role of chance: 126 women who were enrolled in the study were distributed according to their age as followed: under 30 years old (30 patients); 31-34 years old (35 patients); 35-40 years old (45 patients); older than 40 years old (16 patients).

Our results showed that there are lower fertilization rates (71,1% vs. 83,4%, $p < 0.05$) and significantly lower rates of blastocyst formation (23,7% vs. 53,2%, $p < 0.05$) in the group with high values of sperm DNA fragmentation in comparison with the group with normal values of this parameter.

Our data demonstrated that for couples with a female partner under 30 years there is no difference in the rates of clinical pregnancies between the group with high values of paternal sperm DNA fragmentation and the group with normal values of this parameter (63% and 63%, respectively). On the

contrary, for couples with female partners older than 31 the rates of clinical pregnancies were higher for groups with normal values of paternal sperm DNA fragmentation compared to the groups with high values of this parameter: 53% vs. 44% for women 31-34 years old; 32% vs.24% for wome 35-40 years old and 25% vs.0% for women > 40 years.

Limitations, reasons for caution: Our study didn't include a large number of couples, and new bigger studies with extra criteria's of comparison are required.

Wider implications of the findings: Our study shows that high values of sperm DNA fragmentation do not influence the outcomes of the IVF programs only when a female partner is younger than 30 yours old. Thus, we can speculate that oocytes of younger women have an ability to compensate the spermatozoa damaged DNA.

Trial registration number: not applicable.

P-063 Clinical outcomes of microdissection testicular sperm extraction (micro tese) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia (NOA) with the history of cryptorchidism

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Study question: To assess the prevalence and significance including SRR by micro TESE and ICSI outcomes with embryonic development in NOA couples with the history of cryptorchidism.

Summary answer: Micro TESE is particularly helpful for sperm retrieval in UT cases and ICSI outcomes in NOA with UT are equivalent to OA and unexplained NOA.

What is known already: Undescended testis (UT) is associated with impairment of germ cell maturation and subsequent infertility in adulthood because the UT is exposed to a higher temperature compared with the scrotal temperature and there is progressive Leydig and Sertoli cell atrophy. There have been very few studies of ICSI with a focus on, or large enough numbers to examine, the specific outcomes associated with male factor infertility including NOA with the history of cryptorchidism.

Study design, size, duration: This study was conducted in 50 NOA patients post cryptorchidism, 526 unexplained NOA patients without past history (unexplained NOA; not including after orchidopexy, Klinefelter syndrome, cryptozoospermia, mumps orchitis, etc), and 141 OA patients and the ICSI outcomes of their wives are retrospect assessed between September 2013 to December 2017.

Participants/materials, setting, methods: We evaluated SRR of micro TESE, two pronuclei (2PN) oocyte rates, blastocyst development rates, good-quality blastocyst (Grade 3BB and above on day 5 by the Gardner scoring) rates, and clinical pregnancy rates per embryo transfer (ET) in 25 cases the history of UT, 94 cases of unexplained NOA, and 107 cases of OA. The wives age at ICSI after cryptorchidism, Unexplained NOA, and OA were 34.1 ± 3.5 years, 34.6 ± 4.6 years, and 34.9 ± 3.9 years, respectively.

Main results and the role of chance: SRR of micro TESE NOA with the history of cryptorchidism (32/50 = 64.0%) was higher than unexplained NOA (107/526 = 20.3%) ($p < 0.001$). No correlation was found between serum FSH, LH, and T level with the success of sperm retrieval. Testicular volume and patient age at orchidopexy also did not affect the SRR for micro TESE. 2PN oocytes, blastocyst development, and good-quality blastocyst rates were 54.8%, 46.6%, and 38.2% in NOA with the history of cryptorchidism, 55.7%, 34.2%, and 40.9% in unexplained NOA with the history of cryptorchidism, and 62.7%, 37.7%, and 42.6% in OA, respectively (no significant differences). Clinical pregnancy rates per ET were 31.6% in unexplained NOA with the history of cryptorchidism, 30.8% in unexplained NOA, and 40.4% in OA, respectively (no significant differences).

Limitations, reasons for caution: In the present series, we could not assess whether the children born had a cryptorchidism or not. It would be worthwhile evaluating, in a larger series, the prevalence of cryptorchidism in boys born from fathers with an undescended testis.

Wider implications of the findings: However testicular function is considered to be severely impaired, orchidopexy for undescended testes in even adulthood could be considered to have any effect on spermatogenesis. Objective counselling of the NOA patinets with the history of cryptorchidism for the chance of having a healthy baby is of utmost importance.

Trial registration number: N/A.

P-064 Raman spectroscopical assessment of human sperm capacitation in vitro

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Study question: Is it possible to assess human sperm function using a label-free and non-destructive method.

Summary answer: Raman microspectroscopy (RM) can enables label-free, non-invasive, accurate and reproducible assessment of sperm function, including the discrimination of capacitated from un-capacitated spermatozoa.

What is known already: Current methods for evaluating sperm function are indirect and of limited clinical applicability since they render the analysed spermatozoon unfit for IVF. We applied RM as a spectroscopic method to provide detailed biochemical maps of cells, including spermatozoa, without affecting their viability, revealing DNA fragmentation, nuclear composition (such as discrimination of X and Y-bearing bovine sperm cells) and protein/lipid oxidation. Can RM could be used for the biochemical assessment of sperm physiology?

Study design, size, duration: Raman spectra and images of capacitated versus untreated human spermatozoa were analysed. A concentration of 10 million of sperm/ml was divided into four aliquots: the untreated serving as a control; the others were added with a 100 mg/ml of heparin and incubated at 37°C for different times: 1, 2, 3 and 4 hours. A totality of 5 samples from 5 different patients were analysed through CTC assay and Raman spectroscopy.

Participants/materials, setting, methods: Semen samples were obtained from men (age: 35-48 years) attending the Centre for Assisted Fertilization (Naples). The ejaculates were collected after 3–5 days of sexual abstinence and processed. Following a routine spermiogram and washing on a Percoll density gradient, capacitation was induced in vitro using heparin, and the percentage of capacitated spermatozoa was evaluated using a CTC staining assay. Finally, control and treated samples were analysed by RS at the Institute of Protein Biochemistry (Naples).

Main results and the role of chance: The average spectra acquired from different regions of the human sperm head (acrosome, middle region) for both control cells and those treated at different timeswith heparin were analysed. Capacitated spermatozoa reveal important spectral variation in the Raman bands associated with proteins and lipids content (1200-1600 cm^{-1}), reflecting the protein/lipid migration and scrambling especially in the acrosoma region. On the contrary, the average spectra from the mid-head region (mainly nucleus) did not show any significant changes in lipid peak intensity, as expected. Interestingly, the Raman peaks at 2850 cm^{-1} and 2885 cm^{-1}, assigned to cholesterol decreases in the Raman spectra of capacitated spermatozoa, coinciding with the cholesterol efflux that triggers capacitation. All these variations increase with increasing the incubation time with heparin. Finally, multivariate statistical analysis (PCA, principal component analysis) was used for revealing the main specific Raman biomarkers associated with capacitation, and to use them for the efficient identification and sorting ofcontrol and capacitated sperm cells.

Limitations, reasons for caution: Raman analysis was performed on fixed spermatozoa. The results demonstrated only proof of principle of using RS as a method to study sperm function without clinical validation. The complexity in interpreting our results make the translation of RS technique into clinical practice challenging.

Wider implications of the findings: Assessment and classification of male infertility based on sperm function, useful for choosing the best technique for ART.

Complete sperm quality assessment for selecting a fertile spermatozoon for IVF.

Evaluating or comparing media or conditions used to handle sperm in vitro or in cryopreservation processes.

Trial registration number: This in basic science.

P-065 A multicenter study to evaluate oxidative stress by oxidation–reduction potential, a reliable and reproducible method

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Study question: Investigate the reproducibility and reliability of the ORP measurement as a marker of sperm quality across different fertility centers.

Summary answer: ORP provided equal or less variability than the current semen analysis measures across 9 different fertility centers.

What is known already: Discrete measures of free radicals, antioxidant activity, and oxidative damage suggest an ambiguous relationship between the redox system and male fertility. Static oxidation-reduction potential (sORP) measures the balance between all oxidants and antioxidants providing a comprehensive status of the redox system. In a previous study, ORP and semen analysis data were compared between two andrology laboratories, in which ORP remained consistent in both datasets individually or in combined data.

Study design, size, duration: This prospective study was carried out jointly by nine participating fertility centers on 1644 subjects. The study was approved by the institutional ethics committee and subjects were consented prior to participation. Subjects were grouped into those that had all normal semen parameters (concentration, total cell, total motility, progressive motility, and morphology) according to WHO 2010 guidelines and those who failed to meet one or more criteria. ANOVA/t-test measures were used to determine significant differences.

Participants/materials, setting, methods: Exclusion criteria included azoospermia, presence of sexually transmitted disease or chronic disease, use of prescription, OTC medications or antioxidants. Samples were collected and semen parameters assessed using the WHO 2010 guidelines. ORP was measured (mV) using the MiOXSYS system and normalized to sperm concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration >0.999 × 10^6 sperm/mL were included.

Main results and the role of chance: The results of ORP reflects the oxidative relationship between the sperm cell and its environment - the expulsion of oxidants, a by-product of cellular metabolism, into the seminal environment and the deactivation of them by extracellular antioxidants. The resulting ORP measurement reflects the average of the final ten (10) seconds (or twenty readings) of the sample. Of the 1644 samples, 138 were found to have normal semen parameters and 1506 were found to have abnormal semen parameters. The mean ORP value (mV/10^6 sperm/mL) in the semen of the abnormal group was 5.07 mV/10^6 sperm/mL whereas that of the normal group was 0.88 mV/10^6 sperm/mL ($p = 0.001$). The SD for ORP was equal to or better than most measures, with exception to morphology. However, it should be noted that morphology is the parameter with the highest variability typically found between laboratories.

Table I Sperm parameters and ORP values (mv/10^6 sperm) in patients with at least one abnormal semen parameter (n = 1506) versus patients with normal semen parameters (n = 138) [values are presented as mean ± SEM].

	Group	Mean	SEM
ORP	Normal	0.88	0.14
	Abnormal	5.07	0.37
Progressive Motility	Normal	48.08	0.93
	Abnormal	15.81	0.39
Normal Morphology	Normal	6.76	0.30
	Abnormal	4.87	0.19

Limitations, reasons for caution: Study enrollment of an even number of healthy controls with proven fertility was limited in comparison to the male infertility group.

Wider implications of the findings: ORP remains stable even with measurable differences in sperm parameters, and it therefore can be used as a supplementary test to semen analysis to confirm oxidative stress and poor semen quality.

Trial registration number: There are no competing interests.

P-066 Protein Kinase A modulation by nitric oxide during human sperm capacitation

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Study question: To further examine how nitric oxide (NO) modulates Protein Kinase A (PKA) activity during the *in vitro* capacitation of human spermatozoa.

Summary answer: We provide further evidence that NO plays a role in regulating specific phosphorylation events during human sperm capacitation.

What is known already: Several studies have identified important factors involved in the regulation of sperm capacitation, a physiological process necessary to achieve the fertilization ability. Reactive Oxygen Species such as NO are generated during this process and are beneficial in low concentrations for its progress. It has been reported that NO can modulate PKA-dependent phosphorylation events linked to the capacitation in different species. NO can activate the sAC-cAMP-PKA pathway either directly or by increasing the cGMP levels. A rise in the cGMP concentration may inhibit cAMP degradation, which subsequently leads to PKA activation.

Study design, size, duration: Semen samples were obtained from normozoospermic donors (n = 7) by masturbation after 3–5 days of sexual abstinence. Spermatozoa were incubated for 4 hours in capacitating and non-capacitating conditions. The media were supplemented with 100 µM S-Nitrosoglutathione, a NO donor, and two inhibitors of NO synthesis: 10 mM N^G-Nitro-L-arginine Methyl Ester Hydrochloride and 10 mM Aminoguanidine hemisulfate salt. The experiments were performed in absence and presence of 10 mM L-Arginine monohydrochloride, the substrate for NO production.

Participants/materials, setting, methods: The protein phosphorylation pattern on Serine and Threonine residues (i.e. PKA activity) was evaluated by Western blotting (WB). Proteins were separated by electrophoresis on 4-15% SDS-polyacrylamide gels and electrotransferred to PVDF membranes. The latter were treated with the following antibodies: rabbit monoclonal antibody anti-protein kinase A (1:2000) and goat anti-rabbit IgG-HRP (1:10000). The relative amount of signal in each membrane was quantified using the ImageQuant TL v8.1 software (GE Healthcare, Life Sciences, Buckinghamshire, UK).

Main results and the role of chance: Our results indicated that in the presence of Nitric Oxide Synthase inhibitors, spermatozoa showed a lower Serine and Threonine phosphorylation pattern than those capacitated with the NO donor ($p < 0.05$) when quantifying the signal corresponding to the whole WB lane. Moreover, we observed a specific phosphorylation pattern for two PKA substrate species, ~ 87 and ~ 62 kDa, which showed a higher degree of phosphorylation in the presence of S-Nitrosoglutathione ($p < 0.05$). The inhibitory effect on PKA activity when blocking NO synthesis was again evident in the ~ 87 and ~ 62 kDa species.

Our data showed that the presence of L-Arginine had no significant effect when analyzing the signal corresponding to the whole WB lane. However, similarly to the experiment where L-Arginine was not used, the ~ 62 kDa species showed a lower amount of phosphorylation when using NOS inhibitors ($p < 0.05$).

These effects were not observed under non-capacitating conditions.

Limitations, reasons for caution: The number of samples is small and should be increased. Also, the study should include infertile men in the future.

Wider implications of the findings: We identified specific PKA substrates such as the species of approximately 87 and 62 kDa, which show a distinct Serine and Threonine phosphorylation pattern. These bands might include key proteins in modulating the events downstream of NO-mediated signaling and could be differently regulated in infertile men.

Trial registration number: Not applicable.

P-067 Determination of aneuploidy in spermatozoa by flow cytometry and its relationship on reproductive results

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Study question: Is it possible to link the determination of semen aneuploidy by flow cytometry to the reproductive results of IVF?

Summary answer: The degree of semen aneuploidy is not related to reproductive outcomes and should not be routinely included before IVF.

What is known already: Previous studies have confirmed an inverse correlation between aneuploidy in sperm and conventional seminal parameters. Furthermore, the presence of aneuploidy in sperm is associated with failed spermatogenesis, and may cause oligospermia or azoospermia. The study of semen aneuploidy can be performed quickly and sensitively by flow cytometry. This technique allows to determine sperm subpopulations with different DNA contents and, therefore, with different aneuploidy levels. This methodology analyzes a greater number of cells than in a conventional FISH and takes into account the entire DNA content of the cell.

Study design, size, duration: This is a prospective cohort study of 481 couples who have undergone a first IVF cycle with embryo transfer between 2014 and 2017. The patients underwent a semen analysis and a study of sperm DNA using propidium iodide staining (PI/RNase, BD Pharmingen) by flow cytometry (MACSQuant Analyzer, Miltenyi Biotec).

Participants/materials, setting, methods: Sperm populations were classified according to fluorescence intensity as: subhaploid, haploid, diploid and polyploid. A univariate analysis has been carried out in which the different results obtained from the sperm analysis and the study of ploidies have been compared according to the reproductive results (positive / negative): clinical pregnancy, ongoing pregnancy and live birth. The statistical analysis were carried out by Student's t-tests, considering $p < 0.05$ as statistically significant.

Main results and the role of chance: No statistically significant differences were observed in the levels of subhaploidies between patients with positive and negative reproductive results for clinical pregnancy (35.0% vs. 65.0%, $p = 0.77$), ongoing pregnancy (22.5% vs. 77.5%, $p = 0.79$), or live birth (22.2% vs. 77.8%, $p = 0.83$). There were also no differences in the haploidy rate (35.0% vs. 65.0%, $p = 0.45$, 22.5% vs. 77.5%, $p = 0.21$, 22.2% vs. 77.8%, $p = 0.25$); nor in the diploidy rate (35.0% vs. 65.0%, $p = 0.42$, 22.5% vs. 77.5%, $p = 0.29$, 22.2% vs. 77.8%, $p = 0.32$). However, in the polyploidy percentage, statistically significant differences were detected for ongoing pregnancy and live birth (35.0% vs. 65.0%, $p = 0.21$, 22.5% vs. 77.5%, $p = 0.01$, 22.2% vs. 77.8%, $p = 0.02$), as the percentage of polyploidies was somewhat higher in the group of pregnant patients, but without clinical relevance.

Limitations, reasons for caution: The population included in this study was part of infertile couples at their first IVF cycles; caution should be exerted when extending these results to other populations with a longer infertility history.

Wider implications of the findings: The degree of semen aneuploidy does not seem to be related to reproductive outcomes; a more conservative approach to semen analysis seems to be best suited for routine assessment before IVF.

Trial registration number: None.

P-068 Characterization of somatic testicular cells in fetal, prepuberal and adult testis. The role of StAR in Leydig cells

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Study question: Is StAR protein present in somatic cells of male fetal gonads in the first trimester?

Summary answer: No StAR expression in Leydig cells from 9-10 weeks of gestation, whereas a weak expression at 12 weeks and high expression at 14 weeks.

What is known already: In humans, StAR protein is known to be express in adult Leydig cells, but also in Leydig cells of fetal testes from 14.5-19 gestational weeks, however there is no data about the expression of StAR in first trimester fetal male gonads.

Study design, size, duration: We collected fetal, prepuberal (Klinefelter Syndrome patients) and adult testis samples to perform immunofluorescence for studying the presence of specific testicular somatic cell markers as StAR, GATA4 and SOX9 during the human development of the male gonads.

Participants/materials, setting, methods: Human fetal samples from 9 to 21 weeks postconception (wpc) were obtained from Leiden Universitary Medical Center (LUMC) (The Netherlands). Human prepuberal patient samples diagnosed with Klinefelter Syndrome and human adult samples diagnosed with azoospermia were obtained from Cruces University Hospital (Spain). Oral and written information was given and informed consent was obtained from all patients. We performed immunofluorescence for detecting VASA,OCT4, SOX9, GATA4 and StAR markers on paraffin sections of the samples previously mentioned.

Main results and the role of chance: StAR expression in male fetal gonads, there was no presence of StAR at 9 and 10 weeks of gestation, however, a weak expression is observed at week 12, being very significant at week 14 up to 21 weeks. As expected, StAR expression only appeared between the seminiferous tubules, in the interstitial space, where Leydig cells are located. GATA4 was clearly present in the Sertoli cells but also in some interstitial cells. SOX9 expression, it was present only in the nucleus of Sertoli cells.

In testis of Klinefelter Syndrome prepuberal patients, StAR expression is in Leydig cells with a spotty pattern. GATA4 was express in somatic cells inside the seminiferous tubules, but also in cells between tubules.

SOX9 expression is present in the nucleus of somatic cells inside the tubules.

Adult patients were separated in two different groups, depending on the presence or lack of spermatozoa (azoospermia). In general there was no significant difference between both groups and was detected StAR protein in the interstitial cells with spotty pattern. GATA4 positive cells were inside seminiferous tubules and in the interstitial space. SOX9 expression was observed only nuclear staining in Sertoli cells.

Limitations, reasons for caution: Due to the limited and difficulty in the obtention of material used in this study, we used few samples of gonads from several gestational stage and few samples of prepuberal testis.

Wider implications of the findings: The results obtained in this study will help to understand the early development of the fetal somatic cells, especially the Leydig cells and the role of StAR protein in steroidogenesis during this fetal development.

Trial registration number: NONE.

P-069 Association of the sperm telomere length and telomerase activity with semen quality and fertilization in IVF

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Study question: Whether the sperm telomere length(STL) and sperm telomerase activity(STA) are related to semen quality and pregnancy outcomes in IVF?

Summary answer: The STA gradually decreased with age. Better semen quality and fertilization outcomes are observed in patients with longer STL or higher levels of STA.

What is known already: Telomeres are functional DNA-protein structures located at the end of the chromosome and maintain the chromosome stability as well as genome integrity. Telomerase is an critical enzyme for maintaining the telomere length(TL). The sperm TL(STL) has been reported to be associated with idiopathic male infertility and early embryo development in IVF.

Study design, size, duration: 42 samples from men with normal semen examination were collected and divided into the group A(≤36 years old, $n = 16$), group B(36-40 years old, $n = 12$), group C(41-45 years old, $n = 7$) and group D(≥45 years old, $n = 7$).

Participants/materials, setting, methods: The STL was measured using quantitative real-time PCR and STA was detected by a modified quantitative-telomeric repeat amplification protocol assay.

Main results and the role of chance: STA levels differed significantly among four groups($P = 0.010$). The STA was highest in group B(between 0.009 to 0.33) and decreased in group A(between 0.002 to 0.05), group C(between 0.005 to 0.029) and group D(between 0.001 to 0.011). No obvious differences were observed in the STL, sperm density, fertilization rate, normal fertilization rate, available embryos or good quality embryos among these groups($P > 0.05$). Sperm density was positively correlated to the STL($r = 0.325$, $P = 0.044$) in our study population($n = 42$). The non-progressive sperm motility was negatively correlated to STL(r=-0.361, $P = 0.024$) and STA(r=-0.318, $P = 0.033$). The normal IVF fertilization rate was positively correlated to STL($r = 0.365$, $P = 0.021$). There were no significant differences in STL or STA between the pregnant samples and non-pregnant samples($P > 0.05$).

Limitations, reasons for caution: Our sample size is not that large enough to see the significant differences of STL among four groups. Further studies with larger sample size are needed to explored the differences of STL as well as other clinical features among these groups.

Wider implications of the findings: Patients with longer STL or higher levels of STA might have better semen quality and fertilization outcomes in IVF. Men who aged over 46 are more likely to have a very low level of STA.

Trial registration number: Not applicable.

P-070 Effect of leucospermia on sperm parameters and Intracytoplasmic Sperm Injection outcomes

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Study question: Is there an association between seminal leucocytes levels, sperm parameters and ICSI outcomes in a large population of infertile patients?

Summary answer: Leucocytospermia had a harmful effect on sperm parameters, including sperm concentration, morphology and chromatin maturity of spermatozoa, but do not influence ICSI outcomes.

What is known already: Leucospermia is highly prevalent among infertile patients even those with negative semen culture. The role played by leukocytes in the human sperm is not totally understood. Activated leucocytes generate excessive reactive oxygene species (ROS) which have been negatively correlated with fertility and semen quality. In fact, there is consensus that leucocytospermia, defined by leukocytes exceeding 1×10^6 cells/mL, impaires sperm quality. In the other hand, there is still much controversy about its correlation with ICSI outcomes.

Study design, size, duration: Prospective observational study including 157 patients who underwent ICSI cycles between February and May 2017, in a university hospital. Patients were divided into two groups according to sperm leucocytes levels: Group 1 ($n = 33$) ≥ 1×10^6/ml and Group 2 ($n = 124$) < 1×10^6/ml.

Participants/materials, setting, methods: Only patients with negative semen culture were included in our study. Patients with azoospermia or cryptozoospermia were excluded. Sperm parameters were evaluated according to World Health Organization 2010 guidelines. The peroxidase method was used to quantify leucocytes, when round cell concentration exceeded 1×10^6/ml on fresh semen. Sperm chromatine condenstation was assessed by aniline blue staining.

The main parameters compared between the two groups were sperm parameters(volume, concentration, motility, morphology and chromatin maturity) and ICSI outcomes.

Main results and the role of chance: The mean age of the patients was comparable in groups 1 and 2 respectively (39.21 years VS 40.86; p = 0.1445). The clinic and paraclinic characteristics, the stimulation parameters and the ovarian response, were similar in both groups.

The number of leucocytes had a statistically significant negative influence on sperm concentration, morphology and sperm maturity when comparing groups 1 and 2 respectively (30,5 ± 26,0 VS 40,5 ± 37,7; p = **0.02); (**3,3 ± 1,0 % VS 4,6 ± 1,7 % p = **0.00); (**57,6 % ± 14,1 % VS 73,2 ± 11,8%; p = **0.0001)**

In contrast, sperm volume and progressive motility were comparable in both groups.

In the other hand, leucospermia has not significantly influence ICSI outcomes. The fertilization rate and the cleavage rate were comparable between the two groups (81.22 VS 83.82%, p = 0.59); (91.37 vs 95.66%, p = 0.25). We also didn't found any significant difference in top embryo rate and blastulation rate. The pregnancy rate were also comparable (37.03 VS 44.76%; p = 0.51).

Limitations, reasons for caution: The observational character of the study, and the reduced size of the samples studied constitute major limitations. In addition, measurement of sperm leucocytes in routine semen analysis appears to be of little prognostic value with regard to male fertilizing potential.

Wider implications of the findings: Further studies are needed to define other factors that increase the risk of sperm damage through elevated number of leucocytes in semen.

Trial registration number: Not applicable.

P-071 Lifestyle and semen variables: a prospective cohort study of men referring to an Italian Fertility Clinic

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Study question: We designed a prospective cohort study to investigate the relation between lifestyle and semen quality in men of subfertile couples undergoing assisted reproductive procedures.

Summary answer: In our study moderate alcohol intake was related to a beneficial effect on semen quality in male partners of infertile couples undergoing ART cycles.

What is known already: Although the causal link between environmental factors and impaired male fertility is still weak, there is growing evidence

suggesting that semen quality may be influenced by lifestyle habits; in particular, smoking, overweight, physical activity, dietary factors and alcohol intake have been suggested to play an important role but evidence is not always consistent. According to a recent meta-analysis of 15 cross-sectional studies, an occasional consumption does not adversely affect semen parameters, whereas daily consumption has a negative association with semen volume and normal morphology. However, these findings could not be controlled for confounders such as smoking and age.

Study design, size, duration: From September 2014 subfertile couples, presenting for evaluation to the Fertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore, Policlinico, Milan, and eligible for assisted reproduction technologies (ART), were invited to participate to an ongoing prospective cohort study on the role of lifestyle habits and diet on ART outcome. The present study reported exclusively on evidence obtained from the male partner. From September 2014 to December 2016, 327 men were enrolled.

Participants/materials, setting, methods: Men of couples who agreed to participate were interviewed to obtain information on: BMI, smoking, caffeine intake, occupational and leisure physical activity (PA) and usual alcohol intake. Information on diet were obtained using a validated food frequency questionnaire (FFQ). Couples were interviewed on the day of oocyte retrieval. On the same day, a semen sample was collected and analyzed to proceed with ART. Semen volume, total sperm count and sperm concentration and motility, were determined.

Main results and the role of chance: We distinguished 3 categories of alcohol intake: a low intake group, who drank ≤ 4.3 g of alcohol per day; an intermediate intake group, who drank 4.4-13.3 g per day; a high intake group, who consumed ≥13.3 g per day. At univariate analysis, sperm volume was significantly associated with age, alcohol intake and leisure PA; sperm concentration with a history of ROD (reproductive organ diseases); total sperm count with a history of ROD and alcohol intake; sperm motility with age, history of ROD and leisure PA. We analyzed the association between alcohol intake and semen variables in a general linear model including age, history of ROD, leisure PA (associated with at least 1 semen parameter), smoking status, caffeine consumption, calories intake (associated with alcohol intake). After accounting for these variables, alcohol intake showed a significant beneficial relation with semen volume, total sperm count and sperm concentration. In analyses performed in strata of age, ROD and leisure PA showed that the positive nonlinear association between alcohol and semen volume was consistently observed in all subgroups, whereas the significant effects on concentration, total count and motility were limited to men who performed less than 2 hours per week of leisure PA.

Limitations, reasons for caution: Study limitations: findings should be referred only to patients of infertile couples, information regarding alcohol use was self-reported. Study strengths: large sample size, analysis of the role of alcohol in men with or without other conditions associated with infertility, adjustment for potential biases reported to be associated with semen quality.

Wider implications of the findings: A moderate alcohol intake exerts a beneficial effect on semen quality. These findings are in line with results of a recent meta-analysis, supporting that opposite effects might be exerted by alcohol on semen parameters and according to the amount consumed. The mechanisms underlying these effects need to be further investigated.

Trial registration number: No trial registration number needed.

P-072 Clinical predictors for selection of surgical sperm retrieval techniques in non-obstructive azoospermia patients

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Study question: Can the clinical findings of patients with non-obstructive azoospermia (NOA) aid in selecting the most appropriate surgical sperm retrieval (SSR) method prior to invitro fertilization (IVF)?

Summary answer: Clinical findings such as testicular size, initial and post-treatment hormone levels and testicular tissue histopathology can aid in selecting the sperm retrieval technique.

What is known already: Testicular sperm retrieval has been practiced over the past few decades as a mean of providing patients with NOA the chance to father their biologic children through IVF. While microsurgical testicular sperm extraction (mTESE) yields higher SSR rates when compared to testicular sperm extraction (TESA), it can also be considered a more invasive procedure with, at times, detrimental effects on testicular spermatogenic and endocrine functions.

Study design, size, duration: This was a retrospective study of 439 patients presenting with NOA who underwent surgical sperm retrieval at a tertiary medical center over a period of 5 years. Data regarding patient demographics, clinical findings, serum hormone levels before and after medical stimulation therapy, chromosomal abnormality, type/outcome of the sperm retrieval procedure and histopathology results was collected.

Participants/materials, setting, methods: All participants received hormonal stimulation prior to a staged SSR procedure starting with TESA (needle aspiration biopsy technique) and progressing to mTESE when no sperm were retrieved after 4 aspirations from each testicle. Collected variables were examined against the sperm retrieval outcome and the method of SSR utilized. X2 and Kruskal-Wallis tests were used to analyze categorical and numerical values, respectively. A p value < 0.05 was considered statically significant.

Main results and the role of chance: Overall, SSR rate was 42.1%. Sperm were retrieved through TESA and mTESE from 99(22.5%) and 140(42.6%) patients respectively. Patients with a positive SSR were divided into 2 groups according to the SSR method used. Testes size, initial LH and FSH levels, post-treatment LH, FSH and testosterone levels and histopathology results significantly varied between the study groups (table 1). Patients with a positive sperm retrieval through TESA had a statistically significant larger right and left testicular size, lower initial and posttreatment LH and FSH levels, and higher posttreatment testosterone levels. The SSR rate with mTESE was higher than TESA in all testicular histopathologies ($p < 0.001$). However, the highest SSR rate in patients who underwent TESA was found in hypospermatogenesis, followed by maturation arrest, tubular atrophy and Sertoli cell only syndrome.

Table I

	TESA +ve (n = 99)	mTESE +ve (n = 140)
Age	37.6 ± 0.8	36.6 ± 0.6
Left testis size	8.8 ± 0.5*	6.8 ± 0.4*
Right Testis Size	9.6 ± 0.6*	7.2 ± 0.4*
(i)Testosterone	15.9 ± 0.7	16.2 ± 0.8
(i)LH	4.4 ± 0.2**	7.5 ± 0.5**
(i)FSH	8.1 ± 0.7**	14.8 ± 1.1**
(i)Estradiol	104.1 ± 4.1	121.7 ± 5.4
(t)Testosterone	23.3 ± 3.6*	17.3 ± 1.04*
(t)LH	8.1 ± 1.4	10.8 ± 0.8
(t)FSH	11.4 ± 1.1	19.9 ± 1.5
(t)Estradiol	134.7 ± 10.8	213.8 ± 87.1
Histopathology		
Hypospermatogenesis	36.4%**	41.5%**
Maturation arrest	15.2%**	29.6%**
Sertoli cell only	4.6%**	30.2%**
Atrophy	14.8%**	51.8%**
Abnormal Karyotype	11.5%	34.6%
Y-chromosome microdeletion	8.3%	8.3%

(*pvalue<0.05, **pvalue<0.01); (i)initial, (t)post-treatment

Limitations, reasons for caution: This was a single center study. Results need to be validated across different centers and study population.

Wider implications of the findings: It is important to recognize the predictors for successful SSR in different SSR methods to aid in selecting the most appropriate technique that can be effective and at the same time least invasive.

Trial registration number: NA.

P-073 Association between blood metal concentrations and human semen quality: a cross-sectional study in Hong Kong

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Study question: Does heavy metal exposure, including essential and non-essential heavy metals, associated with the decline of semen quality and sperm function?

Summary answer: Although high blood lead and mercury level may be associated with poor semen quality, heavy metal exposure may not affect the sperm fertilisation function significantly.

What is known already: Exposure to metals, including essential and non-essential elements, is widespread and may be associated with altered semen quality. However, evidence from previous reports was contradictive. Moreover, there was lack of investigation regarding the effect of heavy metal exposure on sperm function.

Study design, size, duration: This is a cross-sectional study, which included 298 men who underwent routine fertility assessment between November 2015 and June 2016 in Hong Kong.

Participants/materials, setting, methods: Blood and semen samples were collected from participants at the teaching hospital of the Chinese University of Hong Kong. Routine semen analysis and sperm functional test (sperm vitality, sperm DNA fragmentation and acrosome-reaction test) were performed. Thirteen metals (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, and Zn) were measured in whole blood samples by inductively coupled plasma mass spectrometry. Multivariable logistic regression models were employed in data analysis.

Main results and the role of chance: After adjusting for age, abstinence time, current cigarette smoking habits and alcohol drinking habits in the multiple logistic regression analyses, we found blood lead level was significantly and negatively associated with sperm motility (Ptrend = 0.048). Moreover, we found significant dose-dependent trends of blood lead and mercury quartiles with sperm DNA fragmentation (Lead: Ptrend = 0.006; Mercury: Ptrend = 0.009). Furthermore, we found significantly dose-dependent trends of increase blood selenium with increase sperm morphology (Ptrend = 0.001). There were no significant associations between blood metal level and sperm vitality and acrosome reaction after adjustment for multiple demographic and lifestyle factors.

Limitations, reasons for caution: Our study is a cross-sectional study, the association found in our study is not causation between heavy metal exposure and semen quality, which is a common limitation to cross-sectional studies.

Wider implications of the findings: Environmental exposure to lead may be associated with lower sperm motility, and exposure to lead and mercury may associate with higher sperm DNA fragmentation; whereas selenium may have a protective effect for sperm morology. However, heavy metal exposure may not significantly affect the sperm fertilisation function.

Trial registration number: N/A.

P-074 Assessment of the impact of CryoFlex tubing on sperm cryopreservation outcome

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Study question: Does wrapping cryovials with CryoFlex tubing prior to immersion in liquid nitrogen (LN) impact sperm cryopreservation outcome and the post-thaw recovery of motile sperm?

Summary answer: The use of CryoFlex tubing can negatively impact the outcome of sperm cryopreservation. The tubing should be removed prior to thawing to prevent such outcome.

What is known already: Cryovials are widely used to store cryopreserved semen samples. Nevertheless, they are not considered to provide effective biocontainment. Thus, LN penetration in addition to contamination of samples and LN tanks remain a concern. It has been suggested that wrapping cryovials in heat-shrinkable tubing prior to immersion in LN could reduce these risks. The tubing is made of a safe plastic resin polymer, however it has a poor temperature capability, which could affect cryopreservation-thawing protocols. It is not yet known if the use of CryoFlex tubing will impact the outcome of sperm cryopreservation, specifically, cryosurvival rates.

Study design, size, duration: Semen samples (n = 34) were included in this cross-sectional study. Following dilution with cryoprotectant media, each sample was split into 2 equal aliquots: 1) sample with CryoFlex wrapping, and 2) control without CryoFlex wrapping. The CryoFlex tubing remained wrapped during the thawing in 20 samples; while it was removed prior to thawing in 14 samples. All trials were conducted over the course of 3 months.

Participants/materials, setting, methods: Samples were provided by healthy donors. All aliquots were cryopreserved in vials with internal threading and silicon gasket (Nunc CryoTubes, Thermo Scientific). Heat-sealable wrapping (Nunc CryoFlex, Thermo Scientific) was applied to the sample aliquots, vials without wrapping served as controls. Thereafter, aliquots were placed in liquid nitrogen vapor for 20 minutes followed by plunging in liquid nitrogen. Thawing was performed after a minimum of 24 hours by immersion in 37 C water bath for 4 minutes.

Main results and the role of chance: Cryosurvival rates were calculated by dividing total motile sperm post-thaw by total motile sperm pre-freeze X 100. Statistical analysis was conducted using paired t-test with two-tailed test of significance. In samples thawed with the CryoFlex wrapping, the post-thaw sperm percentage motility and cryosurvival rates were significantly lower compared to controls (26.4 ± 6.1 vs. 30.9 ± 8.2, P = 0.003 and 60.6 ± 11.7 vs. 70.2 ± 12.8, P = 0.007, respectively). In samples where CryoFlex wrapping was removed prior to the thawing, no significant differences were noted in post-thaw sperm percentage motility and cryosurvival rates (32.1 ± 12.9 vs. 32.9 ± 12.8 and 69.0 ± 20.4 vs. 71.0 ± 12.5, respectively). Overall, the results showed that whenever the CryoFlex tubing is kept wrapped around the vials during the thawing process, a significant decline in the recovery of cryopreserved spermatozoa post-thaw will occur. No such decline was seen when the CryoFlex tubing was removed prior to thawing.

Limitations, reasons for caution: Our results are solely based on the evaluation of semen samples from healthy donors with normal semen parameters using a single cryopreservation protocol. It cannot be assumed that semen samples with lower quality parameters or the use of other cryopreservation protocol will yield with the same results.

Wider implications of the findings: This pilot provides new insights into the impact on CryoFlex tubing on sperm cryopreservation outcome. While it may alleviate some of the safety concerns associated with the use cryovials in sperm cryopreservation, CryoFlex can also negatively impact the outcome of sperm cryopreservation unless removed prior to thawing.

Trial registration number: Not applicable.

P-075 Nrf2 signaling in experimental cryptorchid mice

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Study question: Whether Nrf2 signaling activates in experimental cryptorchid mice?

Summary answer: The Nrf2 signaling fails to develop in the cryptorchid testis.

What is known already: The nuclear factor erythroid 2 (Nrf2), a transcription factor, involve in cellular protection against oxidative stress. During cryptorchidism, heat stress induces destructive oxidative stress in testis by following deleterious impact on gonadal function which finally can result in decreasing sperm quality and infertility. Several studies report that Nrf2 plays an important role in inhibition the progress of oxidative stress in spermatogenesis. It has shown that the expression level of Nrf2 target genes decrease in men which suffer from low sperm motility. These findings spurred our interest to investigate the role of Nrf2 signaling in experimental cryptorchidism mice.

Study design, size, duration: 48 male mice at the age of 6 weeks were used. Cryptorchidism was induced by pushing left testis via inguinal canal into the abdominal cavity and fixing the testis to the abdominal wall, then gubernaculum was cut and inguinal canal was closed. After cryptorchidism-induction, mice were scarified on one day, one, four and eight eight weeks post operation. Sperm parameters and the expression of Nrf2 target genes such as HO1 and NQO1 were assessed.

Participants/materials, setting, methods: Sperm parameters including count, motility, morphology and viability were analyzed. Total RNA was extracted from the left testis of each mouse. The cDNAs were synthesized. For real time PCR reactions, primers were adapted from other primers (designed by the NCBI website). The quality of each reaction was confirmed by melting curve analyses. Efficiency was determined for each gene using a standard curve. For each sample, the target genes were normalized to a reference gene.

Main results and the role of chance: According to our statistical analysis, in all groups sperm parameters were reduced after cryptorchidism induction. The expression levels of studied genes were time dependent. One day and one week after surgery had similar gene expression pattern, except about the Keap1 expression (no change one day after surgery and decrease one week after cryptorchidism). In the mentioned time, the expression levels of Nrf2 and NQO1 were raised significantly ($p \leq 0.05$), but the level of HO1 expression declined ($p \leq 0.05$). Two weeks after cryptorchidism induction, Nrf2 expression level increased significantly, but the expression of Keap1 and HO1 significantly decreased ($p \leq 0.05$) and the expression of NQO1 showed any changes. Four and eight weeks after surgery, we could not detect any changes at the level of mRNA expression of the studied genes.

Limitations, reasons for caution: The limitation of research is that only gene expression is not strong tool for assessing the signaling and it needs supplementary studies.

Wider implications of the findings: In this study for the first time we showed Nrf2 signaling is activated during cryptorchidism, but it is not enough strong to prevent deleterious effect on the testis tissue.

Trial registration number: N/A.

P-076 Prevalence of Mycoplasma genitalium among infertile men and sperm donors consulting in a French center for Assisted Reproduction

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Study question: Is the prevalence of *Mycoplasma genitalium* (MG) higher in infertile men than in sperm donors? Does MG affect semen parameters?

Summary answer: MG prevalence in infertile men is significantly lower than in fertile men. No significant impact of MG on sperm parameters was evidenced.

What is known already: *Mycoplasma genitalium* is a bacterium, hard to isolate. Its prevalence is estimated between 1% and 3% in the general population. It is likely to cause a sexually transmitted infection and is responsible for 10 to 25% of non gonoccocal urethritis. *Mycoplasma genitalium* infection is usually asymptomatic. The impact of this emerging bacterium on semen parameters is still unknown as well as its role in male infertility.

Study design, size, duration: This study included 5208 infertile men and 162 sperm donors consulting between March 2012 and December 2016 in our Reproduction Center (Cochin hospital, AP-HP). A systematic search for *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Mycoplasma genitalium* was performed on the first urinary stream by a highly sensitive and specific PCR technique (Test Dx CT/NG/MG®, Bio-Rad). This testing was systematically associated to semen parameters analysis.

Participants/materials, setting, methods: Prevalence of MG was calculated for each sub-group. Concomitant ejaculated semen was analyzed according to the 2010 WHO references. Semen parameters (sperm numbers, vitality, progressive motility and leucospermia) of infertile infected with MG were compared to those of infertile men free of any infection and matched according to the age, the abstinence period and date of analysis, using a student test.

Main results and the role of chance: The prevalence of MG was significantly lower in men consulting for infertility than men willing for sperm donation, already fathers (58/5208 1.1%, versus 7/162 4.3% $p < 0.01$). This prevalence among infertile men is similar to that reported in literature among the general population. The proportion of Chlamydia trachomatis infection was comparable in both populations (2/162, 1,2% versus 26/5208, 0,5% $p > 0,5$). Four of the 56 men consulting for infertility with a positive detection of MG (7.1%) exhibited genital-urinary symptoms. No significant difference was found between the sperm parameters of infertile men with or without MG consulting in our university center considering total sperm count (M) 162.6 ± 182.0 for MG+ versus 188.7 ± 215.5, $p > 0.5$, progressive motility (%) 34.6 ± 15.0 versus 34.4 ± 16.5 in MG-, $p > 0.5$, vitality 56.8 ± 15.8 versus 58.4 ± 17.3, $p > 0.5$, or leucospermia (M/mL) 0.8 ± 2.4 versus 0.4 ± 1.1, $p > 0.5$.

Limitations, reasons for caution: The semen parameters of infertile men with a positive PCR for *Mycoplasma genitalium* were not systemically controlled after an efficient antibiotic treatment to evidence a possible improvement of the parameters and eventually the pathogenic role of *Mycoplasma genitalium* on male fertility.

Wider implications of the findings: These findings need to be confirmed taking into account the prevalence and impact of *Mycoplasma genitalium* among the female partners of these patients and the outcomes of Assisted Reproduction therapies in couples carrying MG.

Trial registration number: Not applicable.

P-077 Euploid rate of embryos derived from aspirated and ejaculated sperm

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Study question: What is the euploid rate relationship between aspirated and ejaculated sperm-sourced embryos?

Summary answer: TESE sperm may be more effective to treat male patients with azoospermia.

What is known already: Micro-surgical sperm aspiration procedures TESE (testicular sperm aspiration) and PESA (Percutaneous Epididymal Sperm Aspiration) followed by ICSI and PGS are all suitable approaches for treating azoospermia (Weng, 2014). The choice of surgical procedure is based on the cause of azoospermia: non-obstructive azoospermia (NOA) or obstructive azoospermia (OA). NOA often requires extracting testicular spermatozoa, which are fragile, non-motile, and have not undergone chromatin condensation. OA can be treated with a less invasive procedure to extract sperm from the epididymis, which are more durable, mostly motile, and have completed or are currently undergoing meiotic maturation.

Study design, size, duration: This is a retrospective cohort study that spans from 1/2016 until 12/2017. Overall 992 embryos were biopsied and analyzed for this study. High quality blastocyst embryos exhibiting tightly bound inner

cellular mass (ICM) and good quality trophectoderm were subsequently biopsied. Trophectoderm biopsy and PGS screening were performed on Day 5, 6 and 7 embryos. Chi-square statistical analysis was performed to determine significant differences between groups.

Participants/materials, setting, methods: Trophectoderm biopsy and PGS screening were performed on Day 5, 6 and 7 of high quality blastocyst embryos. Overall 992 embryos were biopsied (751 ejaculated sperm, 114 TESE, and 127 PESA sperm-sourced embryos). Biopsied embryos were segregated into based on sperm source: ejaculated sperm, TESE, or PESA. Biopsied samples were analyzed using next generation sequencing (NGS) to determine euploid status. Embryos were vitrified using Vit Kit (Irvine Scientific) for subsequent transfer.

Main results and the role of chance: Euploid rates per group were 50% (374/751) for ejaculated sperm, 57% (65/114) for TESE and 43% (54/127) for PESA. Chi-square statistical analysis displayed that PESA sperm embryos resulted in significantly lower euploid rate when compared to TESE sperm rates ($p = 0.0246$). Ejaculated sperm euploid rate was not found to be significantly different between TESE and PESA. Complex abnormal rates were 9% (18/196) for ejaculated sperm, 7% (5/67) for TESE, 14% (15/104) and for PESA; these results were not significantly different from each other. Embryos sourced from testicular sperm resulted in a significantly higher euploid rate than those from epididymal sperm. These data suggest that, in some patients, maturation of sperm through the epididymis may alter DNA during chromatin condensation. This, in turn, can cause an abnormal random assortment during fertilization causing a potential higher propensity of aneuploidy in the developing embryo. Our findings suggest that sperm retrieved prior to further maturation in the epididymis may be more effective to treat male patients with azoospermia.

Limitations, reasons for caution: This study was limited by not grouping age of female patient providing the oocytes, which can impact euploid rate. It also does not provide specific reasons for requiring aspirated sperm.

Wider implications of the findings: Literature suggests that ejaculated sperm would have a higher euploid rate than that of aspirated sperm and no difference between TESE and PESA. This data completely contradicts published literature in that TESE sperm had a significantly higher euploid rate than that of PESA.

Trial registration number: not applicable.

P-078 Is human semen more sensitive than blood for assessing environmental impact on health? EcoFoodFertility Project

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Study question: The aim of this study is to evaluate if human semen can be considered more than blood early biomarker of environmental pollution exposure and useful for innovative prevention program.

Summary answer: Semen RedOx status, motility, DFI and sperm telomere length (STL) can be considered as an early markers of environmental pollution, more sensitive than blood.

What is known already: High environmental pressure may impair male infertility by affecting semen quality, but the real effects remains controversial. Some studies have supposed that specific factors present in some areas, but not in other, might cause a reduction of semen quality. In a previous EcoFoodFertility study, we have demonstrated that sperm DNA fragmentation is significantly higher in men who live in high environmental polluted areas respect of those who live in low polluted areas and, others studies, have hypothesized that air pollution could be affects the length of sperm telomeres.

Study design, size, duration: Pilot biomonitoring study (EcoFoodFertility Project) conducted in Campania (Italy) on blood and semen of clinically healthy men living in the 'Land of Fires" (High Environmental Impact-HEI) and, as control group, in 'Alto-Medio Sele" (Low Environmental Impact-LEI), to assess environmental impact on fertility and human health. 220 men were recruited, from July to December 2015, for analysis of: 22 trace elements, sperm DNA fragmentation (DFI), total antioxidant capacity (TAC), antioxidant enzyme activity and telomere length (TL).

Participants/materials, setting, methods: Partecipants were divided into two groups:

Group A: 110 males from HEI ($n = 60$) and LEI ($n = 50$);

Group B: 112 males from HEI ($n = 57$) and LEI ($n = 55$).

In group A 22 trace elements were analyzed in blood and semen by optical emission spectrometry, DFI by Sperm Chromatin Dispersion test, TAC by Glutathione peroxidase/reductase (GPX-GSR).

In group B telomere length (TL) was assessed by quantitative Real-Time PCR on genomic extracted from leukocytes (LTL) and sperm (STL).

Main results and the role of chance: In Group A, HEI subjects showed significantly higher values ($p < 0.05$) for Al, Mn, Cr, Mg, Li, Co, Ca in blood, as well as for Cr, Cu, Zn in semen, while Fe was lower in semen ($p < 0.05$). Immotile sperms and DFI were both higher ($p < 0.026$ and $p < 0.01$ respectively) in HEI-group. TAC in blood showed no differences, while TAC, GPX and GSR in the seminal plasma were significantly lower in the HEI-group ($p < 0.05$). In group B, a significant negative correlation was found between age and LTL ($r=-0.024$, $p = 0.01$), as aspected, but no correlation was found between STL and age ($p = 0.7$). No significant LTL differences was observed between low and high exposure groups (0.99 ± 0.33vs 0.90 ± 0.38, $p = 0.2$), while STL was significantly higher in HEI-group compared to LEI-group (1.15 ± 0.51vs 0.90 ± 0.26, $p = 0.04$). Age-adjusted analysis confirmed a higher STL in HEI-group compared to control group LEI (1.10 ± 0.36vs 0.90 ± 0.32, $p = 0.05$). When STL was analyzed according to the 75th percentile distribution, HEI-group emerged as a significant risk predictor of longer STL (Odds Ratio: 3.1, 95% confidence intervals: 1.1-10.2; $p = 0.02$).

Limitations, reasons for caution: Several limitations need to be considered: no direct ambient measures of air pollution were available and some of the differences in the associations could be due to the relatively small sample size of the groups. Even if analysis were adjusted for potential covariates, we cannot exclude other unidentified confounding factors.

Wider implications of the findings: Semen RedOX status, DFI and STL can be considered as early markers of environmental pollution and human semen seemed a more early sensitive source of biomarkers than blood to monitor high environmental pressure on human health, hence useful for innovation prevention programs and health surveillance, especially in risk areas.

Trial registration number: This work was supported by institutional funds.

The authors declares.

Not required because it is a pilot biomonitoring observational study.

P-079 Oxytocin expression in spermatozoa is positively associated with their motility, whereas expression of oxytocin receptor is negatively associated with their morphology

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Study question: Is oxytocin (OXT) and oxytocin receptor (OXTR) mRNA expression in spermatozoa different between normal sperm samples and samples with at least one abnormal parameter?

Summary answer: Compared to normal sperm samples, OXT mRNA expression is significantly lower, whereas OXTR mRNA expression is significantly higher in abnormal samples (at least one parameter).

What is known already: OXT is synthesized in the penis, testis, epididymis and prostate, whereas its receptor has been detected throughout the male reproductive tract. Furthermore, OXT promotes the conversion of testosterone into dihydrotestosterone. During orgasm, a burst of OXT is released into the systematic circulation to stimulate contractions of the reproductive tract. Various studies have shown that exogenous administration of OXT increases the spermatozoa concentration of ejaculated sperm. OXT seminal plasma levels have been shown to be lower in fertile men compared to men with oligoasthnoteratozoospermia. However, OXT and OXTR has never been assessed in spermatozoa.

Study design, size, duration: A prospective study was performed in 2016-2017, including a total of 105 samples for OXT and 70 samples for OXTR. All samples had > $1*10^6$ spermatozoa/ml and <$1*10^6$ leukocytes/ml. Sperm samples' analysis was performed according to WHO 2010 criteria and mRNA was extracted to assess the mRNA expression levels of OXT and OXTR. mRNA expression levels were compared between different categories of sperm samples, classified according to their concentration, total number, motility and morphology.

Participants/materials, setting, methods: All sperm samples were subjected to mRNA extraction, cDNA synthesis and quantitative Real-Time PCR (RT-PCR) for the expression of the OXT and OXTR genes. The relative standard curve method was selected as the most appropriate due to the nature of human spermatozoa. Each sample was run in duplicate and normalized to β2-microtubulin. Differences in mRNA expression levels were analyzed between different sperm categories using the generalized linear model. Values are expressed as median (95% CI).

Main results and the role of chance: Compared to normal samples, samples with at least one abnormal sperm parameter had statistically significant lower OXT mRNA expression levels [5.27(1.69-13.98) vs. 0.93(0.53-1.64), p = 0.019] and higher OXTR mRNA expression levels [0.91(0.42-3.3) vs. 4.79(1.02-8.43), p = 0.000). Compared to samples with total number of spermatozoa >39 × 10^6/ml, oligozoospermic samples had statistically significant higher OXT mRNA expression levels [1.64(0.99-2.94) vs. 0.65(0.24-2), p = 0.002] and lower OXTR mRNA expression levels [3.3(0.86-4.71) vs. 16.03(0.07-63.89), p = 0.047). In addition, compared to samples with normal motility, asthenozoospermic samples had statistically significantly lower OXT mRNA expression levels [1.96(1.15-4.4) vs. 0.4(0.21-0.79), >60% immotile spermatozoa, p = 0.000 and 1.97(1.16-4.6) vs. 0.32(0.09-0.75), progressive motility <32%, p = 0.000]. Furthermore, teratozoospermic samples had statistically significant lower OXT mRNA expression levels [4.19(1.66-9.5) vs. 0.79(0.52-1.59), p = 0.049] and higher OXTR mRNA expression levels [0.94(0.44-3.3) vs. 5.46(1.22-10.16), p = 0.000]. Interestingly, OXT mRNA expression was positively associated with the rapid progressive (a) (spermatozoa with a speed >25μm/sec at 37°C, p = 0.008), the slow progressive (b) (spermatozoa with a speed <25μm/sec at 37°C, p = 0.021) and the total progressive (a + b) motility (p = 0.000), and negatively associated with the percentage of immotile spermatozoa (p = 0.001). On the contrary, OXTR mRNA expression was negatively associated with the percentage of normal forms (p = 0.000) and positively associated with the percentage of head defects (p = 0.01).

Limitations, reasons for caution: Given that the method used for the analysis was the relative standard curve method, it would be interesting to define the absolute levels of OXT and OXTR in spermatozoa and confirm the results with the protein expression of both genes.

Wider implications of the findings: We have shown for the first time that OXT mRNA is downregulated in samples with lower motility and OXTR is upregulated in samples with abnormal morphology compared to normal sperm samples. OXT and OXTR mRNA expression in spermatozoa could be used as novel and unbiased diagnostic tool of male infertility.

Trial registration number: NOT APPLICABLE.

P-080 Paternal age and semen quality: Does the association of those two factors affect the embryo development?

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Study question: Does the association of advanced paternal age and low sperm count affect the embryo development?

Summary answer: The paternal age, sperm count or association of them do not affect blastocyst formation rate or clinical pregnancy rate in ICSI cycles.

What is known already: Many studies exist on the impact of female age on fertility and success of ART. More recently, male age has been highlighted and there are reliable scientific data, which confirm decline of fertility related to advancing male age and in the risk of genetic diseases for the offspring. The age at which men are often classified as advanced age is commonly after age 50 years, although many of the risks continue to increase with age. Semen analysis remains a standard component of a male fertility evaluation, and several studies have reported general decline of semen parameters with advancing age.

Study design, size, duration: We hypothesized the association of advancing paternal age and decreased sperm count can affect the embryo development potential. Then, we developed a retrospective cohort study, which reviewed 3063 ICSI cycles using ejaculated sperm performed during the last 15 years in a private ART center. Semen analysis were performed following World Health Organization manual and controlled ovarian stimulation used standard protocols.

Participants/materials, setting, methods: Cycles were split into four groups according to paternal age and sperm count: (1) young men (age < 50 years) and normal sperm count (≥15 × 10^6 sperm/ml) (Group young-normal, n = 2101); (2) young men (age < 50 years) and low sperm count (<15 × 10^6 sperm/ml) (Group young-oligo, n = 796); (3) ageing men (age ≥50 years) and normal sperm count (≥15 × 10^6 sperm/ml) (Group old-normal, n = 87); and (4) old men (age ≥50 years) and low sperm count (<15 × 10^6 sperm/ml) (Group old-oligo, n = 79).

Main results and the role of chance: Men age varied from 21 to 84 years old (young-normal: 38.4 ± 4.8; young-oligo: 38.4 ± 5.1; old-normal: 54.4 ± 5.4; old-oligo: 56.7 ± 7.0; p < 0.001). Sperm concentration were similar for young-normal (50.0 ± 33.3 million/ml) versus old-normal (52.6 ± 37.6 million/ml; p = 0.490) and for young-oligo (5.9 ± 4.4 million/ml) versus old-oligo (5.4 ± 4.5 million/ml; p = 0.351). There was not a correlation between men age and sperm concentration (Pearson's correlation: r=-0.013, p = 0.469). For the 1127 cycles in which the embryos were cultured until blastocyst stage, the blastocyst formation rate was not affected by the men age (young-normal: 29.8%; young-oligo: 27.4%; old-normal: 32.8%; old-oligo: 28.3%; p = 0.457). The association of men age or sperm concentration or interaction of both on the clinical pregnancy was evaluated by multiple regression analysis adjusted to women age and we did not find any significant association (men age: OR = 0.995, p = 0.432; sperm count: OR0.998, p = 0.162; interaction of both: OR = 0.648, p = 0.254). The clinical pregnancy rates of groups were: young-normal: 32.7%; young-oligo: 34.8%; old-normal: 26.7%; old-oligo: 22.8% (p = 0.080).

Limitations, reasons for caution: Retrospective design is a limitation of this study. Despite of large casuistic of this study, the number of cycles included in the group of age ≥50 years is small. The multiple regression model was used to adjust the outcomes for the women age; however, other female factors were not considered.

Wider implications of the findings: We investigated the association of men age and sperm count in outcomes of ICSI cycles and showed no association. Our findings are in line with those authors presenting the paternal age is not a determinant factor of the ICSI cycles, even if the sperm count is declined.

Trial registration number: not applicable.

P-081 The impact of isolated teratozoospermia on the outcome of intrauterine insemination after ovarian stimulation with gonadotropin

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Study question: What are pregnancy outcomes after intrauterine insemination (IUI) in couples with isolated teratozoospermia (<4% of normal form with normal count and motility)?

Summary answer: Clinical pregnancy rate and abortion rate after IUI in couples with isolated teratozoospermia are comparable with those in couples with normozoospermia.

What is known already: Decreased sperm count and motility before and after sperm preparation are known to be associated with IUI outcomes, but the impact of sperm morphology on IUI outcomes are still controversial.

Study design, size, duration: A retrospective study was conducted using data from 466 couples who underwent 858 stimulated IUI cycles from January 2015 to December 2016.

Participants/materials, setting, methods: Couples with endometriosis and tubal factor infertility were excluded. All couples showed normal count and motility in the semen analysis. Ovarian stimulation was performed by gonadotropin injections. The couples were divided according to the percentage of normal form; group A(<1.0%), group B(1.0–1.9%), group C(2.0–2.9%), group D(3.0–3.9%), and group E(≥4.0%). Subgroup analysis was performed in women with normal ovarian reserve(serum AMH level 1.5–4.0 ng/mL, female age ≤40 years).

Main results and the role of chance: Overall clinical pregnancy rate was 15.3% (131/858). Clinical pregnancy rate was 13.0% (32/247) in group A, 24.0% (44/183) in group B, 13.0% (16/123) in group C, 10.4% (8/77) in group D, and 13.6% (31/228) in group E. Clinical pregnancy rate was significantly higher in group B compared with group E (P <.05). In women with normal ovarian reserve, clinical pregnancy rate was similar among five groups; 16.0% (13/81) in group A, 18.3% (11/60) in group B, 15.9% (7/44) in group C, 3.8% (1/26) in group D, and 11.1% (8/72) in group E.

Limitations, reasons for caution: Size and numbers of mature follicles at triggering day was not included.

Wider implications of the findings: These results suggest that IUI which is less invasive and cost-effective may be the first-line option in couples with isolated teratozoospermia before moving on to in vitro fertilization/ Intracytoplasmic sperm injection (IVF/ICSI).

Trial registration number: Not applicable.

P-082 Human sperm lipid profiling in men with asthenospermia

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Study question: Is sperm lipid profile in asthenospermia different from normozoospermia?

Summary answer: Asthenospermic semen samples showed an alteration in cholesterol sulphate/seminolipid ratio.

What is known already: Lipid composition of spermatozoa is important in determining the functional characteristics of the spermatozoa, in particular on motility, acrosomal exocytosis or fusogenic properties of the sperm.

Study design, size, duration: Sperm samples from 33 men undergoing IVF/ICSI treatment were collected of which **9** with normozoospermia (group A; control) and 11 with asthenospermia (group B) were included in this study. The total progressively motile sperm cells/ejaculate were 166.4 + 44.3 millions and 6.4 + 5.9 millions in group A and B respectively. Semen samples were collected, frozen, and stored at -80 °C until processed.

Participants/materials, setting, methods: We used Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) in the negative ion mode combined with Thin Layer Chromatography (TLC) to elucidate the lipid composition.

The signals of the MALDI mass spectra (3 replicates/sample) were exported in a final matrix in a compatible format for the multivariate analysis with *ClinProTools 3.0* software and *t*-test was used to confirm significant differences between the two lipid patterns. Peaks with $P < 0.05$ were considered statistically significant.

Main results and the role of chance: Our data on the lipid composition of whole ejaculate give novel information on lipid molecular species and components. In contrast to previous literature reports, we have found only traces of cardiolipin; the only cardiolipin species found in sperm has four palmitic acid chains (corresponding to the MS peak at m/z 1352.3). The mass spectrometry and chromatographic (TLC) comparative analyses of control and asthenospermic samples suggest an alteration in cholesterol sulphate/seminolipid ratio. As regards sulfolipids, TLC and MALDI-TOF mass spectra replicates showed that the content of the cholesterol sulphate (corresponding to the MS peak at m/z 465.4) and of the sulphoquinovosyl acylalkyl glycerol (namely S-QDG or seminolipid corresponding to the MS peak at 795.8) were significantly different between the two groups ($P < 0.05$). The mean intensity of the cholesterol sulphate MS peak and of the seminolipid MS peak were 40.0 ± 6.8 and 278.9 ± 70.6 in group A and 156.4 ± 53.9 and 59.7 ± 25.5 in group B respectively.

The specific lipid composition of seminal plasma and of spermatozoa is under investigation.

Limitations, reasons for caution: The seminolipid is exclusively present in the spermatozoa whereas the cholesterolsulphate is a component of the seminal fluid and of blood, too. In future studies, it may be interesting to measure the cholesterol levels as well as its derivative cholesterolsulphate in the blood in order to verify patient specific differences.

Wider implications of the findings: Our results suggest that the MALDI-TOF/MS lipid profile of sperm may represent a diagnostic tool for the assessment of asthenospermic conditions in the clinical practice.

Trial registration number: None.

P-083 Fibronectin and human sperm selection

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Study question: Does Fibronectin protein as a new biomarker can be used for human sperm selection in ART?

Summary answer: Fibronectin can be used for selection of sperm with suitable quality.

What is known already: Detection of these molecules on the sperm surface and separation of sperm according to them are dependent on the availability of a specific ligand for designing a commercial test. Todays, hyaluronic acid as the PICSI dish is the only specific ligand available for sperm selection. In addition specific antibodies, as powerful ligands for detection, separation and measurement of other suggested biomarkers were used in different studies. Fibronectin (FN) is a multifunctional diametric glycoprotein on the surface of sperm plays an important role in sperm-oocyte interaction and fertilization process.

Study design, size, duration: Amount of FN and the sperm quality were assessed in normozoospermia (N) (42 men) and asthenoteratozoospermia (AT) (72 men) groups through Sperm chromatin dispersion (SCD), sperm chromatin structure assay (SCSA) and chromatin maturation index (CMI). Semen samples were collected after 48–72 h of sexual abstinence and assessed according to World Health Organization guideline (WHO)

Participants/materials, setting, methods: Polyclonal antibody against human FN was produced in rabbit. Its quality, purity and immune reactivity were assessed by SDS-PAGE and western blotting (WB). Also presence of FN on sperm surface was assessed through immunocytochemistry (ICC) and flow cytometry (FCM).

Main results and the role of chance: The results showed the FN distribution on the equatorial region of human sperm. Statistically significant differences were found in the FN levels of sperm surface between two groups with 24.64 ± 9.08% in N and 16.90 ± 7.27% in AT ($p \leq 0.0001$). Also, FN level correlated negatively with SCD ($p \leq 0.0001$), SCSA ($p \leq 0.0001$), and CMI ($p \leq 0.001$). A threshold of FN level and DFI percentage respectively were 16 and 30, were identified as a cut-off value to determine N with specificity 83.3% and 81.0%, and sensitivity of 16.8% and 19.0%. A specificity and sensitivity of FN-DFI were 91.2% and 8.8%.

Limitations, reasons for caution: This study implied that the FN levels in sperm could be potentially used as a biomarker in sperm selection and assessing the quality of sperm in ART.

Wider implications of the findings: Our study suggests that FN can be used for selection of sperm with suitable quality although future studies are recommended.

Trial registration number: N.A.

P-084 Prognostic factors of ICSI-success rates in azoospermic male patients

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Study question: Identification of prognostic factors affecting success rates in infertile couples undergoing ICSI procedure after TESE (testicular sperm extraction).

Summary answer: High embryo quality and lower male BMI positively affect ICSI success rate after TESE. In case of tubular-atrophy ≥SIGG4 no pregnancy could be achieved.

What is known already: Azoospermia is defined as complete absence of sperms in male ejaculate leading to infertility. Azoospermia can be divided into obstructive azoospermia, where the vas deferens is affected and non obstructive azoospermia, where spermatogenesis itself is impaired. Testicular atrophy can be classified histologically after its severity into categories after SIGG. Azoospermic men are often recommended to undergo TESE to evaluate, if mature sperms can be found and ICSI-procedure is feasible. To our knowledge little is known about further clinical factors that can affect ICSI success rates in those couples undergoing ICSI treatment after sperm-positive testicular biopsie.

Study design, size, duration: Retrospective analysis of 118 cycles of ICSI-treatment after TESE-procedure because of male azoospermia. Clinical data were selected from our medical records. Statistical analysis including Wilcoxon, Fishers Exact and Chi-Square-Test were carried out with SAS statistic software; statistical significance was set as $p \leq 0.05$.

Participants/materials, setting, methods: 118 cycles of couples undergoing ICSI procedure after TESE with sperm-positive result were analyzed. Of those 66 cycles were the first, 35 the second and 17 the third ICSI attempt. To avoid statistical bias only the first 66 cycles of 66 different couples are demonstrated here. We evaluated, if potential prognostic factors, such as male/female age, male/female BMI, male/female nicotine abuse and histological result affect ICSI-pregnancy-rates (HCG-positivity 14 days after embryo transfer).

Main results and the role of chance: Pregnancy rate of couples with two good quality embryos were 47.62% and 38.1% with one good quality embryo, whereas only 14.29% in case of poor quality embryos. So embryo quality was positively associated with pregnancy chance ($p = 0.0278$), as seen in conventional IVF/ICSI. Males whose mates conceived after TESE/ICSI were significantly leaner than men whose partners did not get pregnant ($p = 0.023$). BMI of women and age of men and women had no effect on pregnancy rate in our population; as well as nonsmoking was not positively correlated in men and women with ICSI-success - maybe due to small numbers of smokers in both groups (only 12 men and 9 women in total). Women whose partners had tubular atrophy ≥ SIGG4 did not conceive in our population even if mature sperms were bioptically found, regardless of the number of cycles that were performed.

Limitations, reasons for caution: Analyzed cycle number (total 118) was low. Prospective analysis of more patients are needed to improve prognostic statements for couples undergoing ICSI procedure after TESE.

Wider implications of the findings: Similar to situation in conventional IVF/ICSI embryo quality is highly relevant for success-rate in ICSI/TESE. Additionally, men´s weight seems to have an effect and weight reduction in overweight men can be discussed before TESE. In case of tubular-atrophy ≥SIGG4 couples should be informed about their low chances conceiving with ICSI-procedure.

Trial registration number: not applicable.

P-085 Investigating the expression of sperm-specific microRNAs in a mouse model of obesity

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Study question: To investigate the relative expression and the epigenetic control of sperm-specific microRNAs in high fat diet (HFD) mice and aged matched controls (AMC).

Summary answer: Sperm-specific MicroRNAs from HFD mice are upregulated, with miR-21a-5p being highly significant. miR-21a-5p expression is regulated by methylation of CpG islands on VMP1 promoter.

What is known already: MicroRNAs (miRNAs) are small non-coding single-strand RNA molecules (17-26 nucleotides). miRNAs are encoded in the genome and transcribed by RNA polymerase II to form long primary miRNA transcripts. After post-transcriptional processing, the mature miRNAs can targets mRNA transcripts inhibiting gene expression through disruption of ribosomal attachment, or via degradation of the mRNA transcript. Sperm contain a unique family of miRNAs, which are responsible for spermatogenesis, sperm function and embryogenesis. Several reports have implicated dysregulation of sperm specific miRNAs in male infertility. High fat diet can alter the expression of miRNAs in sperm, which may impair sperm function and fertility.

Study design, size, duration: C57BL/6 mice were fed on rodent diet with 60% kcal% fat (HFD, n = 12) and rodent diet with 10% kcal% fat (AMC, n = 12) for 24-28 weeks post weaning. After sacrifice, the testes were removed and the sperm were extracted form the epididymis and processed for total DNA and RNA extraction. A panel of 84 sperm-specific microRNA was assessed in addition to the methylation status of the VMP1 promoter.

Participants/materials, setting, methods: Epididymal sperm were removed and isolated by the swim-up method. Total RNA was extracted using the Norgen biotek kit and used for qPCR for 84 sperm-specific miRNAs using custom miScript miRNA PCR array (Qiagen-UK). Sperm DNA was isolated by cell lysis and ethanol precipitation. The methylation status at two CpG sites on the VMP1 promoter region was carried out by bisulfite pyrosequencing using PyroMark Q24 system (Qiagen, UK).

Main results and the role of chance: This study revealed an increase in the relative expression of 28 miRNAs ($p = 0.05$) in the sperm of HFD mice. With miR-883a-3p expression been significantly lower in HFD nice ($p = 0.05$). The remaining miRNAs assessed on the miRNA PCR array showed no significant difference in relative expression in HFD and AMC mouse sperm. After applying a Bonferroni correction, it was demonstrated that miR-21a-5p relative expression was highly significant in the HFD mouse sperm relative to AMC ($p = 0.0006$). We were then interested in the epigenetic regulation of miR-21a-5p. The sequence responsible for the expressions of the pre-miR-21a-5p overlap with the upstream 3'UTR end of the VMP1 gene. Using bisulfite pyrosequencing we showed increased methylation at CpG sites in this region in AMC mice. These data indicate the epigenetic control of miR-21a-5p expression in sperm in relation to high fat diet.

Limitations, reasons for caution: A limited panel of miRNAs were analysed and only mature sperm were assessed.

Wider implications of the findings: This study links obesity with altered expression of sperm miRNAs in a mouse model. In addition, there was a change in the methylation status and expression of miR-21a-5p. These data indicate the impact that paternal high fat diet has on sperm miRNA expression and DNA methylation.

Trial registration number: None.

P-086 Sperm quality affects fertilization but not embryo cleavage and pregnancy rates after intracytoplasmic sperm injection

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Study question: This study deals with correlations between sperm quality and fertilization rate, early embryos development, pregnancy and implantation rates in patients undergoing Intra-Cytoplasmic Sperm Injection (ICSI).

Summary answer: Sperm quality significantly affects fertilization rate but not embryo cleavage in patients who underwent ICSI.

What is known already: Several studies have suggested that differences in sperm quality (i.e., azoospermic, sever oligoasthenoteratozoospermic and normozoospermic) reflect on oocyte fertilization, zygotes and embryos quality during cleavage stages. Also, pregnancy and implantation rates are believed to be independent from sperm quality.

Study design, size, duration: This study considered 1049 ICSI cycles between April 2016 and December 2017. The study excluded those cases with freeze-all embryos and couples with sever female factor (i.e., endometriosis and uterine factor). Sperm quality was classified into four groups according to WHO2010: Group1 - Normozoospermic (sperm/ml≥15 M); Group2 - Moderate Male Factor (5≤sperm/ml<15 M); Group3 - Severe Oligoasthenoteratozoospermia (0<sperm/ml≤5 M); Group4 – Azoospermia (sperm/ml = 0).

Participants/materials, setting, methods: Partner age for the four groups was respectively: Female - 34.71 ± 5.8; 37.72 ± 3.75; 36.73 ± 4.47; 36.38 ± 4.45. Male – 40.3 ± 5.8; 40.1 ± 5.75; 39.97 ± 5.73; 41.24 ± 8.57. A total of 5849 MII oocytes were inseminated. Average value of inseminated oocytes for each group was 5.16 ± 2.89, 5.14 ± 2.76, 5.50 ± 3.15, 6.45 ± 3.55 respectively. Data analysis was carried out using correlation study and ANOVA, which was set with a statistical significance threshold of $P < 0.05$. The statistical Matlab toolbox was adopted for the study.

Main results and the role of chance: Fertilization rate decreased with increasing severity of male factor across the groups. The difference was not statistically significant among Group1, 2 and 3 (77%, 73% and 73% respectively) but was significant (P-value 0.00043) between Group1-3 and Group4 (59%). Instead, no trend was observed for cleavage rate both on day 2 and 3. The difference was not statistically significant among groups (97%, 96%, 99%, 96%, P-value 0.07 on day 2) (87%, 86%, 84%, 85%, P-value 0.128 on day 3). Embryo quality, expressed as number of grade 1-2 and 4-cell embryos on day 2, was not affected by sperm quality. In fact, the difference was not statistically significant (P-value 0.507) among groups (90%, 86%, 87%, 84%). The same trend was observed for embryo quality on day 3 (78%, 75%, 80%, 73%, P-value 0.67). Finally, no significant differences were observed in the pregnancy and implantation rates (16%, 11%, 17%, 19%) (10%, 8%, 11%, 12%) (P-value 0.527) respectively.

Limitations, reasons for caution: Although a very large cohort of patients was enrolled, the study is retrospective. In addition, groups are different in size. Therefore, controlled, randomized and well-designed studies are needed to confirm data.

Wider implications of the findings: Sperm quality impacts fertilization but it does not affect ICSI outcome. Therefore, all measures should be taken to assure maximal performance of ICSI in cases of severely compromised sperm quality.

Trial registration number: Not applicable.

P-087 Peak retrograde flow in adolescents with varicoceles influences sperm DNA integrity

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Study question: Does varicocele diagnosed in adolescents using the peak retrograde flow influence sperm DNA integrity?

Summary answer: The presence of varicocele, particularly with a PRF ≥ 38 cm/sec, tends to increase both total and vital DNA fragmentation as compared to healthy adolescents.

What is known already: Correct indication for varicocele repair in adolescents remains a subject of ongoing debate. Some researchers advocate the use of a high peak retrograde blood flow (PRF > 38.4 cm/s) in combination with a 20% testicular atrophy index (TAI) as criteria for treatment. Currently, there is a growing interest in the measurement of the supine PRF in the varicocele vessels. Conventional semen parameters are evaluated in varicocele studies but the significance is weakened due to lack of standardization. However, the true significance of the presence of the harbinger regarding standard sperm parameters and sperm DNA fragmentation in adolescents is still unclear.

Study design, size, duration: This study was a prospective, cross-sectional observational study. Between February 2017 and January 2018, sixty seven volunteers (mean age 21.5 years) were recruited. A total of 62 participants met the inclusion criteria.

Participants/materials, setting, methods: Every participant had a scrotal ultrasound to calculate testicular volumes (using the Lambert formula) and TAI. If varicocele was present, the grade and PRF in supine position was measured. All participants provided semen samples. Standard semen parameters were analyzed according to the WHO 2010 guidelines, and sperm DNA fragmentation test was performed using TUNEL assay in total and vital fractions of the semen. For statistical analysis, primarily ANOVA, Kruskall-Wallis, t-tests and Mann-Whitney tests were performed.

Main results and the role of chance: Of the 62 participants included, 35 were without (56.5%) and 27 (43.5%) with a varicocele. There was no significant difference in the semen parameters (sperm concentration, motility and morphology) and sperm DNA integrity between these two groups. Taking into account the 20/38-harbinger criteria, progressive and total sperm motility was significantly ($p = 0.009$ and $p = 0.027$, respectively) lower than in those who did not meet the harbinger-criterium. Sperm concentration, morphology and sperm DNA were not significantly different. The varicocele group was subdivided into one with a low PRF (21/27, 77.7%) and those with a high PRF (6/27, 22.2%). The descriptive semen parameters were not significantly different in the healthy control and in the varicocele group with high/low PRF. Total sperm DNA fragmentation was significantly higher ($p = 0.021$) with high PRF than with low PRF as compared to the controls. Vital sperm DNA fragmentation was higher in high PRF group than in the low PRF group ($p = 0.029$). TAI and different grades of varicocele did not influence semen parameters and sperm chromatin integrity.

Limitations, reasons for caution: Study is still ongoing. Results are preliminary observations on a limited number of participants.

Wider implications of the findings: The findings in this study might have implications in proving the presence of the 20/38-harbinger in varicocele patients as a valid criterium for surgical treatment.

Trial registration number: B300201730926

P-088 Severe male factor infertility – is there a role for pre-implantation genetic screening (PGS)?

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Study question: Will male factor infertility bring about higher incidence of Embryo Aneuploidy? Will PGS help us pick euploid embryo and optimize reproductive outcome in cases with severe male factor infertility?

Summary answer: Severe male factor does'nt bring about higher incidence of embryo aneuploidy and there seems positive trend for use of PGS to optimize reproductive outcomes.

What is known already: Sperm has an equal contribution in reproductive outcomes. Severe male factor is shown to affect reproductive outcomes. There is an increasing concern that lower sperm counts, advancing paternal age and use of testicular sperm might increase embryo aneuploidy and negatively affect the reproductive outcomes. There is also a potential risk for epigenetic changes in the off spring with severe male factor infertility. Hence, we wanted to look for incidence of aneuploidy in severe male factor cases through PGS and justify the role of PGS to optimize reproductive outcomes in such cases.

Study design, size, duration: This was a retrospective study of all of couples in the year 2014-2016 with severe male factor infertility and undergoing PGS for failed implantation as an indication.

All couples during study period with two-failed IVF/ICSI attempts (fresh or frozen transfers) were offered PGS at our centre.

All couples were subjected to trophectoderm biopsy on day 5-post insemination. All embryos were vitrified post biopsy. Biopsied tissue was subjected to comprehensive chromosomal screening with Next Generation Sequencing.

Participants/materials, setting, methods: Male partners were divided into three groups based on age (<40 yrs (n = 43) and >40 yrs age (n = 20)), sperm counts (<5millions/ml (n = 14) & >5millions/ml (n = 31)) and source of sperm (Ejaculate (n = 31) &Testicular sperm (n = 33)).

Mean aneuploidy was calculated. Only couples with female age < 35years were included to ensure we do not have bias with advanced maternal age. Euploid embryos were transferred in a frozen embryo replacement cycle. Live birth rate in all groups were calculated.

Main results and the role of chance: Mean aneuploidy and Live birth rates in each group respectively were as follows:

<40 yrs age – 54.58% & 68.75%
>40 yrs age – 56.66% & 25% (p = 0.0013)
<5millions/ml -52.63% & 50%
>5millions/ml – 54.45% & 59%
Testicular sperm- 56.41% & 64%
Ejaculate sperm – 54% & 59%

All the groups of severe male factor infertility showed similar incidence of aneuploidy and there was no statistical significance. LBR across all groups was comparable except advanced paternal age group – 25% (p = 0.0013).

Young couples seem to have the highest LBR compared to advanced age. Though the advanced paternal age group had similar aneuploidy rates, still LBR were statistically low. Sperm counts and source of sperm for IVF/ICSI seem to have comparable aneuploidy rates and LBR.

Limitations, reasons for caution: Retrospective data, Small sample size.

Wider implications of the findings: Advanced Paternal age and reproductive outcomes needs further research. Use of testicular sperm for IVF/ICSI seems encouraging. Advanced techniques seem to offer better reproductive outcomes in severe male factor infertility. Third party reproduction using donor sperm should not be offered as a first line of treatment in such cases.

Trial registration number: Not Applicable.

P-089 Protective effects of Cinnamtannin B-1 (CINB-1) on human sperm cryostorage

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Study question: Could the antioxidant CINB-1 protect sperm during cryopreservation and preserve its physiological parameters after thawing?

Summary answer: CIN B-1 at 100 μM preserves sperm viability and mitochondrial activity during sperm cryopreservation.

What is known already: The process of sperm freezing and thawing may be related to an excessive production of reactive oxygen species (ROS), resulting in adverse changes in membrane lipid composition, acrosomal status, motility and viability. Adding antioxidants to the freezing extender could reduce these negative effects. CINB-1 is a natural A-type of proanthocyanidin that is present in few plants, which has a large number of cellular actions, mostly derived from its antioxidant properties. The protective ability of CINB-1 in thawed sperm samples from deer has been demonstrated before.

Study design, size, duration: Forty four amples were evaluated from eleven sperm healthy young donors. Sperm was collected with a period of abstinence between 48-72 h. Samples were cryopreserved following the WHO manual (2010) guidelines, supplementing with 0, 10 and 100 μM of CNB-1. Samples were evaluated after thawing and after incubation (37 °C, 4 h). Motility was evaluated by CASA. Viability, mitochondrial membrane potential, acrosomal status, lipoperoxidation, intracellular ROS and DNA integrity were assessed by flow cytometry.

Participants/materials, setting, methods: Sperm donors were selected according to ESHRE guidelines for gamete donation. After liquefaction, we washed the sperm samples with PBS and diluted them in freezing medium (Irvine Scientific). Extended spermatozoa were loaded into 0.25-mL plastic straws and cooled down, equilibrated to 5 °C (40 min) and frozen in nitrogen vapours. The straws were thawed at 37 °C for 7 min. Data were analyzed by linear mixed-effects models using R (http://www.r-project.org).

Main results and the role of chance: CNB-1 did not affect sperm motility parameters. There were significant effects in sperm viability, CINB-1 100 μM showing a positive effect ($p < 0.05$) on the percentage of live sperm cells at 0 h (19.27% ± 1.26) comparing to the control (15.11% ± 1.38) and CNB-1 (10 μM) (16.23% ± 0.74). Our results also show that the highest concentration of CNB-1 (100 μM) preserved better the mitochondrial activity after 4 h of incubation (10.60% ± 0.48) than the control (8.27 ± 0.54) ($p < 0.05$). There were no effect of CINB-1 on the rest of sperm parameters evaluated ($p \geq 0.05$). There were no significant effects of CINB-1 on other sperm parameters, including DNA integrity, either after thawing or after 4 h of incubation ($p \geq 0.05$).

Limitations, reasons for caution: We believe that sample size might be a limitation of this study as well as the use of normozoospermic samples. Further studies should be carried out to verify CINB-1 protective effect during cryopreservation in samples of low seminal quality.

Wider implications of the findings: Given the beneficial effect of Cinnamtannin B-1 *in vitro*, its use could also be considered as a feeding supplement especially in infertile men.

Trial registration number: not applicable.

P-090 Ability of SOD1 and SOD3 in seminal plasma to distinguish between two different azoospermic subgroups (mixed testicular atrophy and Sertoli cell-only syndrome)

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Study question: Are there marker proteins in seminal plasma that can predict the presence of testicular sperm before an invasive surgery in men suffering from non-obstructive azoospermia?

Summary answer: SOD3 (Superoxide dismutase 3) protein in seminal plasma has potential to predict the success of micro-dissection sperm retrieval in non-obstructive azoospermia men (NOA).

What is known already: Men with NOA have zero sperm in the ejaculate.

Testicular sperm suitable for assisted reproductive technology (ART) could be present in NOA patients.

Sperm is collected by micro-testicular sperm extraction (M-TESE). However, no diagnostic test exists to predict the presence of sperm in the testis biopsy

before surgery. About 50% of azoospermic men are unable to have sperm retrieved at M-TESE and therefore have surgery unnecessarily. The ability to predict the presence of sperm in the testis prior to TESE would improve sperm retrieval rates and avoid unnecessary surgery.

Study design, size, duration: Identification of specific proteins related to spermatogenesis in seminal plasma(SP) could provide a novel proteomics assay for sperm presence prior to biopsy. SP is a rich, easily-accessible and promising source of spermatogenesis biomarkers. This study was designed to compare the SP proteomes of two different groups of NOA.

(1) Men with mixed-testicular atrophy(MA) with a positive sperm retrieval at M-TESE.
(2) Sertoli-cell-only(SCO) patients with a negative sperm retrieval at M-TESE.

Results also compared with the control-group.

Participants/materials, setting, methods: SP was collected from MA and SCO patients (n = 8/group). Samples were compared by label-free Liquid chromatography-mass spectrometry/MS proteomics. Differentially expressed proteins were defined as those with a fold-change >2.0 and a significant difference between the groups($p < 0.05$).

Among the significant proteins identified, SOD3 and SOD1 were selected for further validation by 3 different methods:Western blotting, immunohistochemistry and ELISA (n = 15/group). To compare with SP, the protein concentrations of SOD3 and SOD1 were measured in the blood-serum of participants.

Main results and the role of chance: SOD3 immunoreactivity (ELISA) in SP from SCO men was 2.3 fold lower than controls ($p < 0.05$), and SP from MA men was 4.6 fold lower than controls ($p < 0.0001$). The SOD3 MA/SCO protein ratio was 0.49 ($p < 0.05$) by ELISA and 0.16 ($p < 0.01$) by mass spectrometry. SOD3 gave the expected band of 30 kDa by Western blot of SP samples, but no significant difference between MA and SCO groups was observed.

In normal human testis, SOD3 staining was predominantly observed in Sertoli cells, while in the testis of azoospermic patients staining was more variable.

SOD1 immunoreactivity (ELISA) in SP from SCO and MA men was decreased significantly compared with controls ($p < 0.05$). The MA/SCO ratio of SOD1 was significantly different by mass spectrometry but not by ELISA or quantitative Western blot. In normal human testis, SOD1 was predominantly localized in spermatogonia, while in the testis of azoospermic patients variable SOD1 staining was noted.

There were no significant differences in the levels of SOD1 and SOD3 in the blood-serum of MA compared to SCO patients.

Limitations, reasons for caution: Two different antibodies were used for quantitation of protein (in both cases of SOD1 and SOD3) by Western-blotting compared to ELISA which may explain the variation in results. Moreover, the selected antibodies may not target the same sequence region that was detected by the mass spectrometry.

Wider implications of the findings: SOD3 was significantly different in SP from MA versus SCO azoospermic patients by at least 2 different proteomics-methods (mass spectrometry and ELISA), and thus has the potential to predict the presence of sperm in the testis prior to biopsy.

Trial registration number: The trial registration number for this project is IRTG-62280541.

P-091 Predictors of positive sperm retrieval in azoospermic men

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Study question: Are there reliable factors able to predict the sperm retrieval rate (SRR) after conventional testicular sperm extraction (c-TESE)?

Summary answer: Testicular volume and histopathological diagnosis are related with the SRR while age, months of infertility, FSH level and body mass index are not good predictors.

What is known already: Obtaining sperm from the testis in combination with ICSI has become a routine in the assisted reproduction technology. However, it is still controversial which is the most effective sperm retrieval technology, the accuracy of different factors in predicting the SRR and if uncoupled TESE/oocyte pick-up (OPU) should be considered to prevent possible unnecessary ovarian stimulation and OPU when no sperm cells are detected.

The ability to predict those patients with a high probability of achieving a successful sperm retrieval would be of great value in counselling the patient and his partner.

Study design, size, duration: This is a retrospective cohort study which includes 96 consecutive azoospermic patients, 17 with obstructive azoospermia (OA) and 79 with non-obstructive azoospermia (NOA) who underwent c-TESE between 2004 and 2017.

The origin of the obstructive azoospermia was epididimal obstruction (n = 13), vas deferens obstruction (n = 3) or vasectomy (n = 1).

Participants/materials, setting, methods: All patients, whose azoospermia was confirmed by two semen analyses, underwent c-TESE by the same surgeon. The specimens were examined by an embryologist, where all the tubules were teased and analysed for the presence of sperm. A histopathology specimen was sent for analysis.

Factors such as male age (years), duration of infertility (months), FSH level (mUI/mL), body mass index (BMI), testicular volume (mL) and the histopathological diagnosis were correlated with the presence of sperm.

Main results and the role of chance: OA patients were older than NOA males (39.5 (95%CI 34.3-43.8) vs. 36.03 (95%CI 35.0-37.0)) ($p = 0.015$). However, the infertility duration (27.5 (95% CI 20.5-34.4) vs. 32.35 (95%CI 27.2-37.5)) ($p = 0.028$) and FSH levels (5.6 (95%CI 4.2-7.1) vs. 16.8 (95%CI 14.0-19.7)) ($p = 0.001$) were lower.

The SRR was 100% in the OA patients and 29.1% in the NOA group. No complications after the surgery were seen.

In the NOA group, the area under the ROC curve of male age, months of infertility, FSH level and body mass index respect the chance of finding sperm after c-TESE were 0.59, 0.51, 0.36, 0.45, respectively.

In 45.6% of the NOA patients, the volume of the biopsied testicle was diminished (<14 mL). The chance of positive SRR was higher in normal vs. low volume testicles (73.9% vs. 26.1%, $p = 0.03$).

Histopathological findings included four patterns: hypospermatogenesis (reduced number of normal spermatogenetic cells), MA (maturation arrest, only spermatogonia, spermatocytes and spermatids were detected), SCO (Sertoli cell only) and tubular sclerosis. The incidence of each pattern in NOA patients with negative SRR was 7.1%, 39.3%, 44.6% and 9.3%, respectively. That means that in 92.9% of the cases where the embryologist did not found any spermatozoa, the histopathological diagnosis confirmed the absence.

Limitations, reasons for caution: A low number of patients were analyzed because most of the azoospermic males visiting in our center are foreigners and their diagnostic TESE is performed abroad.

Our SRR is in the low range of what has been published, but the absence of sperm was mostly confirmed with the testis histopathology.

Wider implications of the findings: Although the testicular size seems to be related to the SRR, the most reliable parameter predicting the presence of sperm is the histopathological examination of testicular fragments. Despite having higher predictive value, it would not prevent the patient from undergoing a testicular biopsy.

Trial registration number: Not available.

P-092 Impaired fertility in a rat model of spinal cord injury and the role of inflammasome complex

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Study question: Activation of inflammasome complex affects male fertility following spinal cord injury.

Summary answer: We found that there is a relationship between inflammasome activation in rat testis and low sperm parameters.

What is known already: Infertility is a common feature of men with spinal cord injury (SCI). Inflammation is one of the most important abnormalities in semen of these patients. Previous studies showed some inflammasome components in semen of SCI men. Inflammasome activation pattern was found in many tissues and disease but about testis is still unclear.

Study design, size, duration: There are 20 Wistar male rats in this study. They were divided into four groups (n = 5): spinal cord injury was induced at T10 level of the spine, at three groups and one group remained intact (control group). Rats in SCI groups were sacrificed at three different time points: one day, three days, and 7 days after surgery. Testes were removed and protein analysis was done.

Participants/materials, setting, methods: In this experiment, Male Wistar rats weighing 200-250 g were used. Testis sampling was done at three specific time points (1, 3 and 7 days after injury). Protein expression (Caspase-1 and ASC) was assessed by immunofluorescence (IF). Data were analyzed by a parametric test of One-way ANOVA, and the comparison between the groups was taken by the Tukey post hoc test ($p < 0.05$).

Main results and the role of chance: Immunofluorescence showed a significant increase of inflammasome complex components from day 1 after injury. The protein level of Caspase-1 increased significantly on day 1, 3 and 7 after injury in comparison to control ($p < 0.001$).The peak of Caspase-1 expression was on day 7. ASC protein was raised significantly on day 1, 3 and 7 after injury, versus the control group ($p < 0.001$). The highest level of ASC protein was on day 3

Limitations, reasons for caution: We had some ethical considerations about the number of rats used in the study. Our data are experimental and should be proven in human studies.

Wider implications of the findings: The reason of diminished sperm parameters in patients with spinal cord injury is not clearly defined. Our data revealed a pattern for inflammasome activation in the rat testis following spinal cord injury. In later step, Inflammasome antagonists should be used to eliminate inflammasome complex and improve sperm parameters.

Trial registration number: not applicable.

P-093 Novel mutations in the CFTR gene associated congenital absence of vas deferens in China

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Study question: What are the novel cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations and mutation spectrum in Chinese patients with congenital bilateral absence of vas deferens (CBVAD)?

Summary answer: 23 novel CFTR mutations were detected in 192 Chinese patients with CBAVD. 66% (127/192) patients have CFTR mutations.

What is known already: CBAVD is an autosomal recessive disease and occurs in 1–2% of infertile men. CFTR mutations or IVS8-5T polymorphism were the most common causes of CBAVD. The most common CFTR mutation in many populations is a deletion named F508del. The 5 T variant in intron 8 (IVS8-5T) is common in the different populations, but a higher than normal frequency of this allele has been found in CBAVD patients.

Study design, size, duration: A total of 192 male probands of congenital bilateral absence of the vas deferens were collected from the Andrology Clinic of the Reproductive Medicine Center in the First Affiliated Hospital of Sun Yat-sen University between 2014 and 2016. Twenty males with normal vas deferens were included as a control.

Participants/materials, setting, methods: CFTR mutations were searched for using Sanger sequencing to analyze genomic DNA samples from 192 probands with CBAVD. The primers used to amplify the 27 coding exons, the adjacent intronic regions, and the promoter. Potential pathogenic variants were filtered by the multiple-step bioinformatics analysis and further evaluated in 20 normal controls by Sanger sequencing.

Main results and the role of chance: 66% (127/192) patients have CFTR mutations. 35% (67/192) patients have two or more mutations (common CFTR mutations or IVS8-5T polymorphism). 31% (60/192) have a single detectable CFTR mutation. Of these detected mutations, 23 mutations were novel. 7 novel mutations were detected in promoter. 11 of 16 novel mutations in exons were predicted to be pathogenic by bioinformatic analysis. 30 (16%) homozygous and 73 (38%) heterozygous IVS8-5T polymorphism were found. But it was not found in controls. The most common CFTR mutation (F508del) in many populations were not found in our subjects. This suggested the IVS8-5T polymorphism was the major effect for Chinese male patients with CBAVD. The other frequent mutation in the studied cohort, G970D, was found in 9 probands. This mutation was on shared haplotype and thus evidence of a founder effect in the population. Furthermore, the severity of phenotypes may affect by the number or type of CFTR mutations in those patients.

Limitations, reasons for caution: Over 34% (65/192) of the patients without identified mutations might carry other pathogenic variants in the intron of CFTR, or may be caused by mutations in novel genes yet to be identified.

Wider implications of the findings: This study analyzed the relation between genotype and phenotype in Chinese CBAVD patients with CFTR mutations. A comprehensive analysis of the CFTR gene expanded the mutation spectrum in Chinese patients with CBAVD, and supplied the clues for discovering novel causative CBAVD genes and the foundation for gene therapy.

Trial registration number: not applicable.

P-094 Improvement of ICSI outcome using sperm selection by thermotaxis

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Study question: We tested if human and mice spermatozoa selected by thermotaxis show higher quality than spermatozoa selected by swim-up.

Summary answer: We found that spermatozoa selected by thermotaxis show much higher quality than those selected by swim-up and that mice thermotaxis-selected spermatozoa improved ICSI outcome.

What is known already: Sperm selection occurring *in vivo* within the female reproductive tract is bypassed when some artificial reproductive techniques (ART) are used, such as *in vitro* fertilization or intracytoplasmic sperm injection (ICSI). This could be in part behind the relative low success of fertility treatments. Thus, improving sperm selection has been pointed as primary strategy in order to improve ARTs outcome. Processes operating within the oviduct to guide the spermatozoa to the fertilization site are candidates as mechanisms of selection. This is the case of thermotaxis that guides the spermatozoa in response to a temperature gradient stablished within the Fallopian tube.

Study design, size, duration: Mice and human spermatozoa were selected by swim-up or by thermotaxis and the DNA integrity of the spermatozoa after both selections was analyzed (n = 5 for mouse and n = 8 for human, 50-100 spermatozoa per determination). ICSI was conducted with mice spermatozoa selected by swim-up or by thermotaxis (260 total ICSIs per sperm type). Resultant embryo were cultured *in vitro* up to two cells or blastocyst stage and transferred to recipient females.

Participants/materials, setting, methods: Sperm samples from B6D2 mice and 15 normospermic patients were used. Swim-up was performed following a standard procedure and thermotaxis using a method of sperm migration between two drops of medium connected by a capillary. Between the two drops a temperature gradient was stablished in a way that one drop was at 35°C (were spermatozoa were loaded) and the other at 37°C (were spermatozoa were collected after 1 h). DNA quality was analyzed by comet assay.

Main results and the role of chance: In both species we clearly observed lower DNA fragmentation in spermatozoa selected by thermotaxis. Thus, mice swim-up showed 12 ± 0.6% and thermotaxis 3 ± 0.5% ($P = 0.0001$, Student's *t*-test) of DNA fragmentation and human swim-up showed 15 ± 3% and thermotaxis 5 ± 0.8% ($P = 0.001$, Student's *t*-test). Using spermatozoa selected

by thermotaxis for ICSI we produced higher percentage of embryo reaching to blastocysts stage (expanded and hatched) than using swim-up spermatozoa [73 ± 6% vs 40 ± 11% respectively ($P = 0.01$, Student's *t*-test)]. Furthermore, significantly higher implantation rates (analyzed 15 days after ICSI) were recorded for thermotaxis-derived than swim-up-derived embryos both when two cell embryos (75 ± 8% *vs* 27 ± 7%, $P = 0.01$, Student's *t*-test) or blastocysts (77 ± 9 *vs* 43 ± 18, $P = 0.03$, Student's *t*-test) were transferred. For transfers with 2-cell embryo, we also recorded higher rates of development to term for the spermatozoa selected by thermotaxis (5 and 13 pregnancies to term out of 24 transfers per group for swim-up–ICSI and thermotaxis–ICSI respectively) and higher rates of born pups for the thermotaxis-ICSI derived embryos (12 ± 2 and 29 ± 4% for swim-up- and thermotaxis separated spermatozoa respectively; $P = 0.027$ two-tailed Student's *t*-test).

Limitations, reasons for caution: Our results of ICSI are limited to mice and are a proof of concept. At this stage we cannot conclude that sperm selection by thermotaxis would improve ICSI outcome in humans. Clinical trials with human patients are being done now.

Wider implications of the findings: Sperm selection by thermotaxis represents the first approach available to isolate a specific subpopulation of capacitated spermatozoa for their characterization. This would allow to reveal basic aspects of sperm biology and seed light on the contribution of the spermatozoa in the process of fertilization and on early embryo development.

Trial registration number: not applicable.

P-095 Predictive value of static oxidation-reduction potential in detecting high levels of sperm DNA fragmentation in infertile men

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Study question: Can sperm oxidative stress, measured as static oxidation-reduction potential (sORP), predict high levels of sperm DNA damage?

Summary answer: sORP was positively correlated ($p = 0.002$) to DNA damage and a sORP cut-off of 2.37 mV/10^6 sperm/mL was predictive of high levels of DNA damage.

What is known already: High levels of sperm DNA damage have been associated with decreased pregnancy rates and an increased risk of miscarriage. While the causes are multifactorial, sperm oxidative stress has been shown to play a key role in the etiology of DNA damage. The MiOXSYS system uses sORP, a global measure of the balance between oxidants and reductants, to determine oxidative stress.

Study design, size, duration: This prospective, institutional REB approved cross-sectional study was performed on 52 male, infertile patients presenting to the Andrology laboratory for routine semen analysis after 2-5 days of abstinence at the CReATe Fertility Center between July 2017 and January 2018.

Participants/materials, setting, methods: Male infertility patients with leukocytospermia or sperm concentration <1 million/mL were excluded. Semen analysis was conducted as per WHO 2010 guidelines. sORP was measured using the MiOXSYS system. DNA damage was evaluated by standard flow cytometric acridine orange-based assay as DNA Fragmentation Index (DFI). sORP was compared between a high DFI (≥30%) and low DFI (<30%) group and correlated to semen parameters. Receiver Operator Curve (ROC) analysis determined the sORP cut-off predictive of high DFI.

Main results and the role of chance: The mean paternal age was 39.2 ± 6.1 years (age range: 28 – 54 years). The mean sperm concentration was 43.0 ± 37.2 M/mL, total motility was 55.2 ± 14.5 %, morphology was 6.5 ± 4.2 %, and DFI was 21.7 ± 10.1 %. A significant positive correlation was found between sORP and DFI ($r = 0.43$, $p = 0.002$). A significant negative correlation was found between sORP and concentration ($r=-0.46$, $p = 0.0006$) and between sORP and morphology ($r=-0.33$, $p = 0.02$). Mean sORP was significantly higher ($p = 0.03$) in the high DFI group (4.29 ± 2.52 mV/10^6 sperm/mL) compared to the low DFI group (1.87 ± 2.17 mV/10^6 sperm/mL). ROC analysis indicated that a sORP cut-off of 2.37 mV/10^6 sperm/mL had a sensitivity of 75.0%, a specificity of 77.2%, a positive predictive value (PPV) of 37.5% and a negative predictive value (NPV) 94.5%. The area under the ROC (AUROC) was 0.84 (95% CI: 0.72 – 0.96, $p = 0.003$) and the diagnostic accuracy was 76.9%.

Limitations, reasons for caution: No additional confirmatory DNA damage methodologies were assessed. The DNA damage assay used in this study is generally considered the gold standard, however several alternatives are also routinely used. Larger standardized studies are needed to confirm the performance characteristics of sORP in predicting high DFI.

Wider implications of the findings: Requiring as little as 5 minutes to complete and a drop (30 uL) of semen, the MiOXSYS system provides a rapid, cost-effective measure of global oxidative stress easily integrated into clinical workflows. It can be used to specifically identify patients with oxidative stress induced DNA damage for anti-oxidant therapy.

Trial registration number: not applicable.

P-096 Vitamin D levels and sperm parameters

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Study question: Is it possible to evaluate a potential impact of vitamin D on male fertility through the measurement of routine sperm parameters?.

Summary answer: Routine seminal parameters are not a good predictor of the possible deleterious effects of low levels of vitamin D on male fertility.

What is known already: Vitamin D has physiological functions beyond those related to bone metabolism. As far as male fertility is concerned, it has been shown that vitamin D receptors are expressed in germ cells, ejaculatory duct and mature sperm cells. Vitamin D deficit has been described to a greater or lesser extent in all human populations studied. Deleterious reproductive effects of such deficit have been shown in animals. There is, however, contradictory evidence in humans, which makes this a controversial issue.

Study design, size, duration: Prospective observational study carried out in males coming to our institute between April 2013 and May 2015, seeking subfertility testing, who accepted to participate in the study. Vitamin D levels were tested and routine sperm parameters were recorded from the same sample used in the fertilization cycle.

Participants/materials, setting, methods: Caucasian males were included in the study. Subjects with systemic diseases were excluded. Vitamin D serum levels were tested and routine semen quality parameters were recorded. Semen samples were obtained by masturbation after an abstinence of 2-5 days. They were analyzed following 2010 WHO criteria. Association among variables was analyzed using linear regression and a Student´s t-test.

Main results and the role of chance: The study included 175 subjects (18 were smokers), with an average age of 39,7 ± 5,8 years. Average BMI was 26,3 ± 3,6. Average vitamin D was 25,6 ± 8,9 ng/ml (51% of the samples were collected between May and September). All semen parameters (volume, concentration/count and % progressive motile) showed a trend towards values indicating better quality with higher vitamin D levels. However, when correlations among vitamin D and the different semen parameters were analyzed a significant positive correlation was found only with sperm counts ($p = 0,045$). This correlation was lost after adjusting for confounding factors (age, BMI, seasonality and smoking). From 122 subjects with vitamin D levels< 30 ng/ml, 43 (35%) had alterations in semen. This proportion diminished to 28% (14 out of 53) in males with vitamin D levels ≥ 30 ng/ml ($p = 0,25$).

Limitations, reasons for caution: Age was relatively high in our study subjects, so that results may not be extrapolated to younger populations.

Wider implications of the findings: Our population included a few males with vitamin D levels≥ 30 ng/ml, above which the pleiotropic effects are evident. Studies with comparable subject samples with adequate/deficient vitamin

D levels are needed. Longitudinal studies comparing sperm quality in males with vitamin D deficit before and after supplement ill be useful.

Trial registration number: Not applicable.

P-097 Levels of p53 protein in human spermatozoa, embryo quality and pregnancy rate. EcoFoodFertility project (preliminary data)

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Study question: The aim of this study is to evaluate if different concentrations of p53 protein in human spermatozoa could influence embryo quality and pregnancy rate.

Summary answer: High concentrations of p53 in human spermatozoa is associated to low percentage of embryos at 6-8 cells stage in third day and lower pregnancy rate.

What is known already: Frequently seminal fluids with normal parameters of evaluation, according to the WHO laboratory manual for examination and processing of human semen (2010), show variations on the capacity to fertilize oocytes and also the quality of obtained embryos is very variable.

Protein p53 is well known as "The guardian of genome"; it changes its concentration in human spermatozoa DNA in relation to the damage of the latter. It has been suggested that the role of the p53 ancestral gene was to ensure the integrity of the genomic germline and the fidelity of the development process.

Study design, size, duration: From July 2013 to June 2017 we have examinated retrospectively 79 couples with 2-5 years of infertility history.

We have divided the couples on the basis of p53 levels:

Group A: 0,35–1,65 ng/mil (21 males);

Group B: 1,66–3,57 ng/mil (32 males);

Group C: 3,58-14,53 ng/mil (26 males).

We have evaluated the number of embryos at stage of 6-8 cells, obtained at the third day of embryo development, in these three different groups.

Participants/materials, setting, methods: Male partners of examinated couples had an average age of 27 ± 7,5 years, sperm concentration of 33,8 ± 6,2 mil/ml, progressive motility of 41,4 ± 8,3% and a typical morphology of 16,5 ± 3,5% according to Kruger's method.

In order to evaluate the concentration of p53 protein, we first proceeded to a DNA extraction with forensic method and then to a quantification p53 protein with ELISA-immunoenzymatic assay, expressed in ng/million of spermatozoa.

Main results and the role of chance: We have observed different percentage of embryo development at stage of 6-8 cells in the third day and different pregnancy rate (PR):

Group A: 101 embryos at 6-8 cells/ 147 total number of obtained embryos in this group (68,4%) and PR = 52,38%

Group B: 128/240 (53,5%); PR = 37,50%;

Group C: 79/216 (36,1%); PR = 7,69%.

These results support the hypothesis that an high concentration of p53 in human sperm DNA is associated to a low percentage of embryos able to reach the stage of 6-8 cells in the third day of development and also to a lower pregnancy rate.

So p53 levels can be considered as a predictive value to embryo development and pregnancy rate.

Limitations, reasons for caution: This study is preliminary. The number of couples examinated is limited. Some unknown confounding factors could not be considered. Further data are necessary.

Wider implications of the findings: Protein p53 is a sequence-specific transcription factor that responds to a wide variety of stress signals. Particularly quantitative research of p53 could be considered as a novel biomarker of sperm quality, able to predict the success of ART techniques, and could open a new road for infertility diagnosis.

Trial registration number: JHRS-01-18

P-098 Redox potential in human semen: validation and qualification of a quantitative electrochemical assay for measurement of oxidative stress

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Study question: Does an electrochemical assay using the MiOX^{sys} CE-marked analyser, consistently meet the requirements and specifications to reliably measure static oxidation reduction potential (sORP) in human semen?

Summary answer: The MiOX^{sys} assay is robust, performing according to expectations and has been standardised and validated to reliably and accurately measure sORP in human semen.

What is known already: Low levels of reactive oxygen species (ROS) are prerequisite for sperm function. Physiological levels of ROS are maintained by antioxidants (reductants) in the local environment. A disturbance in the balance between oxidants and antioxidants leads to oxidative stress (OS), impairing sperm function and genetic integrity. A novel method for assessing OS in semen involves measuring sORP using the MiOX^{sys} galvanostat-based technology analyser. ORP measures levels of oxidants and reductants, providing a more comprehensive measure of OS than measurement of ROS alone. Although the MiOX^{sys} assay effectively measures sORP in semen, it has yet to be fully validated.

Study design, size, duration: This study was a technical validation of an assay for measuring sORP using the MiOX^{sys} CE-marked analyser. It included 40 replicate measurements of control samples on different days and on two separate analysers. Intra- and inter-technician variability was determined from 40 replicates. The characteristics of sORP in semen and the effects of independent variables were measured in 6 replicate samples from 19 men. Variables included time from ejaculation, temperature, mechanical agitation and freeze-thaw action.

Participants/materials, setting, methods: The assay requires applying 30 µl sample onto a disposable sensor which is inserted into the MiOX^{sys} analyser and a current is applied. sORP is displayed in mV after 2-4 min. Values were recorded for different operators, days and analysers using multipurpose handling medium (Irvine Scientific, CA, USA) as a control. Semen samples were obtained from consenting men attending for routine semen analysis to determine the stability of seminal ORP under different conditions.

Main results and the role of chance: Measurement of sORP (mV) using the CE-marked MiOX^{sys} platform (Aytu Bioscience, CO, USA) demonstrated repeatability between 40 replicates of the same control sample with no significant difference between measurements (mean sORP (mV) ± SEM = 281.5 ± 4.8; CV = 0.1). Readings remained consistent across multiple operators ($p = 0.67$), separate analysers ($p = 0.95$) and on different days ($p = 0.09$). Additionally, there was no significant difference between sORP values for 40 replicates of a single semen sample (mean sORP (mV) ± SEM = 45.1 ± 1.0; CV = 0.1). Although readings remain relatively stable in semen up to 45 min post ejaculation ($p = 0.06$), there are significant variations in seminal sORP values across all samples by 60 min post ejaculation ($p < 0.005$). sORP in semen remains stable after freezing and thawing as values do not change significantly ($p = 0.47$). This was demonstrated across biological replicates ($n = 6$). There was a significant difference between sORP in semen after mechanical agitation using a bench-top vortex ($p = 0.005$) and between samples incubated at different temperatures (2-6°C, 20-24°C and 36 + 1°C; $p = 0.003$).

Limitations, reasons for caution: The validation of the MiOX^{sys} platform was carried out in one laboratory only. A multicentre validation would assist in confirming the reproducibility and reliability of the test.

Wider implications of the findings: This simple to use, cost effective assay could be implemented in conjunction with semen analysis providing a welcomed

addition to routine diagnostic testing for male infertility. It may be particularly relevant to men with unexplained infertility, whose partners experience a delay in conception, multiple assisted conception failures or miscarriages.

Trial registration number: Not applicable.

P-099 The addition of porcine oviductal fluid (OF) in swim-up media improves the selection and modifies motility patterns and capacitation potential of boar spermatozoa

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Study question: Could the OF improve the sperm selection in swim-up and modify sperm parameters?

Summary answer: Porcine OF improve sperm selection, modulates motility patterns and increase the percentage of spermatozoa with high calcium response after incubation in capacitated media.

What is known already: Previous studies have demonstrated that the use of porcine OF from late follicular phase or specific OF proteins provides sperm membrane changes modulating capacitation process and motility pattern (Coy et al, 2010 Theriogenology).

When OF was added to swim-up procedure and the oocytes were incubated in OF before IVF, embryo development was improved, DNA methylation and gene expression patterns were closer to *in vivo* embryos (Cánovas et al, 2017 eLife). However, the specific role of the OF addition in swim-up media on sperm parameter is not well known.

Study design, size, duration: NaturARTsPIG sperm swim-up media (Embryocloud, Murcia, Spain) were supplemented with different amounts of BSA and OF. As a control we used swim-up medium with 5 mg/ml of BSA (5BSA) and was compared to 1 mg/ml of BSA (1BSA), 1 mg/ml of BSA plus 1% of Porcine OF (NaturARTsPOF-LF, Embryocloud) (1BSAPOF) or 1% Porcine OF (POF). Then we analysed sperm recovery, morphology, motility and vitality and calcium levels as capacitation signal after samples were incubated in a capacitating media.

Participants/materials, setting, methods: Swim-up was prepared adding 1 ml of NaturARTsPIG sperm swim-up medium in a conical tube and 1 ml of sperm sample of boar with proven fertility at the bottom of the tube. After incubation 500 µl from the top were recovered. Sperm recovery rate and morphology were evaluated. Motility and motion parameters were assessed by CASA and simultaneously sperm vitality and calcium levels were measured by flow cytometer with propidium iodide and Fluo-3AM (14 replicates).

Main results and the role of chance: Swim-up resulted in a suitable sperm selection, increasing normal morphology from 83,5 ± 1,3% before swim-up to >90% after. Morphology selection was worse in 5BSA (90.6 ± 2.2%) than other groups (1BSA: 96.4 ± 1.5%; 1BSAPOF: 96.3 ± 0.9%; POF: 95.7 ± 1.1%, $p = 0.02$). In addition, changing BSA concentration and adding OF modified motility patterns and calcium response. These motility differences have been found in all motility parameters which were represented in a study of motility subpopulation, with lower values for OF groups than others (motility %, velocities). In this sense, after a subpopulation analysis, the POF group showed a lower percentage of high motility population than others (5BSA: 14.1%, 1BSA: 9.4%, 1BSAPOF: 9,9%, POF: 4,5%). On the other hand, sperm vitality (mean 68.1 ± 2.4%) was not affected by groups ($p = 0.50$). Regarding the calcium response after incubation in a capacitating medium, the percentage of spermatozoa with high calcium level was higher in groups with OF (1BSAPOF: 32.2 ± 3.3%; POF: 34.6 ± 3.4%) than without OF (5BSA: 20.8 ± 2.7%; 1BSA: 26.7 ± 2.4%) with significative differences ($p = 0.01$). These results confirmed OF can modulate sperm function, leading to a better status for fertilizing after the swim-up procedure.

Limitations, reasons for caution: Modulating capacity of the OF could be affected by concentration of some proteins in the system and further studies are necessary to optimize the sperm selection procedure, in terms of viably and healthy embryos after IVF.

Wider implications of the findings: Our results agree with other studies demonstrating that OF reduces motility parameters and protect spermatozoa from early capacitation. The control capacitation patterns could be involved in the improved embryo development. This information could be used as a model to improve fertilization rate and embryo quality in humans.

Trial registration number: not applicable.

P-100 The effects of melatonin on possible damage that will be occured on epididymis and sperm parameters with the coadministration of fructose and bisphenol a (BPA)

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Study question: Does melatonin have effects on epididymis and sperm morphology with the coadministration of fructose and BPA?

Summary answer: Administration of BPA, fructose, and concurrently both of these causes damaged to epididymis and sperm samples and melatonin were partially successful in preventing this damage.

What is known already: Nowadays, it is notable that the increase in the incidence of metabolic syndrome (MetS) and related clinical cases correlated with the increased of fructose rich food consumption and environmental and oral exposure to chemical substances known as endocrine disruptors. Fructose feed has recently increased and higher fructose consumption has associated with the increased of diseases such as dyslipidemia, insulin resistance, hypertension, hyperuricemia and infertility. BPA exhibits estrogen-like activity that cause toxicity in reproductive organs suppresses spermatogenesis, decreases sperm production and fertility rates.

Study design, size, duration: We used 42 adults, Sprague Dawley rats that were divided into seven groups. The groups were arranged as follows, Group 1 (n = 6, Control), Group 2 (n = 6, 10 % fructose), Group 3 (n = 6, only with 25 mg/bw/day BPA), Group 4 (n = 6, fructose 10% + 25 mg/bw/day kg BPA), Group 5 (n = 6, fructose 10 % +20 mg/bw/day melatonin), Group 6 (n = 6, 25 mg/bw/day BPA + 20 mg/bw/day melatonin), Group 7 (n = 6, fructose 10 %+ 25 mg/bw/day BPA + 20 mg/kg/day melatonin.

Participants/materials, setting, methods: After 8 weeks epididymal tissue was removed and analyzed by using histochemical (Hematoxylen-eosin, Masson's Trichrome and Silver impregnation stain) immunohistochemical (Zonula occludens-1 (ZO-1), Occludin (Occ) and Claudin-1 (Cl-1)) procedure. Sperm samples were counted, sperm morphology, motility and viability were investigated with Diff-quick and Eosin-Nigrosin stains. The apoptotic sperm cells were determined by flow cytometry.

Main results and the role of chance: Histopathologic changes were observed on the epididymis in BPA and BPA + Fructose groups and it was observed that melatonin has a protective effect on the histologic structural and morphological alterations of epididymis and sperm. When we semi-quantitatively compared the groups, ZO-1, Occ and Cl-1 immunostaining were decreased in BPA and BPA+Fructose groups compared to the control group, and these expression increased after melatonin administration. The total sperm count was not significantly different between the all groups ($p = 0.069$). Sperm motility increased significantly in the BPA + melatonin group compared to the BPA group ($p = 0.049$). Viability percentage of BPA + Fructose group was significantly lower than the control group ($p = 0.001$). The percentage of apoptotic sperm with Annexin V-FITC/PI staining in the BPA+Fructose+Melatonin group was lower than BPA+Fructose group ($P = 0,04$). Moreover, BPA and BPA+Fructose induced anomalies of the head and tail of sperm ($p < 0.05$). The anomalies head and tail of sperm were significantly lower in the BPA+Fructose +Melatonin group than BPA+Fructose group ($p = 0.018$, $p = 0.02$, respectively).

Limitations, reasons for caution: The *limitations* of the *study* are being an experimental animal model and immunohistochemical results should be supported quantitatively by using advanced molecular techniques.

Wider implications of the findings: Fructose and BPA have adverse effects on epididymis and sperm parameters. The decreased in immunoreactivity on epididymal tight junction proteins suggests that BPA and fructose can disrupt

the regular mechanism of blood-epididymis barrier and affect sperm maturation. This study have shown that melatonin can reduce the negative effects.

Trial registration number: not applicable.

P-101 Identification of seminal plasma proteins as potential biomarkers in men with primary infertility

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Study question: To identify molecular mechanism(s) and potential biomarkers of male infertility by comparing the seminal plasma proteome profile of fertile donors and men with primary infertility.

Summary answer: Altered protein expression profile in the seminal plasma of men with primary infertility may affect spermatozoa function, its use as a biomarker can be explored.

What is known already: Male factor is estimated to contribute to approximately 50% of all infertility cases. Primary infertility is the inability to father a child after 12 months of regular unprotected sexual intercourse. Basic semen analysis is the first step in the assessment of male potential. However, men with normal semen parameters can be infertile. There is a need for advanced methods to evaluate male fertility. Seminal plasma is a unique biological fluid that contains secretions from sex accessory glands. Therefore, the analysis of seminal plasma can serve as a great source for identification of key biomarkers of primary infertility.

Study design, size, duration: This study included seminal plasma samples from 13 men with primary infertility (without any female factor) and 13 proven fertile men. Following the basic semen analysis, seminal plasma was separated from the spermatozoa by high-speed centrifugation. For the proteomic study a pooled sample (n = 5) for each group was analyzed, and differentially expressed proteins (DEPs) identified. Validation of 7 selected proteins was carried out by Western blot (n = 8).

Participants/materials, setting, methods: Seminal plasma proteins were extracted and quantified. Following LC-MS/MS, Mascot, SEQUEST and X! Tandem tools were used to search the human reference database for protein identification. DEPs were identified based on normalized spectral abundance factor. Functional annotations, pathways and enrichment analysis were performed using GO, UniProtKB, Reactome, Metacore™ and DAVID. Network analysis was performed using both IPA™ and Metacore™ software databases. DEPs were selected for validation by Western blot and compared with proteomic analysis.

Main results and the role of chance: A total of 515 proteins were identified in the two groups, 48 were differentially expressed in primary infertility compared with proven fertile donors. In the primary infertility group, proteins related to diseases such as male infertility ($p < 0.0001$), azoospermia ($p = 0.005$), and oligozoospermia ($p = 0.02$) were underexpressed. Cellular assembly and organization, cell morphology and cell-to-cell signaling and organization were the main biological functions identified as dysregulated in primary infertility group. Proteins involved in cellular movement, free radical scavenging and cell-to-cell signaling and interaction such as: annexin-A2 ($p = 0.02$), cell division control protein 42 ($p < 0.0001$), peroxiredoxin-2 ($p = 0.03$), CD63 ($p = 0.005$) and transferrin ($p < 0.0001$) were overexpressed in the primary infertility group. On the other hand, proteins such as semenogelin-1 ($p = 0.001$) and semenogelin-2 ($p < 0.00001$), implicated in liquefaction and regulation of flagellated sperm motility, were underexpressed in the primary infertility group. The aforementioned DEPs were chosen to be validated by Western blot, however only annexin-A2 ($p = 0.03$) showed overexpression in the primary infertility group. This protein is a calcium regulated membrane-binding protein with high affinity to calcium. Dysregulation of calcium levels can be detrimental for key sperm processes such as capacitation, hyperactivation, acrosome reaction, and motility, which in turn may result in a failure of fertilization.

Limitations, reasons for caution: The number of samples used in this study is a major limitation. The use of only 13 different samples for each group is not enough to overcome the biological variability seen in the fertile and infertile male population.

Wider implications of the findings: The different seminal plasma proteomic profile of proven fertile men versus men with primary infertility suggests the important role of these proteins in the impairment of fertility. These proteins could be developed as biomarkers for diagnostic purposes. However, larger cohort studies are needed to justify their value as biomarkers.

Trial registration number: 'not applicable'

P-102 Exosomes, a bio-compatible delivery platform for mammalian sperm cells: A non-invasive approach for the transfer of therapeutic compounds

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Study question: Can naturally-synthesised exosomes rival the efficacy of engineered nanoparticles to mediate the delivery of compounds into gametes for potential clinical use?

Summary answer: In vitro exposure of sperm to in vivo-synthesised exosomes did not significantly affect motility. Thus, exosomes may represent an effective delivery-tool for compounds into sperm.

What is known already: Over recent years, exosomes have become widely utilised as an efficient tool to mediate directed delivery of nucleic acids, peptides, antibodies, fluorescent compounds and other small molecules into cells and tissue in a non-invasive manner. However, the potential application of exosomes for the delivery of therapeutic compounds to mammalian gametes has not yet been elucidated.

Study design, size, duration: This study aimed to develop a mammalian cell line (HEK293T cells) from which exosomes can be synthesised, isolated and characterised for their ability to deliver compounds to mammalian sperm. A fluorescent dye (BODIPY) was used to test the efficiency of association between exosomes and sperm and to evaluate internalisation and safety aspects.

Participants/materials, setting, methods: BODIPY-labelled exosomes were synthesised and characterised by Nanoparticle Tracking Analysis (NTA) and Western blotting. Different exposure times (1 h, 2 h and 4 h) were tested by incubation with pre-activated boar sperm (n = 3). Sperm motility was then assessed by Computer Assisted Sperm Analysis (CASA), sperm-exosome association was quantified by Metafer-4 analysis and localisation/internalisation was assessed by confocal microscopy.

Main results and the role of chance: NTA showed that synthesised exosomes had a mean diameter of 152.5 ± 5 nm and concentration of 4.55 × 108/mL. Furthermore, Western blotting showed bands of the expected sizes for the characteristic exosome markers: Alix, syntenin and CD9. The association rate of BODIPY-exosomes incubated with boar sperm increased in a time dependent manner (1 h, 19.0%; 2 h, 24.9%; 4 h, 26.8%). Moreover, a consistent trend was observed for both internalisation and multiple association to the sperm with increased incubation (internalisation: 1 h, 12.5%; 2 h, 18.0%; 4 h, 55.0%; multiple association: 1 h, 0.83%; 4 h, 1.63%). Association to the tail declined progressively (tail association: 1 h, 50%; 2 h, 46%; 4 h, 35%). Interestingly, no exosome-surface head attachment was observed after 4 h. Metafer-4 analysis indicated a relationship between association and the length of incubation (1 h, 5.33%; 2 h, 6.6%; 4 h, 7.38%). Finally, exposure to labelled-exosomes did not significantly influence sperm motility after 2 h of incubation ($p = 0.244$).

Limitations, reasons for caution: The data presented here is preliminary in nature and derived from only a small sample size, thus limiting statistical analysis. Furthermore, Metafer-4 analysis is unable to quantify sperm tail association, and association rates may have been under-represented.

Wider implications of the findings: Our preliminary data demonstrated the successful synthesis, labelling and characterisation of exosomes. *In vitro* exposure of labelled-exosomes to mammalian sperm showed encouraging patterns of association and did not affect sperm motility. An exosome delivery-platform represents a multifaceted tool for reproductive biology with which to investigate gamete structure, physiology and preservation.

Trial registration number: not applicable.

P-103 The diet quality of couples undergoing IVF

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Study question: Is the diet quality of men and women undergoing IVF adequate and is it correlated between partners?

Summary answer: The average diet quality of couples undergoing IVF does not meet Belgian dietary recommendations and is highly correlated between partners.

What is known already: Evidence on the association between reproductive health and dietary patterns of infertile men or infertile women is increasing. Infertility is a couples' condition. Yet, little is known on the correlation between the diets of partners undergoing IVF. Multiple methods to evaluate diets exist. Using a diet quality index has the added value of considering the complexity of food consumption patterns. It is an overall index to assess compliance with food-based dietary recommendations based on the quality, balance and variety of foods consumed.

Study design, size, duration: A cross-sectional observational survey was conducted between October 2016 and October 2017. Valid and reliable food frequency questionnaires (FFQ's) were disseminated online through the hospital e-platform to 396 Dutch speaking heterosexual couples about to start an IVF-cycle at a Belgian University fertility clinic. A total of 105 couples visited the e-platform. No reminders for filling out the questionnaire were sent.

Participants/materials, setting, methods: A total of 57 couples fully completed the FFQ, assessing portions and frequencies of foods consumed and their quality, balance and variety. Diet quality was described with an overall score between 0-100 and a cut-off <70/100 warranting need for dietary advise. Spearman correlations described inter-partner correlations. Clinical pregnancy (with fetal heartbeat) after one IVF and corresponding cryo-cycles was registered. Logistic regressions evaluated if the diet quality of couples was associated with their clinical pregnancy rate.

Main results and the role of chance: Participating men had a mean age of 36 (±6.7) and mean body mass index (BMI) of 26.6 kg/m^2 (±4.8). Participating women had a mean age of 33 (±4.8) and mean BMI of 23.6 kg/m^2 (±4.3). The overall diet quality was 70.5/100 (±12.7) for men and 73.2/100 (±10.1) for women. The diet quality of partners was positively correlated ($r = .537$, $p = .001$). On food (group) level, subfertile men and women did not meet the Belgian dietary recommendations. The vegetable intake (recommendation of 300 g/day) was inadequate, respectively on average 154 g/day in men and 146 g/day in women. The intake of processed foods (i.e. cookies, soft drinks, fast food) exceeded the recommendation of 10% of daily food intake, respectively 23.4% in men and 20.4% in women. One in three couples achieved a clinical pregnancy. After controlling for BMI and age, the average couples' diet quality of this small sample was not associated with their clinical pregnancy rate (OR 1.12, 95% CI .88-1.57).

Limitations, reasons for caution: Selection bias by health conscious couples is likely, which means that the diet of the average couple might be even more suboptimal. The small sample size and cross-sectional nature of this study does not allow causal inference.

Wider implications of the findings: Subfertile couples are in need of dietary advise, as was already reported for the general Belgian population. It would be interesting to examine whether advise improves reproductive health in large scale randomized controlled trails, which should include couples as partner's diet quality is correlated.

Trial registration number: Not applicable.

P-104 Impact of male partner chlamydia trachomatis seropositivity on infertility in unselected subfertile population

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Study question: Does *C. trachomatis* infection affect male fertility and pregnancy outcome? Is there association in lifestyle factors of male partners and female fertility?

Summary answer: We found a link between male chlamydial seropositivity and tubal factor infertility (TFI) in the female partner but no significant association in male semen parameters.

What is known already: The study results on the role of *C. trachomatis* and male fertility have been variable. *C. trachomatis* persistent infection might cause scarring of the ejaculatory ducts and loss of stereocilia or induce an autoimmune reaction inducing antisperm antibodies or autoantibodies. DNA fragmentation in sperm caused by *C. trachomatis* could affect the quality of the embryo and implantation. Fragmented DNA is a common finding in couples having a history of recurrent miscarriage. It has been reported that *C. trahcomatis* IgA and IgG but not heat shock protein 60 antibodies in male serum correlate negatively with semen characteristics and lower pregnancy rates.

Study design, size, duration: Altogether 224 subfertile couples were recruited in the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland, between July 2007 and December 2010 and follow-up continued until June 2014. Infertility work-up was performed according to our standard protocol after at least one year of unprotected intercourse. Data on lifestyle factors (smoking, alcohol consumption), previous illnesses, and medication was collected.

Participants/materials, setting, methods: Humoral immune responses to *C. trachomatis* were studied by measuring *C. trachomatis* MOMP specific IgG and IgA and CHSP60 specific IgG antibodies using ELISA kits (Medac Diagnostika, Hamburg, Germany) according to manufacturer's instructions. Results were obtained as a mean absorbance of duplicated samples at 450 nm. Less than 10% variation was seen in doublets (OD > 0.2). Semen samples of the male partners were analyzed according the criteria of World Health Organization (WHO).

Main results and the role of chance: The age of all male participants ranged from 21 to 49 (median 32) years with no difference between *C. trachomatis* seropositive or seronegative males. The prevalence of *C. trachomatis* IgG in male population was 10.3% (23/224), IgA 8.5% (19/224) and CHSP60 IgG 16.1% (36/224). The duration of infertility was significantly longer in couples with IgA seropositive males ($p = 0.04$) when compared to seronegative males. History of miscarriage was more common in couples with CHSP60 and IgA antibody positive male partners than antibody negative male partners ($p = 0.04$ and $p = 0.05$, respectively. No significant difference on live birth rate or need for IVF occurred between couples of chlamydial seropositive and seronegative males. There was no significant association in male semen parameters and chlamydial seropositivity. Male seropositivity correlated with TFI in the female partner ($p = 0.007$), but not with male factor infertility ($p = 0.43$). Alcohol consumption by a male partner was associated with a history of *C. trachomatis* infection ($p < 0.001$) and with TFI of the female partner ($p = 0.003$), while male smoking was associated with unexplained infertility of the couple ($p = 0.01$).

Limitations, reasons for caution: The rate of *C. trachomatis* antibodies were low in our study population leading to small study groups. The results on semen parameters were based on one sample. Knowing that the quality of semen can vary within the same individual this can have effect on the results.

Wider implications of the findings: In our study, we found significant association between male lifestyle factors and TFI, thus couples should be informed on the effect of healthy lifestyle on fertility. According to our results, not only female but also male chlamydial infection has an important effect on fertility of the couple.

Trial registration number: not applicable.

P-105 Open testicular mapping: a new and less invasive testicular sperm extraction (TESE) procedure

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Study question: The aim of this study was to evaluate the successful sperm recovery rate (SSR) of open testicular mapping (OTEM) in non-obstructive azoospermic men.

Summary answer: Testicular sperm viable for Intracitopalsmic Sperm Injection (ICSI) was found in 50 (54%) of 92 azoospermic men.

What is known already: In 1999, Schlegel *et al.* reported on novel microsurgical techniques for TESE.

In Schlegel's hands this technique of microdissection resulted in an improvement of sperm retrieval rates (SRR) from 45 to 63%

Despite initial good results, the wide opening of the tunica albuginea and potential vascular damage are the controversial aspects of this technique.

Testicular microdissection SSR range from 34% (Japanese Nationwide Survey) to 63% (initial Schlegel results)

Study design, size, duration: Retropective study of laboratory files from patients presenting with non obstructive azoospermia from 2008 to 2016 evaluating 92 patients submited to OTEM for ICSI or cryopreservation.

Participants/materials, setting, methods: After delivered, the testis were mapped for sperm production without albuginea opening.

Patients were divided in two groups according to sperm recovery for purpose of analysis: group 1 (G 1), men with sperm found, and group 0 (G 0), men with no sperm recovered.

Age, FSH level and testicular volume (TV) were compared between groups 1 and 0 using the Statistical Package for Social Sciences (SPSS), 21.0 version, with significant values below five percent ($p < 0.05$)

Main results and the role of chance: SSR was 54% (50/92). Statistical difference was found for male age, mean 38 years for G 1 and 34 for G 0. FSH and TV had no statistical difference between the groups.

Limitations, reasons for caution: Histologic evaluation and laboratory exams were done in different laboratories may leading to non uniform readings and a wide range of normal values.

Wider implications of the findings: We present a new and less invasive option for sperm retrieval for patients presenting with non obstructive azoospermia with similar results to testicular microdissection.

Trial registration number: not applicable.

P-106 Differential expression of exosome-associated proteins in seminal plasma of infertile men with unilateral varicocele

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Study question: Does compromised delivery of sperm maturation factors by defective seminal exosomes cause sperm dysfunction in infertile men presented with unilateral varicocele?

Summary answer: Altered expression of seminal plasma proteins involved in the exosome-sperm fusion pathway may be a contributory factor for infertility in patients with unilateral varicocele.

What is known already: Exosomes are cell-derived vesicles that are either released from the cell upon fusion of an intermediate endocytic compartment (the multivesicular bodies) with the plasma membrane or directly from the plasma membrane. Seminal plasma provides a favorable environment for spermatozoa and exosomes released from the epididymis, prostate and seminal vesicles. Seminal exosomes carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions. Though proteins associated with exosome function are involved in energy pathways, protein metabolism, cell growth and maintenance, there is paucity of information on the role of seminal exosomes on sperm function in general and varicocele in particular.

Study design, size, duration: Semen samples were collected from 33 infertile patients with unilateral varicocele from March 2012 to April 2014 and from 10 proven fertile healthy male volunteers. Proteomic profiling of seminal plasma was carried out in Finnigan LTQ linear ion trap mass spectrometer LC-MS/MS system. Differentially expressed proteins (DEP) in the unilateral varicocele patients to that of the fertile controls were evaluated.

Participants/materials, setting, methods: We compared the proteome of seminal plasma from unilateral varicocele patients ($n = 5$) with that of fertile men ($n = 5$). Relative quantification of proteins was estimated by spectral counts (SC) and their abundance determined based on the normalized spectral abundance factor. In silico analysis was done using Cytoscape and Metacore platforms to identify the top-most enriched pathways and DEPs involved in regulating the upstream transcription receptors respectively. Exosome-associated DEPs were validated by Western blot (WB) analysis.

Main results and the role of chance: Of the 47 DEPs identified in the seminal plasma, three were unique to the unilateral varicocele group and one to the fertile donor group. Network analysis predicted 13 DEPs (APOD, SERPINF2, ORM1, TSPAN1, CRISPLD2, Rab-27A, CD13, MME, KLK11, TMPRSS2, DPP4, TGM4 and GLOI) to be under the regulation of Androgen receptor affecting normal sperm function. Other transcription factors YB-1 (associated with fertility), SP1 (related with reproductive processes) and NRF2 (antioxidant gene expression) were the principal upstream regulators of most DEPs. Bioinformatic analysis revealed the altered expression of vital proteins involved in regulation of vesicle fusion and positive regulation of endocytosis related to exosomes. We demonstrated the presence of exosomes in the seminal plasma of both, the patients and the control group by WB expression of CD63 (exosomal marker). Annexin A2 (ANXA2), a calcium-regulated binding protein responsible for fusion of exosomes was significantly upregulated (2.49-folds; $P = 0.0016$) and transferrin (TF), involved in positive regulation of endocytosis showed a 1.19-fold ($P = 0.1257$) increase indicating impaired fusion of exosomes with spermatozoa in varicocele patients. Therefore, it is suggested that exosomes were unable to deliver the regulatory factors/elements resulting in impaired maturation of spermatozoa in varicocele patients.

Limitations, reasons for caution:

- We did not purify the exosomes for proteomic studies, thus decreasing the specificity of the technique.
- Samples were pooled to overcome the biological variation in high-throughput experiments.

Wider implications of the findings: Aberrant expression of exosome function associated proteins hinders the transfer of exosomal elements/factors to the spermatozoa essential for maturation process. Validation of additional DEPs may further strengthen our hypothesis. These findings could help clinicians identify the exosomal dysfunction as one of the causes of infertility in patients with unilateral varicocele.

Trial registration number: Not Applicable.

P-107 Taste receptors expression in human sperm: possible chemosensors for sperm-oocyte attraction

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Study question: Are taste receptors (TAS) implicated in the capacitation and reprogramming of spermatozoa during their successful trip toward the oocyte?

Summary answer: Human spermatozoa express a number of taste receptors and their localization is modified by sperm capacitation and ligand interaction.

What is known already: Recent studies showed the expression of several TAS in mammalian testis and sperm, suggesting their possible role in fertility. The taste receptor ligands are single amino acids, nucleotides, sugars, proteins, acids and other molecules; their presence in the female genital tract imply a possible role for taste receptors as chemosensor during the journey of sperm towards the fertilization site. To this regard, it has been demonstrated that glutamate concentration is very high in the uterus but it decreases in the fallopian tube, thus suggesting the possibility that taste receptors may use this gradient to guide the sperm towards the oocyte.

Study design, size, duration: We enrolled for this study a total of 30 patients referring to our Centre for Couple Sterility (median age 36 years; range18–58) undergoing semen evaluation during an infertility diagnostic screening, from October 2016 to February 2017, at the Centre of Couple Sterility, Siena University Hospital.

Participants/materials, setting, methods: Patients have been enrolled after signing an informed consent. Ejaculated sperm were collected and seminal parameters were monitored according to WHO 2010 guidelines. Taste receptor expression/localization was confirmed at the protein level by western blot and immunofluorescence. We analysed, by quantitative RT-PCR, in ejaculated sperm the gene expression profile of the genes involved in the transduction cascade elicited by TAS. Finally, we also evaluated the TAS expression/localization after exposure to TAS ligands, agonist/antagonist.

Main results and the role of chance: In ejaculated human sperm we demonstrated the expression of several taste receptors: TAS1R1, TAS1R2, TAS2R3, TAS2R4, TAS2R14, TAS2R19 and TAS2R43. Immunofluorescence showed a different localization of these receptors in basal and capacitated conditions; the modification of their expression pattern seems to be compatible whit motility hyperactivation and predisposition to acrosomal reaction. Indeed, during their journey through the female genital tract, sperm are subjected to modifications that induce a functionally reprogramming or capacitating enabling the sperm to became able to fertilize. Western blot analysis confirmed these modifications in basal and capacitated conditions, with different protein isoforms detected. Since the molecules involved in sperm capacitation and chemoattraction are still widely unknown, we *in vitro* tested some TAS ligands selected from the literature, such as genistein, daidzein and trihydroxyflavone. Our data demonstrated a time and dose-dependent significant impact on sperm parameters, such as motility and vitality. Therefore, investigations on these molecular pathway could be interesting for increasing the outcome of ART procedures.

Limitations, reasons for caution: In this pilot study we analyzed only normozoospermic patients. The inclusion of patients with altered semen parameters (oligozoospermic, astenozoospermic) is mandatory in order to validate these findings.

Wider implications of the findings: Further studies might contribute in better understanding the physiologic role of taste receptors in sperm-oocyte attraction and recognition. This should be crucial to clarify the mechanism underlying fertilization, thus to provide a chance for ameliorating the in vitro conditions, in order to improve IVF outcome.

Trial registration number: Not applicable.

P-108 To evaluate outcome of methylxanthine theophylline in frozen thawed testicular sperm in intra cytoplasmic sperm injection cycles

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Study question: To evaluate whether methylxanthine theophylline can be used routinely in frozen thawed testicular sperm in intra cytoplasmic sperm injection (ICSI) cycles.

Summary answer: Theophylline increases sperm motility, which may contribute towards higher fertilization, improved embryo formation and better pregnancy rates.

What is known already: Cryopreserved thawed spermatozoa are used to avoid further damage to the testis. While reduced motility of extracted sperm and affect of cryopreservation represent a persistent problem in finding a viable sperm for ICSI. Over the years, many chemical agents have been tried to over come this problem like: caffeine and other methylxanthines. Pentoxifylline turned out to be an effective tool in stimulating motility in fresh and cryopreserved human sperm. Interestingly, theophylline, a closely related molecule, has been investigated for this purpose to a much lesser extent and no randomised control study is reported to its show benefit.

Study design, size, duration: A randomized controlled study was conducted from 1st January 2015 to 31st September 2017 where normal responder women undergoing frozen testicular sperm ICSI cycles were randomized using computer generated list and closed opaque envelops into 2 groups, group (A) sperm treated with theophylline and group (B) not treated with theophylline. All A grade embryos were vitrified at day 3 (7–9 cells) or day 5 (Blastocyst) and transferred in the subsequent frozen embryo transfer cycle.

Participants/materials, setting, methods: Eighty women were randomised in group A (n = 36) and B (n = 34),rest 10 women were excluded due to <4 oocyte retrieved or left the cycle. All A grade embryos in both groups were vitrified and warmed using same protocol by same embryologist on 7-9 cells and blastocyst stage.Primary outcome of the study was clinical pregnancy rate and secondary outcome was fertilization rate. Statistical analysis was performed using SPSS for Windows version 20.

Main results and the role of chance: Descriptive characteristics of the patient in group A and group B like Age, BMI, total dose of FSH, total dose of HMG, days of stimulation, Metaphase II oocytes were found to be comparable. The sperm selection was easier in the group A than group B due to increased motility in thawed testicular sperm. The fertilization rate in group A were 67.2% vs. group B 46.4% (95% CI 13.6–27.7%, $p < 0.001$) and mean Grade-A embryo formation rate in group A were 2.97 ± 1.424 vs. group B 2.24 ± 0.741 (95% CI 0.191–1.283, $p = 0.023$) were significantly higher in group A compared to group B. Results were comparable for both the groups for clinical pregnancy rate 44.4% vs. 29.4% (95% CI -7.4% - 35.4%, $p = 0.193$) however, it was higher in the group A than group B.

Limitations, reasons for caution: Need large sample size.

Wider implications of the findings: Theophylline increase selection of viable sperm due to increased motility. This may contribute towards higher fertilization, improved embryo formation and increased clinical pregnancy rate. This can also reduce the burden on embryologist for finding viable sperm. Further studies may be required for better understanding of potency of theophylline.

Trial registration number: MCDH/2015/7.

P-109 The relevance of Annexin A5 in male fertility

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Study question: The study aimed to investigate the relationship of the haplotype M2/ANXA5 and male fertility parameters.

Summary answer: Our study does not show any biologically relevant effect of lower levels of annexin A5 on male fertility parameters.

What is known already: Annexin A5, which functions as an anticoagulation protein, is coded by the ANXA5 gene. The haplotype M2/ANXA5 decreases the ANXA5 gene promoter region activity and mRNA expression. The carrier status of the haplotype M2/ANXA5 is an important risk factor for recurrent pregnancy loss during the early stages of pregnancy.

In rabbits, annexin A5 is the main protein of the seminal plasma and influences all sperm parameters. Yet, the role of annexin A5 in men is currently underresearched.

Study design, size, duration: This study consists of a retrospective selection and analysis of the clinical data from our patient cohort (n = 340; 165 severe oligozoospermic and 175 normozoospermic), taking into consideration a new genetic diagnostic evaluation.

Participants/materials, setting, methods: Genomic DNA from blood was sequenced with Sanger sequencing. The frequency of the haplotype M2/ANXA5 was then compared between the groups and compared with the general population. The clinical data from the patient groups was evaluated according to their genetic variant of the promoter region for ANXA5. The hormonal parameters were FSH, LH, testosterone, SHBG, free-testosterone, prolactin and estradiol. Sperm parameters evaluated were ejaculated volume, concentration, total count, motility, morphology, vitality and accessory glands markers.

Main results and the role of chance: Association of M2 carrier status with oligozoospermia was first assessed in the two comparably sized groups of oligozoospermic and normozoospermic patients. Both groups were in HWE at $p = 0.773$ and $p = 1.000$, respectively. The M2 carrier rate was 24.2% for oligozoospermic and 17.7% for the normozoospermic patients (table 1). The comparison of both groups yielded an OR of 1.5 (95%CI 0.9 to 2.6), not achieving significance. When comparing the oligozoospermic patients with the general population control group, the OR was 1.8 (95% CI 1.1 to 2.8), $p = 0.009$. The only statistically significant differences between the carriers and non-carriers of the haplotype M2/ANXA5 could be observed in the prolactin and α-glucosidase levels when looked in the normozoospermic patients. The prolactin levels mean in the non-carriers and M2 carriers was 196 mU/l and 167 mU/l, respectively. The α-glucosidase levels mean was 78.3 mU and 113 mU in the non-carriers and M2 carriers group, respectively. In the oligozoospermic patients, there is also a decrease in prolactin levels and an increase in α-glucosidase levels. However, these variations are small and not statistically significant. Selection of patient cohorts may account for the observed differences or lack of them.

Limitations, reasons for caution: The study was carried out with patients attending a human reproduction service, thus the results may not reflect the differences present in the general population.

Wider implications of the findings: The patients carrying the haplotype M2/ANXA5 do not present a clinical profile, so genetic screening is advisable to avoid recurrent pregnancy loss.

Trial registration number: not applicable.

P-110 Timing of intrauterine insemination in relation to human chorionic gonadotropin trigger following superovulation with clomiphene citrate

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Study question: Does the timing of intrauterine insemination in relation to ovulation trigger with human chorionic gonadotropin (hCG) affect the outcomes of clomiphene citrate cycles?

Summary answer: Intrauterine insemination (IUI) performed 19–23 hours after the administration of hCG has comparable clinical pregnancy rates with those performed 24–36 hours after trigger.

What is known already: Several factors affect IUI outcomes, including female age, total number of inseminated motile spermatozoa (TM), number of recruited follicles, and infertility diagnosis. The timing of IUI is also important given the limited fertilization window of the ovulated oocyte(s). Yet, the optimal insemination timing is not well defined.

Study design, size, duration: It is a retrospective cohort study at an academic medical center. All IUI cycles performed between 2004 and 2013 were reviewed. To control for female age and stimulation protocol, we included only clomiphene citrate cycles of women ≤35 years old with normal uterine cavity and patent fallopian tubes.

Participants/materials, setting, methods: A total of 741 clomiphene citrate/IUI cycles were included. Cycles were divided into two groups based on the number of motile inseminated spermatozoa ($<15 \times 10^6$ and $\geq 15 \times 10^6$). Within each group, the clinical pregnancy rates (CPRs) were compared between IUI performed within the first 23 hours after hCG to those who had their IUI ≥24 hours after hCG. χ^2 and Fisher's exact tests were used for categorical variables. Values were expressed as mean ± standard deviation.

Main results and the role of chance: Within the group of patients with an adequate number of motile inseminated spermatozoa ($\geq 15 \times 10^6$) (n = 618 cycles), the CPRs were comparable between those who underwent IUI within the first 23 hours after hCG (range: 19–23 hours) (n = 71 cycles) and those ≥24 hours (range: 24–36 hours) (n = 547 cycles) (15.5% vs. 20.2%, respectively, $P = 0.3$). There was no significant difference in terms of female age (32.4 ± 2.9 vs. 31.8 ± 2.7 years old, $P = 0.2$), number of dominant follicles larger than 18 mm at the time of trigger (1.7 ± 0.8 vs. 1.8 ± 0.1, $P = 0.1$), or peak endometrial thickness (7.9 ± 2.3 vs. 7.9 ± 2.3 mm, $P = 0.9$) between cycles of the two timing groups. Similarly, among the cycles in which the number of inseminated spermatozoa was $<15\times10^6$, IUI performed within the first 23 hours after hCG had comparable CPR with those ≥24 hours (7.6% vs. 14.4%, $P = 0.3$). Female age (31.7 ± 2.1 vs. 31.7 ± 2.7 years old), number of dominant follicles (1.8 ± 0.8 vs. 1.9 ± 0.9), and peak endometrial thickness (7.5 ± 2.1 vs. 7.8 ± 2.3 mm, $P = 0.6$) were not significantly different between the two timing groups.

Limitations, reasons for caution: Although this is a retrospective study, the adjustment for all potential confounding factors such as female age, number of dominant follicles, stimulation protocol, peak endometrial thickness, and number of inseminated motile spermatozoa strongly supports the accuracy of our findings in investigating the influence of IUI timing on pregnancy outcomes.

Wider implications of the findings: Timing the IUI as early as 19 hours after hCG administration yields comparable clinical pregnancy rates with IUI performed as late as 36 hours after ovulation trigger. This reassuring wide range of IUI timing grants providers and patients more flexibility when scheduling IUI without impairing the outcomes.

Trial registration number: N/A.

P-111 Differential sperm proteomic profile between sperm samples achieving pregnancy or not in intracytoplasmic sperm injection (ICSI) cycles in oocyte donation program

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Study question: Are there any differences between sperm samples that failed vs. those achieving pregnancy regarding the sperm proteomic profile after ICSI cycles?

Summary answer: This study reveals a differential protein profile between sperm samples that failed vs. those achieving pregnancy, able to be used as sperm fertility biomarkers.

What is known already: There is a lack of sperm fertility markers, and several studies demonstrated that idiopathic male infertility is multifactorial. Although is believed that sperm are cells translational and transcriptionally silent, their functionality can be studied using proteomic approaches. Many proteins have been already identified having an important role in sperm motility, sperm-oocyte's zona pellucida interaction, metabolism, apoptosis, cellular cycle, meiosis, membrane transport, and ribonucleotide acid regulation.

Furthermore, some previous works from our group described differential molecular factors in sperm samples from which a pregnancy was obtained compared with those failing, defining molecular characteristics for physiologically competent sperm.

Study design, size, duration: Descriptive, prospective and non-randomized biomedicine study evaluating the proteomic profile of spermatozoa from patients' ejaculates where pregnancies were (Group pregnant (P), n = 4) or were not (Group non-pregnant (NP), n = 4) achieved after ICSI in an oocyte donation program aiming to standardize female factor. Aliquots from the same samples employed in ICSI procedures were collected, frozen until the reproductive results were known, and then assigned to their corresponding group and analysed.

Participants/materials, setting, methods: Eight sperm samples from infertile males undergoing ICSI cycles in oocyte donation program with normal sperm parameters and total progressive motility >5mill were included in our study, 4 of them failed to achieve pregnancy and 4 who succeeded. Proteins were separated and analysed by means of SWATH-MS (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra). Proteins are available via ProteomeXchange: identifier PXD006309. All proteins abundances were compared statistically between P and NP groups.

Main results and the role of chance: We identified 2228 proteins, 53 of them were found in different abundance between sperm samples, being 37 significantly more abundant in sperm samples in P group, and 16 less abundant in this same group compared with NP. We applied Elastic-Net statistic method to identify the 27 proteins best performing to differentiate both groups. Also, we use PLS-DA analysis (Partial Least Squares Discriminant Analysis) verifying that the samples are classified accordingly to their group. Finally, we applied a *VIP-score* test over Elastic-Net and PLS-DA, in order to determine which proteins have more importance in achieving pregnancy. We obtained 27 proteins with high *VIP-score* in Elastic-Net, and 300 in PLS-DA.

Then we applied functional analysis over the proteins obtained in the analysis PLS-DA+*vip-score*, obtaining 5 proteins more abundant in P group with reproduction functions (NME5, SPATA20, DNAI1, ARMC4 and AK7) and 10 less abundant in NP group (GNB2L1, PDIA4, YWHAZ, SYNGR1, FNTB, LCP1, NDUFS4, COG3, STOM and GIT1) with metabolic functions.

Finally, we identified 11 proteins present in PLS-DA+*VIP-score* analysis, located in membrane, susceptible to be selected by MACS (Magnetic-activated-cell-sorting) to develop a new system to select the best spermatozoon.

Limitations, reasons for caution: Other molecular factors not included within this analysis could be involved in sperm fertility given that sperm function has been demonstrated multifactorial.

The low number of samples studied make us be cautious about our findings, but lead us to design a focused research on the main findings with bigger sample-sizes.

Wider implications of the findings: The description of proteins linked to sperm fertility, open new possibilities regarding the development of male fertility diagnostic tools, culture media formulations, or, to design new sperm selection tools based on these molecular traits, using MACS of spermatozoa exhibiting specific proteins associated with the best reproductive results.

Trial registration number: This study is not considered as a trial study.

P-112 Sperm DNA fragmentation index (DFI) does not increase with increasing number of treatment cycles

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Study question: Do men undergoing numerous IVF cycles have higher amount of sperm DNA strand breaks than those who experience only one cycle?

Summary answer: Men in couples who have undergone numerous cycles do not have higher mean DFI then those who have undergone one.

What is known already: Sperm DNA strand breaks, measured as DNA fragmentation index (DFI) by sperm chromatin structure assay (SCSA) is a clinically useful marker of male infertility, and a couple's chance of pregnancy. However, it is still unclear if high DFI is more common in men going through many IVF treatment cycles compared to those achieving pregnancy in the first.

Study design, size, duration: Prospective, clinical study, in which n = 2968 men were consecutively enrolled during the period 2007–2018, at Reproductive Medicine Centre, Malmö, Sweden. Sperm samples were collected and analysed before each cycle. In total, DFI from 4894 fresh treatment cycles ranging from cycle number 1-7 were included.

Participants/materials, setting, methods: Approximately 5000-10 000 sperm cells were analyzed by SCSA. Analysis of the flow cytometric data was carried out using dedicated software.

Main results and the role of chance: In the first cycle there were 2460 DFI values, second cycle 1402 DFI values, third cycle 755 DFI values, fourth cycle 190 DFI values, and in cycles 5-7 87 DFI values were available. The mean DFI value for the first, second and third cycle was 16%, respectively, the fourth cycle 19%, and the 5^{th}-7^{th} 17%. There was no statistically significant difference in DFI between the cycles (p trend = 0.058).

Limitations, reasons for caution: The number of men was decreasing with numbers of IVF cycles completed.

Wider implications of the findings: Men who have undergone many IVF/ICSI are not presenting with higher DFI than those who enter the assisted reproduction treatment.

Trial registration number: Not applicable.

P-113 Intrauterine insemination: predictive factors regarding pregnancy outcomes for women aged 35 and over

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Study question: From what age pregnancy rates in intrauterine insemination (IUI) decrease? Among these women, what sperm parameters are necessary to get pregnancy in IUI?

Summary answer: There is an interest of IUI in women aged 35 and over when spermatic parameters allow to reach the needed thresholds.

What is known already: As women's age increases, specialists of assisted reproductive technologies (ART) tend to offer an in vitro fertilization (IVF) whereas the necessary conditions for an IUI were sometimes reunited. There is no threshold concerning women's age. Furthermore, the number of inseminated motile spermatozoa definitely has an impact on the pregnancy outcome after IUI, but with variable thresholds. The sperm morphology impact of is more questionable, and only the initial morphology (on total sperm) has been studied.

Study design, size, duration: We have performed a prospective study in Poissy Hospital Center, between January 2015 and June 2017. A multivariate logistic regression analysis was used to determine the predictive value of each parameter in clinical pregnancy outcome after 12 weeks. A p value of 0.05 was considered as statistically significant.

Participants/materials, setting, methods: We have studied couples with primary infertility and benefits from an IUI cycle after ovarian stimulation with follicle-stimulating hormone (FSH) and mono-follicular answer. For each IUI, sperms count and motility of post-wash sperm preparation (sperm preparation for IUI) were performed with computer-assisted method. A smear of this preparation was stained to establish percentage of typical spermatozoa (David classification). The number of inseminated motile spermatozoa (NIMS) and the number of inseminated normal spermatozoa (NINS) were calculated.

Main results and the role of chance: 433 couples with 990 IUI cycles were included in the study. The clinical pregnancy per cycle was 11.8%. According to a multivariate logistic regression analysis, women's age is the only predictive factor of clinical pregnancy (p <0.004) in this population. A 35-year threshold was shown to be predictive for success, with higher rates of pregnancy among women under 35 (14.1% vs. 8.1%, p = 0.0038).

The second part of the study focuses on women 35 years and older (383 cycles). According to the multivariate logistic regression analysis, the NIMS and the NINS are the only predictive factors of the occurrence of a clinical pregnancy in women over 35 years-old. In this population, regardless of women's age, women's body mass index (BMI), and the number of attempts, the rates of clinical pregnancies are statistically higher:

- if NIMS > 7.25 million (11.4% vs. 5.3%, OR adjusted 2.21 [1.04, 4.93], p = 0.04)
- or if NINS > 1.56 million (10.6% vs. 4.8%, OR adjusted 2.33 [1.05, 5.69], p = 0.04)
- and if NIMS and NINS are above the previously described thresholds (3.9% vs. 10.7%, OR adjusted 3.37 [1.22, 7.97], p = 0.02).

Limitations, reasons for caution: It's a monocentric study, and we only included mono-follicular cycles. Only a few centers use David classification to improve sperm morphology.

Wider implications of the findings: We demonstrated the value of IUI in women aged 35 and over when spermatic parameters allow to reach the thresholds previously found, with rates of progressive pregnancy per cycle higher than 10%, which is the equivalent of pregnancy rates, reported in the general population in IUI, regardless of the age.

Trial registration number: not applicable.

P-114 New computational approach for the analysis of quantitative proteomics: Identification of patient-specific altered pathways in seminal fluid from infertile men

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Study question: Could novel computational methods to analyze quantitative proteomic data be designed to seek for patient-specific altered pathways in seminal fluid?

Summary answer: Patient-specific altered protein pathways in seminal fluid were determined in individual infertile patients, by identifying deregulations in the stable-correlated proteins established in the control population.

What is known already: The current classification of infertile men is limited to the evaluation of seminal parameters. However, a wide range of factors alters both sperm and seminal fluid compositions, leading to a high heterogeneity within patients sharing the same phenotype. Quantitative proteomics analyses provide a valuable tool in the field of male reproduction to identify proteins in semen as biomarkers to better stratify the different subgroups of infertile patients. Nevertheless, no candidates were validated to date, probably due by the misclassification of the patient groups. Novel approaches for data analysis are demanded to overcome these limitations.

Study design, size, duration: Quantitative proteomic data derived from the TMT-10Plex isobaric labeling followed by mass spectrometry identification of 16 samples of seminal plasma were used for this computational methodology. The samples were separated in four groups according to the World Health Organization criteria: 4 normozoospermic patients with normal seminal parameters (NZ), 4 oligozoospermic patients with low sperm concentration (OZ), 4 asthenozoospermic patients with altered sperm motility (AS) and 4 azoospermic patients (AZ) without presence of sperm cells.

Participants/materials, setting, methods: Stable-correlated proteins were determined in each group of patients by applying the following statistical principle: 2 proteins (with more than 1 peptide quantified each) were highly-correlated when ≥90% of their peptides has a Pearson correlation coefficient ≥0.9. The repetition of this analysis with control group samples (NZ) by adding a case sample once a time allows determining changes on the control profile of stable-correlated proteins, leading to the identification of deregulated pathways in individual samples.

Main results and the role of chance: A total of 1175 correlations comprising 78 different proteins were identified in the control population (NZ). Interestingly, BASP1, a protein required for the activation of the oocyte, showed the highest number of correlations with other proteins (58). In contrast, the number of identified stable-correlated proteins was drastically reduced in patients presenting altered seminal parameters (126 in AS, 0 in OZ and 6 in AZ). These results reflect the high heterogeneity present in infertile patients, as well as the multiple causes that might be affecting to the seminal parameters. To overcome these limitations we used the methodology mentioned above to assess potential specific-altered pathways in individual patients. The repetition of the analysis after adding patient AS-2 to the control group resulted in few changes in the stable-correlated proteins profile. This suggests that AS-2 contains a seminal plasma protein signature similar to the control group. Interestingly, this patient was taking MACA, an herbal supplement considered to improve male fertility. In contrast, when this analysis is repeated by adding other patients one by one, deregulations in specific pathways are revealed: glycolysis for AS-1, OZ-1, AZ-1 and AZ-2, the response of oxygen reactive species for AS-4, and the complement system for OZ-3 and AZ-4.

Limitations, reasons for caution: This computational statistical study has been conducted with a small sample size. It is considered a first pilot study to design the algorithms that will be used in a wider sample population.

Wider implications of the findings: This study represents the first step for the generation of new methodologies leading to the identification of patient-specific molecular deregulations. This would contribute to an improvement in the classification of the infertile patients, as well as to the achievement of a personalized medical care in the treatment of infertility.

Trial registration number: N/A.

P-115 Comparison of ICSI outcomes with hypo-osmotic swelling (HOS) reactive sperm and motile sperm on sibling oocytes in patients with severe asthenospermia

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Study question: Is there any difference between motile sperm and immotile HOS reactive sperm from fresh/frozen and ejaculate/testicular sperm samples in terms of embryo development?

Summary answer: Since the effect of using HOS reactive or motile sperm on fertilization rates, HOS reactive sperm can be used in asthenospermic patients.

What is known already: Considering that the prevalence is approximately 18% for isolated asthenospermia and abouth 63% for asthenospermia associated with oligospermia or teratospermia; this cases have a great importance for ART centers. It is known that the motility and viability of spermatozoa and their origin from ejaculate or testicular biopsies are important predictors in terms of fertilization, pregnancy and live birth rates. In order to improve the outcome of ICSI in these cases, various methods have been described for the selection of the immotile but viable spermatozoa.

Study design, size, duration: A retrospective cohort study was performed using database of University affiliated Assisted Reproductive Technology Center from 2011 to 2017. The inclusion criteria were patients with isolated male infertility etiology (total motile sperm count<10% from ejaculate or testicular biopsies) under the age of 39 (male/female) and ICSI cycles in which the total number of oocytes was higher than the total motile sperm counts in order to compare both motile and immotile sperm results in sibling oocytes.

Participants/materials, setting, methods: 491 oocytes obtained from the 49 cycle were evaluated. Considering that the injected sperm was motile (Group 1) or immotile HOS reactive(Group 2) study groups were formed. Each groups divided in 4 subgroups according to sperm sample type (fresh or frozen) and origin (ejaculate or TESE). If the absence of motile sperm, HOS reactive sperm had to chosen for ICSI. Fertilization, implantation, live birth, cleavage (CQS) and blastocyst quality score (BQS) were compared throughout individual embryos.

Main results and the role of chance: The total fertilization rate was 43.1% (n = 211) and there was no differences in group 1 (47.5%, n = 105) compared with group 2 (39,4%, n = 106) including comparison between ejaculate and

TESE subgroups. When fresh or frozen sperm samples in Group 2 were considered, the fertilization rate of fresh ejaculate sperm was significantly higher than frozen ejaculate sperm (46.2% vs 10.0%, p = 0.02), while there was no significant difference in the fertilization rates between fresh and frozen testicular sperm (50.4% vs 43.3%, p = 0.42). When quality scores of embryos obtained by using motile or immotile HOS reactive sperm with randomized sibling oocytes were evaluated, there was no significant difference between day2 CQS, day3 CQS and day5 BQS between the two groups. According to the number of transferred embryos (n = 74), there was no difference between group 1 (51.4%, n = 38) and group 2 (48.6%, n = 36) (p = 0.26). The implantation and live birth were only achieved when fresh testicular sperm had been used. 4 live birth were achieved with fresh/immotile HOS reactive/TESE sperm and 1 pregnancy was achieved with fresh /motile /TESE sperm.

Limitations, reasons for caution: The limitations of the study were the small sample size and HOS test could not be confirmed with different methods of selecting viable sperm from among immotile sperms. The main disadvantages of HOS test is related to false positive as well as false negative results.

Wider implications of the findings: There is no difference between motile sperm and HOS reactive sperm in terms of clinical outcome in ICSI cycles. In contrast to testicular sperm, HOS test could not predict spermatozoa viability following freezing / thawing procedure of ejaculate sperm.

Trial registration number: The study is not a clinical trial.

P-116 Human sperm protein extraction for proteomic analysis: Effect of different lysis buffer on quality and quantity of extracted proteins

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Study question: Are there quantities and qualities variation in human sperm proteins extracted with different lysis buffer?

Summary answer: Lysis buffer containing Urea/triton yielded higher levels of the proteins with better quality in human sperm cell.

What is known already: Molecular studies in protein level of sperm is increasing due to the crucial role of proteins in normal functions of spermatozoa. High purity and concentration of extracted protein is essential for protein analyses. Based on the types of the cells and intended proteomic approaches, proper lysis buffer must be selected. The specific lysis buffer should be considered for sperm protein extraction because of the special features of sperm such as high amounts of membrane associated proteins and intensive nuclear compaction.

Study design, size, duration: In this experimental study, the semen samples of 70 normozoospermic men who referred to Royan institute for assisted reproductive treatments were collected between April 2017 and November 2017.

Participants/materials, setting, methods: This study comprised two experiments. At the first, the pooled semen samples were divided to the seven equal groups and diluted in urea/ thiourea, urea/ nonidet P-40 (NP40), urea/ sodium dodecyl sulfate (SDS), urea/triton, RIPA, Trizol and Qiagen kit lysis buffers. The extracted proteins were quantified through the Bradford assay and SDS-PAGE. In second experiment, the subcellular localization of specific proteins which extracted with high yielded lysis buffers, were quantified by western blotting.

Main results and the role of chance: In the first experiment, the protein concentration was significantly high in urea/ thiourea, urea/ NP 40, urea/ SDS and urea/triton lysis buffers in compared to the others. The highest yield of the proteins was related to urea/triton which was not significantly different between four mentioned lysis buffers. In the second experiment, the expression of cytoplasmic (β actin, α tubulin, GAPDH, Lactate dehydrogenase C, heat shock protein 60) and mitochondrial (Malate dehydrogenases, and ATP synthetize 5 A) proteins were not significantly different, although the expression level of nuclear protein such as histone H3 and membrane associated protein like pan cadherin were higher in urea/SDS and urea/triton lysis buffers respectively.

Limitations, reasons for caution: Lower concentration of the extracted proteins in some lysis buffers such as urea/SDS, was a limited factor for the number of possible replicates in the western blot technique.

Wider implications of the findings: According to our results, urea/triton lysis buffer could be a proper suggestion for extraction of whole sperm proteins. However, other lysis buffers would be suitable for extraction of specific proteins that are localized in the particular subcellular regions.

Trial registration number: 0

P-117 Testicular sperm retrieval in azoospermic male cancer survivors

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Study question: Can testicular sperm extraction offer azoospermic cancer survivors a chance for fertility and a possible route to biological fatherhood?

Summary answer: Successful sperm retrieval can be achieved in men who have received intensive chemotherapy treatment, however larger studies are required to determine predictors of success.

What is known already: Sperm cryopreservation is routinely offered to men with cancer prior to undergoing gonadotoxic treatments such as chemotherapy. For children, young adolescents and azoospermic men, cryopreservation prior to treatments may not be possible, and hopes for biological children rest on recovery of sperm following treatments. Azoospermia in adults following treatment modalities can be treated with surgical sperm retrieval techniques (TESE), however success rates in this patient group are unknown and there is not enough data to confirm patient selection for this invasive procedure. We present our experience.

Study design, size, duration: A retrospective case note analysis of 28 patients with a history of past cancer treatment who underwent surgical sperm retrieval for azoospermia at a tertiary fertility clinic within a 3 year period.

Participants/materials, setting, methods: Patients with a history of past cancer treatment who underwent TESE for azoospermia at a tertiary Fertility clinic.

Main results and the role of chance: 28 patients aged 22-66 years (median 37) underwent TESE. The diagnoses included 11 patients with testicular cancers, 6 patients with lymphomas, 3 patients with leukaemia and the remaining 8 patients had other types of cancers. 11/28 (39.2%) had mature sperm successfully retrieved, 16/28 (57.1%) were unsuccessful and 1/28 (3.6%) had partial success (2 grossly abnormal sperm seen). In the testicular cancer group 5/11 (45%) had successful TESE with 3 showing normal spermatogenesis, 1 showing maturation arrest and 1 showing sertoli cell only syndrome (SCOS) upon histological analysis. In lymphoma group, 2/6 (33%) had successful TESE, with histology showing 1 patient with SCOS and 1 with normal spermatogenesis. None of the 3 patients with history of acute leukaemia, total body irradiation and bone marrow transplant had successful TESE. However, 1 patient with history of rhabdomyosarcoma had a successful procedure, indicating the potential for fertility in men surviving intensive treatment modalities. Testicular histology in all unsuccessful TESE showed SCOS in all but 2 patients, the remaining having fibrotic or totally sclerosed tissue. 4 patients with normal spermatogenesis and 1 with maturation arrest indicates that azoospermia in the post-chemotherapy male may be attributed to complications of cancer treatment other than gonadotoxicity.

Limitations, reasons for caution: Although TESE is widely used for patients with cancer, there is little published research studying the outcome of TESE in azoospermic men following chemo/radiotherapy. Larger studies will be required to help in defining the determining predictors of success. Outcome data for use of retrieved sperm in fertility treatments were unavailable.

Wider implications of the findings: Our data shows 39.2% success with TESE for this patient group. This signals an opportunity for this patient group to achieve fertility, including those who have received intensive treatments.

Prospective patients should be counselled for the undetermined success rates, which may remain low in some groups.

Trial registration number: not applicable.

P-118 Adipokines expression in seminal fluid of normal-weight men

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Study question: The aim of the study was to investigate expression profile of adipokines in seminal fluid of normal-weight men and compare their seminal and plasmatic concentrations.

Summary answer: Adipokines profile expression in seminal and blood plasma is different: many adipokines, including some inflammatory markers, are present in seminal fluid in higher concentrations.

What is known already: Infertility affects 14% of childbearing-age couples and a male causal factor is involved in 20-50% of cases. Unlike women, little data is available about relation between body-mass index and spermatic parameters. It is however known that obesity is associated with impaired male fertility through a decrease of sperm quality. We hypothesized that adipokines, produced by adipose tissue, could interfere with gonadal function. Leptin's role in the interaction between energy metabolism and male reproductive system is well-known. Recently, some novel adipokines have been identified in seminal fluid and their seminal concentration is likely to be correlated with some morpho-functional spermatic parameters.

Study design, size, duration: Seminal and blood samples from 70 healthy men consulting for couple infertility were collected in two Assisted Reproductive Centers (Tours, FERTIPROTECT protocol, n = 32, and Paris, METASPERME protocol, n = 38) between 2015 and 2017. Subjects aged between 20 and 40 years, had normal BMI (20-24,5 kg/m^2) and normal semen analysis according to 2010 World Health Organization guidelines. Men with metabolic disorders were excluded. Informed consent was signed by each participant.

Participants/materials, setting, methods: Adipokines were analysed in seminal fluid and blood plasma using three different methods. First, human adipokine-arrays, testing simultaneously 58 adipokines, were performed on seminal and blood samples from 6 patients enrolled in Tours. The identified adipokines of interest were then quantified by enzyme-linked immunosorbent assay (ELISA) and Western-Blot analysis in the samples from Paris Centre (n = 38) and Tours Centre (n = 20), respectively.

Main results and the role of chance: Preliminary adipokine-arrays (n = 6) showed that blood concentrations of adiponectin, leptin, chemerin and resistin were significantly higher than seminal ones. Interestingly, others adipokines, such as visfatin, vaspin, fibrinogen, IL-8, CCL-2, FGF-19, calgranulin and angiopietin-1, were significantly more expressed in seminal fluid than in blood plasma. These results were confirmed by successive analyses. Western-Blot analysis evidenced that visfatin, FGF-19, VEGF, IL-8, HGF and CCL-2 concentrations were significantly higher in seminal fluid than in blood plasma ($p<0,0001$). Similarly, significantly higher visfatin and IL-6 concentrations in seminal fluid were confirmed by using ELISA assay ($p<0,0001$). On the other hand, adiponectin, leptin, chemerin and resistin were predominantly expressed in blood plasma ($p<0,0001$ for all adipokines, except for resistin, $p = 0,01$), then confirming different adipokines profile expression in seminal fluid and peripheral blood.

Limitations, reasons for caution: Our study shows that adipokine expression in seminal fluid is different from blood plasma. However, the meaning of a predominant expression of some adipokines in seminal fluid, notably including inflammatory markers, remains to be elucidated. We can only speculate that adipokines may play a role in male fertility.

Wider implications of the findings: Different adipokines profile expression in seminal and blood plasma suggests the possibility of a gonadal production or a compartment-specific regulation of adipokines in reproductive tract and peripheral blood. Immunohistochemistry characterisation of adipokines, currently in progress in spermatozoa and different testicular cellular types, could allow us to elucidate adipokines metabolism.

Trial registration number: METASPERME Protocol: AOM 10020 – NI 10033 – ID-RCB 2011-A01052-3

FERTIPROTECT Protocol: approved by Ethical Committed of Leonard de Vinci Clinic.

P-119 Association between sperm DNA damage extent, fertilisation and cumulative live birth rates among infertile couples undergoing assisted reproduction treatments

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Study question: Is there any association between sperm DNA Fragmentation Index and cumulative live birth rate (CLBR) following up to three IVF/ICSI cycles?

Summary answer: If the first treatment was IVF, couples with DFI $\geq^3$20% at initiation of treatment had statistically significantly lower CLBR as compared to those with DFI<20%.

What is known already: Several studies have shown that high DFI is associated with lower chance of live birth, mainly in IVF and, to a less degree in ICSI cycles. However, these observations are made on single IVF/ICSI cycles. From a clinical point of view, it seems most relevant to look at the CLBR following all treatments offered to a couple. In many countries where the IVF/ICSI treatment is supported by the public health service, the couples are offered up to 3 fresh cycles and frozen embryo transfers emerging from these treatments.

Study design, size, duration: The study is a large cohort longitudinal analysis of retrospective data from August 2007 to August 2017 in a tertiary medical centre, at the Reproductive Medicine Centre at the Skåne University Hospital, Sweden. In total 5443 cycles in 2730 eligible consecutively included couples with 1280 children born were analysed.

Participants/materials, setting, methods: All IVF/ICSI treatments (up to 3 fresh plus frozen) offered by the public health care were included. DFI value used for analysis was, assessed by SCSA at the initiation of the treatment. The couples were divided into them starting with IVF and those with ICSI as first treatment. Switching between IVF and ICSI, during the treatment course, were based on standard laboratory parameters, sperm number after gradient centrifugation and fertilisation/good quality embryo in previous cycle(s).

Main results and the role of chance: Using linear regression we found negative association between DFI and fertilization rate for IVF ($p<0.0001$) but not for ICSI ($p = 0.89$).

Using Kaplan-Meier analysis with subsequent log rank test, adjusted for the female age, we found statistically significantly higher CLBR for those with DFI<20% at initiation of treatment as compared to couples with DFI^3 ≥20%, if

the first method used was IVF (56% vs 51%; p<0.02), whereas it was not case if the first treatment was ICSI (54% vs 53%, p = 0.97).

These calculations based on the assumption that patients who did not return for IVF/ICSI cycles would have the same chance of a pregnancy resulting in a live birth as patients who continued treatment. were confirmed by calculations based on assumption that none of the women who did not return for a subsequent cycle would have had a live birth.

Limitations, reasons for caution: Despite our robust methodological approach, the presence of biases related to retrospective design cannot be excluded.

Wider implications of the findings: Our results, demonstrate that selection of the first method for assisted reproduction, based on the results of SCSA analysis, has a statistically significant impact on the CLBR.

Trial registration number: Not relevant.

P-120 Is fertility preservation justified in male patients with congenital adrenal hyperplasia (CAH)?

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Study question: Is sperm cryopreservation relevant in patients with congenital adrenal hyperplasia (CAH), especially in those having testicular adrenal rest tumours (TARTs)?

Summary answer: Men with CAH should benefit from a sperm cryopreservation at the onset of puberty, before the occurrence of TART and/or before alteration of sperm parameters.

What is known already: CAH is a genetic autosomal recessive defect in the adrenal steroidogenesis pathway. 21-hydroxylase deficiency is the most common form, leading to glucocorticoid and in more severe cases, mineralocorticoid deficiency associated with androgen excess. The clinical phenotype is classified classic (with or without salt-wasting) for the severe form or non-classic for the mild form. In men with CAH, infertility is an important complication in which (TARTs) are the most frequent cause. TARTs can strongly contribute to the occurrence of oligospermia or azoospermia through an obstructive or a deleterious paracrine effect. Therefore, several authors have recommended sperm cryopreservation in patients with CAH.

Study design, size, duration: This was a retrospective and observational study including CAH male patients, addressed to the CECOS (sperm bank) of Paris Cochin Hospital between 2008 and 2017 for sperm cryopreservation. Clinical, hormonal and testicular ultrasound data were collected as well as sperm parameters before freezing and after thawing.

Participants/materials, setting, methods: Twenty CAH patients between 15 and 37 years old, with or without TARTs were included. Initial sperm analysis was performed in all patients. Sperm cryopreservation was performed in 15 men and sperm parameters before and after freezing were analysed. Data were compared between the group of patients with TARTs or without TARTs. All samples were analysed according to the WHO 2010 recommendations. The potential use of straws in assisted reproductive technology (ART) was evaluated.

Main results and the role of chance: Mean age of the patients was 22.1 +/- 5.6 years old. 85[i1] % of them had a 21-hydroxylase deficiency, and 75% classified as salt-wasting TARTs were present in 65% of the patients. Median total sperm count (33.8×10^6) and sperm concentration (12.1×10^6 /ml) was low according to the WHO criteria. 25% (5/20) of the patients showed azoospermia and 25% (5/20) showed oligospermia while 50% (10/20) had normal sperm. All patients who were azoospermic had TARTs (5/5). Severe oligozoospermia (<5 M/ml) was significantly more frequently observed than moderate oligozoospermia (between 5 and 15 M/ml) or normozoospermia (>15 M/ml) in patients with TARTs compared to patients without TARTs (p = 0 .04). Sperm concentration and total sperm count were significantly lower in patients with TARTs than in patients without TART (respectively p = 0.02 and p = 0.03). The[DV2] post-thawing sperm progressive motility was significantly lower in all patients (p = 0.001) and particularly in patients with TARTs compared to sperm progressive motility before freezing (p = 0.01). The mean total number of motile spermatozoa per straw was low, at 0.7×10^6, allowing their use only in ICSI in 73% of the patients in case of a future fertility project and regardless to the fertility check-up in the female partner.

Limitations, reasons for caution: CAH is a rare disorder, explaining the low number of patients included. The same id observed in the scientific litterature. Our study was a retrospective observational study and a prospective longitudinal study with a larger sample would be necessary to confirm these first results about sperm cryopreservation in CAH patients.

Wider implications of the findings: We have confirmed that patients with CAH and especially those with TARTs, could have severe altered sperm parameters, with high rate of azoospermia. Sperm cryopreservation should be proposed as soon as possible, before the occurrence of TARTs and/or alteration of sperm parameters, in order to improve their chance to procreate.

Trial registration number: Not applicable.

POSTER VIEWING
Embryology

P-121 Effects of the oral oxytocin receptor antagonist nolasiban on early embryonic development

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Study question: Nolasiban, an oral oxytocin receptor antagonist, may increase rates of implantation and live-births among patients undergoing ART. The embryonic safety of nolasiban was demonstrated using rat and rabbit models.

Summary answer: Nolasiban showed no teratogenic or embryotoxic effects in rats and rabbits, nor effect on postnatal development and learning abilities of rats at the doses tested.

What is known already: Antagonism of oxytocin receptors may enhance uterine receptivity by decreasing uterine contractions and by improving endometrial perfusion. A meta-analysis of 7 studies showed significant improvements in clinical pregnancy rate after administration of oxytocin receptor antagonist (OTR) atosiban. To enable safe clinical testing of nolasiban, a novel, oral OTR, it is critical to evaluate its effects on early embryonic development.

Study design, size, duration: We administered nolasiban to rats and rabbits from implantation and throughout embryonic development at doses of 0, 37.5, 75 and 125 mg/kg/d and of 0, 125, 200 and 300 mg/kg/day, respectively, to assess fetal safety.

Participants/materials, setting, methods: Purpose-bred pregnant rats (50/group) and rabbits (20/group) were treated daily from gestation day (GD) 6-17 and 7-19, respectively. Females underwent cesarean section before term and external, visceral and skeletal examinations of fetuses were performed.

Additional rat groups were allowed to litter and offspring was assessed for mortality, behavior, learning abilities and reproductive performance.

Main results and the role of chance: Nolasiban had no effect on pregnancy parameters of pre- and post-implantation loss or the number of implantations or live fetuses per female. There were no effects on fetal weight or fetal sex ratio and no malformations. Treatment had also no effect on clinical observations including body weight, food intake developmental hallmarks such as vaginal opening and testes descent, behavior and learning abilities and mating and reproductive performance. The number of observed litters were sufficient to exclude nolasiban effects and in line with international testing guidelines.

Limitations, reasons for caution: The translation of animal findings to man should be made with caution.

Wider implications of the findings: In addition to previously reported peri-implantation safety data, these studies confirm the absence of harmful nolasiban effects on early fetal development, paving the way for developing nolasiban for use in ART. Our findings raise a potential to administer nolasiban beyond the current single-dose administration on the day of embryo transfer.

Trial registration number: N/A.

P-122 Time intervals from the hCG trigger: analysis of different checkpoints and their impact on embryo development, implantation and pregnancy

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Study question: To analyse the impact on reproductive outcome of different time intervals at each stage of an ART procedure, from triggering of final oocyte maturation.

Summary answer: In ICSI cycles, we observed better pregnancy and implantation rates when interval between oocyte retrieval and denudation was < 2.5 hours.

What is known already: Previous reports have observed a greater number of available embryos and a significant increase in fertilization and clinical pregnancy rates when oocyte collection was close or even after 36 h from the hCG trigger. Other authors have also proposed delaying oocyte retrieval to optimize oocyte maturation.

Modifying oocyte incubation time could also affect reproductive outcomes and synchronise nuclear and cytoplasmic maturation. Some authors concluded that the incubation of oocytes approximately 1.5-2 hours between collection and denudation significantly influences the implantation rate.

Study design, size, duration: An initial set of 843 patients performing ART treatment with their own oocytes during a one-year period was reviewed. After exclusion of 145 patients with less than 3 oocytes retrieved, a cohort of 679 patients was analysed. Among them, 336 underwent ICSI and 343 conventional IVF insemination. We analysed the number of oocytes retrieved, the maturity rate, fertilisation rate, and blastulation rate, as well as implantation and clinical pregnancy rates at different time intervals.

Participants/materials, setting, methods: Patients were grouped into the following time-intervals:

(1) hCG trigger to oocyte retrieval (<35 and >35 h; early and late retrieval)
(2) oocyte retrieval to denudation (<2.30 and >2.30 h)
(3) denudation to ICSI (<10 and >10 min)
(4) oocyte retrieval to IVF (<2 and >2 h) and.
(5) administration of hCG to IVF or ICSI (<38 and >38 h).

Continuous variables were compared by T-test; categorical variables were compared by X^2 test.

Main results and the role of chance: One hundred and eighty seven patients (early retrieval group) and 492 (late retrieval group) were compared, showing similar age (37.7 ± 4.0 vs. 37.3 ± 3.8, p = 0.17) and total number of eggs retrieved (8.4 ± 6.2 vs. 9.2 ± 6.1, p = 0.12). In ICSI cycles, we observed a higher number of good quality embryos on day 3 (54.2% vs. 63.1% p = 0.002), and better blastocyst development (41.4% vs. 48.9% p = 0.0006) in the late retrieval group, which also showed a trend towards better oocyte maturity rate, although not significant (p = 0.06). Also in ICSI cycles, there was an increase in clinical pregnancy (42.3% *vs.* 23.2%, p = 0.005), ongoing pregnancy (34% *vs.* 19.6%, p = 0.03) and implantation rates (28% *vs.* 16%, p = 0.007) when time between oocyte collection and denudation was less than 2 h 30 min. These differences between groups were not present in IVF cycles, were both groups showed comparable outcomes. This could be related to the fact that the exact timing of fertilisation events and gamete fusion are difficult to evaluate and cannot be determined precisely.

Limitations, reasons for caution: This is an analysis of time intervals in an unselected population, where most retrievals were done in a narrow window between 35 and 36 hours of hCG trigger. Subtle differences between patients or undetected premature luteinization could affect these results.

Wider implications of the findings: Even a slight flexibilization of the trigger timing can have a substantial impact on treatment outcome because it has an influence on oocyte maturation and quality. Nonetheless, extending the culture of the oocytes prior to ICSI could have a negative effect, potentially linked to the premature ageing of the oocyte.

Trial registration number: Not applicable.

P-123 Comparing early embryo morphokinetics between patients with lower and normal ovarian response by using time-lapse microscopy in assisted reproductive technology

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Study question: Are there differences on early embryo morphokinetic parameters and cleavage stage patterns between patients with a low and normal ovarian response to controlled ovarian stimulation?

Summary answer: We did not find any statistically significant difference in early morphokinetic parameters and cleavage patterns between the two patients groups.

What is known already: In the era of time-lapse microscopy (TLM) and elective single embryo transfer, early embryo morphokinetic parameters seem to play a significant role to select the embryo with the highest implantation potency. Patients with a low ovarian response have fewer fertilized oocytes hence fewer embryos for transfer than the normal responders. Little is known about embryo developmental patterns in low responders compared with patients with a normal ovarian response and if a similar morphokinetic model can be applied in both groups.

Study design, size, duration: Retrospective study on early embryo morphokinetics and cleavage patterns in 606 oocytes, where 435 oocytes derived from 47 patients with a normal ovarian response and 171 oocytes derived from 47 patients with a low ovarian response. Data were collected from May 2015 to November 2017 and extracted from our Embryoscope database.

Participants/materials, setting, methods: Number of aspirated oocytes and AMH were used to allocate patients into two groups. Low responders had AMH ≤ 7.8 pmol/L and less than 7 oocytes, while normal responders had AMH 10-35 pmol/L and 8-15 oocytes. Time of PN fading (tPnf), time to two cells (t2), three cells (t3), four cells (t4), asynchrony in 2nd cell cycle (S2), fragmentation, multinucleation, direct cleavages from 1-3 cells, reversed cleavage, rolling and non-tetrahedral shape were recorded.

Main results and the role of chance: Student's t-test was used to compare the timings between the groups and Chi-square test to compare proportions. The parameters that did not follow the normal distribution were analyzed with Mann- Whitney U-test. The timings of the early cell divisions did not differ statistically (p-value >0.05) between the low and normal responder group (t2: 29.0 h vs 28.1 h, t4: 40.4 h vs 39.6 h). Additionally, no statistically significant difference was observed for irregular cleavage patterns (26.5 % vs 35.1 %), multinucleation (53.8 % vs 53.4 %), fragmentation and non-tetrahedral shape rate at the 4-cell stage. The groups did not differ regarding fertilization and cleavage rate as well as the number of embryos that fulfill the ESHRE criteria for top quality characteristics on Day 2.

Limitations, reasons for caution: The retrospective nature of the study and the small number of patients limit the potential power. Prospective studies are required to confirm the validity of our results.

Wider implications of the findings: It is reassuring for patients with lower ovarian response and for clinicians to know that embryos derived from these patients are not prone to more prolonged and abnormal cleavages than the embryos from normal responders. Also, the use of a similar time-lapse morphokinetic model for both groups is possible.

Trial registration number: Not applicable.

P-124 Comparison of neonatal outcomes of births conceived from day 1 rescue ICSI and conventional ICSI cycles

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Study question: Are the neonatal outcomes of births conceived from day 1 rescue ICSI different from births generated during conventional ICSI cycles?

Summary answer: neonatal outcomes of births conceived from day 1 rescue ICSI were comparable with the births generated during conventional ICSI treatment, including the incidence of abnormalities.

What is known already: A limited number of reports on day 1 rescue ICSI children are currently available; the available data and a systematic study are lacking.

Study design, size, duration: The study was a retrospective analysis of 74 singletons born from day 1 rescue ICSI cycles in fresh cleavage-stage embryos transfers, 38 singletons in frozen-thawed cleavage-stage embryo and 71 singletons in frozen-thawed blastocyst-stage embryo.To evaluate day 1 rescue ICSI on neonatal outcome, comparison groups (conventional ICSI) were used: 148 singletons born in fresh cleavage-stage embryos transfers 56 singletons in frozen-thawed cleavage-stage embryo and 142 singletons in frozen-thawed blastocyst-stage embryo.

Participants/materials, setting, methods: Conventional ICSI cycles were chosen as a control group to exclude possible influences of the insemination technique. To eliminate the effects of age, the female ages were matched between rescue ICSI cycles and conventional ICSI cycles (one rescue ICSI cycle was matched with two conventional ICSI cycles).

Main results and the role of chance: No significant differences were found between rescue ICSI cycles and conventional ICSI cycles with respect to the birth weight of singletons (3334.99 ± 338.59 g vs. 3331.52 ± 511.70 g) and gestational age (39.04 ± 1.44 vs. 38.61 ± 1.86 weeks) in fresh cleavage-stage transfers, and there were no significant differences with respect to the birth weight of singletons (3352.14 ± 599.33 g vs. 3406.21 ± 474.17 g) and gestational age (38.69 ± 1.47 vs. 39.09 ± 1.32 weeks) in frozen–thawed transfers and no significant differences with respect to the birth weight of singletons (3526.06 ± 434.15 g vs. 3505.90 ± 421.43 g) and gestational age (39.23 ± 1.54 vs. 38.88 ± 1.54 weeks) in blastocyst-stage transfers. Rate of congenital birth defects of the rescue ICSI group were similar to those in the conventional ICSI groups.

Limitations, reasons for caution: Additional cases are in need of study, especially with respect to the long-term health and development of day 1 rescue ICSI children.

Wider implications of the findings: The current study showed that the neonatal outcomes of births conceived from day 1 rescue ICSI are comparable with conventional ICSI treatment. Additional cases are in need of study, especially with respect to the long-term health and development of day 1 rescue ICSI children.

Trial registration number: The study was approved by the Ethics Committee of Peking University Third Hospital (reference no. 20080612) and all patients signed written informed consent.

P-125 Identifying general markers of human cumulus cells enables precise analysis for predicting human oocyte quality

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Study question: Are flow cytometric analyses useful in detecting the biological features of cumulus cells relevant to predicting oocyte quality?

Summary answer: We successfully identified several general markers of human cumulus cells that could be used to sort and characterize these cells.

What is known already: Many studies have tried to predict oocyte quality by analyzing the surrounding human cumulus cells. For example, transcriptomic analyses identified candidate genes expressed in cumulus cells that were related to oocyte quality. However, the results of such studies remain controversial. As proposed by us in 2017 at the annual ESHRE meeting, several specific markers of cumulus cells must be identified for precise analysis and quality assessment of oocytes to avoid irrelevant information from contaminating peripheral blood cells and dead cells.

Study design, size, duration: From June 2016 to October 2017, cumulus cells were collected from intracytoplasmic sperm injection-treated patients who provided informed consent. The total number of patients was 26.

Participants/materials, setting, methods: The age of patients ranged from 30 to 47 years. After hyaluronidase treatment of the cumulus-oocyte complexes (COCs), cumulus cell samples were collected for staining with antibodies and analysis by flow cytometry (Gallios, Becton-Dickinson, USA). In cases where patients provided more than 2 COCs, the cumulus cells derived from the COCs were pooled. Alternatively, to compare the character of cumulus cells from individual COCs, cumulus samples from each COC were separately prepared.

Main results and the role of chance: The cumulus cell samples collected included 63.1% ± 15.95% dying/dead cells, indicated by staining with propidium iodide (PI). The PI-negative living cells contained10.7% ± 9.52% CD45-positive blood cells and CD235a-positive erythrocytes, suggesting contamination of the suspension by surrounding blood cells during oocyte retrieval. After screening out the blood cells by flow cytometry, the remaining non-hematopoietic cells expressed CD49a, activated leukocyte cell adhesion molecule, CD49f, and CD56 on the cell surface.

By further isolating the $PI^{-}CD45/CD235a^{-/dull}CD49f^{+}$ cells, cumulus cells were purified and confirmed by expression the Cyp19a1 gene using real time RT-PCR. Expression comparisons of these surface proteins on cumulus cells from individual COCs showed no remarkable differences, even in the presence of distinct oocyte developmental outcomes. Furthermore, by using JC-1 dye as a mitochondrial potential sensor, we could stain mitochondria in the cumulus cells.

These results suggest that flow cytometry and cell sorting are useful for precise analyses of cumulus cells.

Limitations, reasons for caution: The current work involves only a small number of samples, and large-scale analyses of cumulus cells should be done in the future.

Wider implications of the findings: Purification of human cumulus cells hold the promise of enabling precise transcriptomic analysis. In addition, flow cytometric analysis itself could provide simple screening of the condition of human cumulus cells associated with prediction of oocyte quality.

Trial registration number: Not applicable

P-126 Laboratory factors affecting the occurrence of multinucleated blastomeres

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Study question: Do clinical and laboratory factors have an influence on the occurrence of multinucleation?

Summary answer: The occurrence of multinucleation among human embryos may be affected by laboratory factors such as culture media.

What is known already: Multinucleated blastomere (MNB) is a nuclear abnormality, and has been reported to be associated with poor embryo development and low implantation rates. Accordingly, embryos with MNB are usually not the first choice for embryo transfer. Furthermore, it has been reported that the frequency of MNB is high in human embryos cultured in vitro, and it

may have an effect on the development of aneuploidy among human embryos. However, it is unclear what kinds of factors have an impact on the development of MNB. We aimed to investigate whether clinical and laboratory factors may affect the occurrence of MNB embryos.

Study design, size, duration: This is a retrospective observational study conducted at Kyono ART clinic Takanawa in Japan from January 2015 to April 2017. A total of 1509 embryos from 302 cycles of 189 patients were included in this study. All embryos were monitored by time-lapse cinematography.

Participants/materials, setting, methods: We compared the backgrounds of the embryos which had at least one MNB between day 1 to day 3 embryo culture for age factor, fertilization method (c-IVF or ICSI), ovarian stimulation protocol (GnRH antagonist, agonist long, agonist short and others) and culture media (two different single culture media). Chi-squared and Fisher's test were used for statistical analysis. A P value of less than 0.05 was considered statistically significant.

Main results and the role of chance: The overall incidence of MNB was 33.7% (509 / 1509). MNB embryos showed lower blastulation rates (50.7%) compared to non-MNB embryos (55.9%), although this was not statistically significant ($P = 0.055$). The good morphology blastocyst (Gardner's grade 3BB or more) formation rate in the MNB embryos was significantly lower (18.3%) than that of the non-MNB embryos (25.1%) ($P = 0.003$). There was no significant difference in the MNB occurrence rate in terms of age factor (patients aged under 40 y.o. vs over 40 y.o.; 35.5% vs. 31.2%), and fertilization method (c-IVF vs ICSI; 33.6% vs. 33.8%). Also, there was no significant difference in MNB rate in terms of ovarian stimulation protocols. Culture media tended to have an impact on MNB incidence rate (media A vs B; 16.2% vs. 27.5%, $P = 0.067$) although this was not statistically significant.

Limitations, reasons for caution: The small sample size may have influenced these results. The details of culture media which have affected MNB is unclear.

Wider implications of the findings: MNB affects subsequent developmental potential of embryos, and these events may be affected by laboratory factors such as culture media. MNB formation may be related to an increase in aneuploidy rate and mosaic formation rate by using different culture media.

Trial registration number: not applicable.

P-127 Blastocyst development rates of embryos with morphokinetic variables and the effect of morphokinetic variables on clinical pregnancy rates in vitrified-warmed single blastocyst transfers

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Study question: How many embryos exhibiting morphokinetics variables could develop up to blastocyst stage? And how much the blastocysts were affected by morphokinetics variables on pregnancy?

Summary answer: The blastocyst development rates of embryos with morphokinetic variables were more than 40% and the clinical pregnancy rates of the blastocysts vitrified-warmed were adversely affected.

What is known already: In time-lapse cultures, morphokinetic variables are used to refer to two concepts; morphological assessment parameters and morphokinetic assessment parameters. Morphological assessment parameters include uneven pronuclei, blastomere, and multinucleation etc. Morphokinetic assessment parameters include direct division, reverse division, and rapid division etc. Morphokinetic variables generally have a fatal influence on the embryo development, implantation and pregnancy rates in fresh cycles. There are a few reports on the impact of various morphokinetic variables on blastocysts developments; and, there are a few reports on the effect of morphokinetic variables on vitrified-warmed blastocyst transfer cycles.

Study design, size, duration: Morphokinetic variables include uneven pronuclei, blastomere, multinucleation, and direct division, rapid and irregular division. Regularly developed embryos which are not observed in any kind of morphokinetic variables were compared with the embryos observed in morphokinetic variables. We conducted a review between January 2014 and November 2017. During the same period, the clinical pregnancy rates of the single blastocysts vitrified-warmed were determined and the effects of each type of morphokinetic variables on pregnancy rates were observed.

Participants/materials, setting, methods: Embryos were monitored in a time-lapse incubation system (embryoscope). Embryos were selected for transfers on day 3 or day 5, and surplus embryos were cultured for blastocyst development. The blastocysts were evaluated according to the blastocyst scoring system, and blastocysts graded 3BB or higher were cryopreserved. Vitrification and thawing processes were performed as lab protocols. In thawed blastocysts, they were graded CC or higher and were used for embryo transfers.

Main results and the role of chance: The blastocyst development rate and freezing rate of regularly developed embryos were 56.6% (2116/3739) and 45.1% (1687/3739) respectively. Uneven pronuclei were 43.4% (189/435) and 31.7% (138/435) respectively. Uneven blastomere were 55.9% (137/245) and 42.4% (104/245) respectively. Multinucleation were 53.5% (423/790) and 40.1% (317/790) respectively. Direct division were 40.2% (146/363) and 27.0% (98/363) respectively. Rapid division were 56.8% (162/285) and 39.3% (112/285) respectively. And, irregular division were 43.9% (94/214) and 24.3% (52/214) respectively. The blastocyst development rates of embryos with morphokinetic variables were lower than regularly developed embryos, but the blastocyst development rates of embryos with morphokinetis variables were more than 40%. The clinical pregnancy rate of regularly developed single blastocyst that vitrified-warmed was 37.7% (60/159). Uneven pronuclei were 20.0% (2/10). Uneven blastomeres were 10% (1/10). Multinucleation were 18.8% (6/32). Direct division was 6.3% (1/16). Rapid division was 0.0% (0/9). And, irregular division was 0.0% (0/5). The clinical pregnancy rates of single blastocyst with uneven pronuclei, blastomere and multinucleation were lower than a regularly developed single blastocyst. The clinical pregnancy rates of single blastocyst with direct, rapid and irregular divisions were much lower than a regularly developed single blastocyst.

Limitations, reasons for caution: In this study, it was possible to assess the effect of morphokinetic variables on pregnancy rates. However, further studies will be required to accumulate sufficient data to determine the effects of each type of morphokinetic variables on pregnancy, delivery, and congenital anomaly rates.

Wider implications of the findings: More than 40 percent of embryos exhibiting morphokinetic variables could develop up to blastocyst stages in the time-lapse system. Therefore, it was important that freezing the embryos were to be done carefully, because the embryos with morphokinetic variables were not easy to implant, even if morphology was good after thawing.

Trial registration number: not applicable.

P-128 A prediction model powered by a Group Method of Data Handling (GMDH) algorithm for selecting patients of a single frozen embryo

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Study question: As frozen embryo transfer (FET) cycles become more common, policies regarding the number of embryos to transfer need to be formulated for individual patients.

Summary answer: Using the most relevant factors relating to patients, embryos and fresh cycle outcomes, this GMDH predictive model can reasonably predict the occurrence of implantation.

What is known already: The introduction of vitrification has improved both embryo survival and FET success and has led to a large increase in routine embryo freezing. The improvement of embryo cryopreservation significantly increases the overall success for superovulation cycle. But a high proportion of twins results after transfer of 2 frozen embryos. Studies from fresh cycles suggested that acceptable pregnancy rates could be

achieved after transfer of a single frozen embryo in selected groups of patients.

Study design, size, duration: A retrospective analysis of all FET cycles carried out at Waterstone clinic between Feb 2012 and Dec 2017 was conducted.

Participants/materials, setting, methods: All embryos in the study had been vitrified on day 5 or day 6. Success rates were compared after transfer of either one or two embryos. SET cycle outcomes were also analysed with the factors which most strongly predicted success after FET. Two statistical models were used: the logistic regression and the GMDH algorithm.

Main results and the role of chance: From Feb 2012 to Dec 2017, a total of 1535 out of 1601 vitrified embryos survived after warming 95.9% and were transferred in 1205 cycles. Two embryos were transferred in 327 FET cycles and one embryo was transferred in 878 cycles. The clinical pregnancy (fetal heart activity at 8/40) rates were 41.9%/ transfer and 33.3%/ transfer for 2-ET vs. 1-ET cycles. The overall implantation rate was 31.4%. The proportion of pregnancies which were twins in the 2-ET group was 37.2%.

A logistic analysis was performed based on SET events to investigate the relationship between the implantation of embryos and different prognostic factors. We identified three prognostic factors that made the most significant contributions to the success of implantation: the woman's age, the quality of the inner cell mass (ICM) and the success or failure of the fresh cycle from which the frozen embryos derived. The predictive ability of the model measured by the area under the receiver operating characteristic (ROC) curve was 0.61 when using logistic analysis. Prediction ability was significantly improved by application of a GMHD algorithm which increased the ROC to 0.67 with the negative and positive predictive value of 70.5% and 76.9%.

Limitations, reasons for caution: This prediction model may be benefited by including uterine factors. Prospective studies are now required to confirm the clinical validity of the model.

Wider implications of the findings: Application of this GMHD algorithm may help in the decision making by accurately predicting success. It should identify patients for whom single embryo transfer is indicated and a smaller number of patients for whom a 2-embryo transfer would be wiser.

Trial registration number: na.

P-129 Polar body transfer in a mouse model to overcome transmission of mitochondrial diseases

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Study question: Can polar body transfer (PBT) serve as an efficient type of germline nuclear transfer (NT), to overcome mitochondrial diseases?

Summary answer: This mouse model study supports the efficient use of first (PB1) polar body transfer for overcoming mitochondrial DNA (mtDNA) disorders.

What is known already: Since mtDNA diseases are difficult to treat and manifest with life-threatening consequences, their prevention is a priority. Different germline NT have recently been proposed as novel technologies for preventing mtDNA mutations transmission, including germinal vesicle transfer (GVT), spindle transfer (ST), pronuclear transfer (PNT) and even PBT. Previous mouse studies have revealed full potential of PB1/2 genomes for embryonic development, by replacing female genome of oocytes/zygotes with PB1/2. However, PB2T requires female PN identification in zygotes, which is arduous in human. Therefore, we tested if MII oocytes can be used for both PB1/2T and compared its efficiency with ST and PNT.

Study design, size, duration: In a first set, PB1/2T were carried out in B6D2F1 mice, by introducing PB1/2 into enucleated oocytes (spindle removal, PB1T/novel PB2T) or zygote (female pronucleus removal, PB2T). For controls, ST, PNT and ICSI groups were implemented. In a second set, ST, PNT and routine PB2T were performed between NZB/OlaHsd and B6D2F1 mice for evaluating effect of heterologous NT on embryonic developmental potential. Oocytes/zygotes from B6D2F1 mice served as recipients, while karyoplasts originated from NZB/OlaHsd mice.

Participants/materials, setting, methods: Oocytes/zygotes were from superovulated B6D2F1 and NZB/OlaHsd female mice (6-8 weeks). NT was performed in KSOM-HEPES (2 µg/ml cytochalasin D, 1 µg/ml nocodazole). Karyoplasts were exposed to Sendai virus for fusion with enucleated oocytes/zygotes. One part of ST-, PB1T- and novel PB2T-oocytes were fertilized (ICSI) and exposed to cytochalasin D (only for novel PB2T) to prevent PB2 extrusion. Another part were examined for *de novo* spindle formation through polarized microscopy, and spindle morphology assessment via confocal analysis.

Main results and the role of chance: For homologous NT between B6D2F1 mice, after ICSI, novel PB2T-oocytes showed significantly lower two-cell formation rates compared to ST and PB1T groups (50.0%, 83.8% and 90.0%), but not different with ICSI control (70.6%). For routine PB2T, blastocyst rates (52.2%) were comparable with PB1T group and ICSI control (77.8% and 75.0%, respectively), but these were significant lower than ST- and PNT-embryos (80.6% and 100%, $p<0.05$ and $p<0.005$ respectively). Remarkably, no blastocyst formation could be achieved in the novel PB2T group. Polarized microscopy showed most reconstructed oocytes after novel PB2T contained a visible spindle (22/27, 81.5%) similar to PB1T-reconstructed oocytes (12/17, 70.6%). Confocal analysis, however, revealed that 61.5% of novel PB2T-oocytes displayed an abnormal spindle-chromosome-complex compared to 33.3% in PB1T-oocytes showing aberrant spindles. After heterologous NT from NZB/OlaHsd to B6D2F1 mice, following fertilization, 38.5% of ST-oocytes developed to blastocysts, significantly lower than ICSI control (B6D2F1-oocytes) (72.7%, $p<0.05$) and the homologous ST group (80.6%, $p<0.05$). In heterologous routine PB2T and PNT groups, two-cell rates were similar (69.2% and 83.3%, respectively), whereas PNT-embryos yielded higher blastocyst rates (27.8% vs 100%, $p<0.001$).

Limitations, reasons for caution: Further investigation is currently ongoing to determine whether these NT techniques would alter the genetic and transcriptomic landscape of resulting blastocysts and to assess the mitochondrial carry over level.

Wider implications of the findings: Intra-strain NT in B6D2F1 mice, routine PB1/2T, ST and PNT, did not compromise embryonic development. Novel PB2T could not support blastocyst formation, probably owing to embryonic aneuploidy. Inter-strain NT between NZB/OlaHsd and B6D2F1 mice resulted in declined development in routine PB2T and ST groups contrast with PNT and ICSI controls.

Trial registration number: Not applicable.

P-130 Predictive value of total number of normal morphology and progressively motile sperm on the fertilization failure of short-term insemination in in vitro fertilization

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Study question: To investigate the predictive value of total number of normal morphology and progressively motile sperm (TNNPS) on the fertilization failure of short-term insemination.

Summary answer: TNNPS may be considered as a potential parameter for predicting the failure of short-term insemination.

What is known already: Short-term insemination combined with early-rescued ICSI has some advantages in decreasing the incidence rate of fertilization failure. However, some studies suggested that short-term insemination can lead to increase the abnormal fertilization. For the patients treated by short-term insemination and early rescue ICSI, they had to withstand greater economic and mental stress. Therefore, it is necessary to predict the rate of fertilization failure of short-term insemination. TNNPS contains numerous information of semen and has shown to closely related with fertilization failure in IVF and IUI. Therefore, it is potential to be a parameter to predict the failure of short-term insemination.

Study design, size, duration: 2 768 cycles of short-term insemination in our fertility center from January 1, 2013 to March 31, 2016 were retrospectively analyzed. All cycles were divided into four groups by the 25th, 50th, and 75th percentiles of TNNPS, and then the cycles whose TNNPS≤3.0×10^6 were divided into six groups according to the difference of TNNPS and the intercept was 0.5×10^6.

Participants/materials, setting, methods: Comparing the differences of basic medical recording information (such as female age, male age, infertility years, average number of oocytes retrieved and female BMI) and fertilization failure rate among the different groups. Logistic regression was used to determine the influence of female age, male age, infertility years, average number of oocytes retrieved,female BMI and TNNPS≤1.0×10^6 on fertilization failure.

Main results and the role of chance: For all the cycles, no significant differences were found in the basic medical record information(P>0.05)among 4 groups. However, the insemination failure rate of group 1(23.27%, TNNPS:0~2.76×10^6) was significantly higher than that in group 2(9.83%, TNNPS:2.76~7.21×10^6), 3(9.83%, TNNPS:7.21~16.31×10^6), and 4(5.92%, TNNPS:16.31~149.61×10^6) (P<0.05). For the cycles that TNNPS≤3.0×10^6, no significant difference was found in the basic medical record information (P>0.05) among 6 groups. However, both the insemination failure rate of group I (43.31%, TNNPS:0~0.5×10^6, n = 157)and group II (28.79%, TNNPS:0.5~1.0×10^6, n = 132)were significantly higher than that in group III (12.84%, TNNPS:1.0~1.5×10^6, n = 148), IV (14.04%, TNNPS:1.5~2.0×10^6, n = 114), V (16.50%, TNNPS:2.0~2.5×10^6, n = 103) and VI (11.34%, TNNPS:2.5~3.0×10^6, n = 97)(P<0.05). Analysis of logistic regression demonstrated that TNNPS≤1.0×10^6(OR: 25.422; 95% CI: 17.702-36.508) had significance differences for predicting the fertilization failure of short-term insemination.

Limitations, reasons for caution: If patients with TNNPS≤1×10^6 treated by short-term insemination, they may have a higher risk of fertilization failure. Thus, it may be more favorable if those patients would be benefited by changing treatment program from IVF to ICSI. However, currently we lack the relevant data to support our conjecture.

Wider implications of the findings: TNNPS may be regarded as an index for predicting the failure of short-term insemination. For the patients treated by short-term insemination with TNNPS≤1.0×10^6, they have a higher risk of fertilization failure.

Trial registration number: None.

P-131 Comparative effectiveness of a universal warming/dilution approach to blastocyst vitrification using two different solutions: A randomized trial

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Study question: Is the efficacy of vitrified human blastocyst post-warming dilution using a universal sucrose dilution approach comparable to standard manufacturer procedures?

Summary answer: Human blastocysts vitrification is equally effective using a simple sucrose step down dilution approach independent of the type of cryoprotective agent used.

What is known already: Blastocyst vitrification is a global standard in the IVF industry. Numerous vitrification systems are being marketed and successfully used. Each commercial company has its own recommended thawing solution. As more patients move their cryopreserved embryos between laboratories, it has become increasingly difficult and cost inefficient to maintain a stock of different thaw solutions. Although proprietary in formulation, it is safe to assume that all manufactured thaw solutions have one thing in common, declining sucrose concentrations. Sucrose is an effective non-permeating solute that safely removes intracellular cryoprotective agents (EG, DMSO, Glycerol) and allows for isotonic equilibration by gradual rehydrating dilution steps.

Study design, size, duration: Research consented, discard slow frozen (SF) embryos (N = 363; n = 328 cleavage stage, n = 35 blastocysts) were thawed, cultured and fair-good quality blastocysts were randomly assigned within batch groups to 1 of 2 vitrification treatments: 1) control Glycerol/EG solution, I.C.E. >7.9 M;or 2)15% DMSO/15% EG solution. All vitrified blastocysts were warmed rapidly according to standard operating procedures (SOP) then randomly assigned to either stepwise SOP dilution or universal sucrose step down dilution (n = 31 embryos/group; 2×2 factorial arrangement of treatments).

Participants/materials, setting, methods: All vitrified blastocysts were rapidly cooled (>1500°C/min) and warmed (>6000°C/min) in an aseptic closed system. All warmed embryos underwent stepwise dilutions by manufacturer SOP or a universal 4-step sucrose dilution (21°C) of 1.0 M for 5 min and 0.5 M, 0.25 M and 0.125 M for 3 min each before isotonic equilibration for 5 min at 37°C. Embryo survival and development was determined and differences in % survival and continued development were statistically compared by Chi-square analysis (p<0.05).

Main results and the role of chance: Sucrose diluted, SF embryos yielded 124 (34.2%) fair to good quality blastocysts for study revitrification, whether derived from cleaved embryos (n = 113, 34.5%) or blastocysts (n = 14; 40%). Day 5 SF blastocysts had superior sustained development (67%, n = 10 of 15) in contrast to Day 6 (20%, n = 4 of 20), as defined by blastocoele re-expansion and cellular fullness/growth. There were no differences in survival or sustained development of vitrified blastocysts between the combined dilution treatments, whether SOP thawing (93.5%) or standard sucrose dilutions (90.3%) was applied. Interestingly, cryoprotective agent treatments were significant, with 98.4% (n = 61) of the Glycerol/EG treated blastocysts being viable post-dilution and culture compared to 86.9% for DMSO/EG exposure (n = 53). Only a single I.C.E.-sucrose treated embryo degenerated, whereas degeneration occurred in 4 DMSO/EG-SOP and 5 DMSO/EG-sucrose dilution treated embryos. Although some DMSO/EG treated blastocysts appear to be more sensitive to osmotic injury, the overall viability of the intact blastocysts was excellent based on cellular fullness, clarity and growth/re-expansion.

Limitations, reasons for caution: Possible differences in blastocyst quality may have contributed to VTF survivability of blastocyst exposed to the EG/DMSO vitirification solutions, as DMSO exposure to the lipid bilayer of vulnerable trophectoderm cells may be more sensitive to osmotic stress. Alternatively, 30%EG/DMSO solutions may be unstable under closed vitrification/warming conditions, being less reliable.

Wider implications of the findings: This study has demonstrated that a cost-effective "universal" approach involving stepwise sucrose dilutions is an acceptable thawing method for vitrified blastocysts independent of the vitrification solution used. This standardized procedure allows for continuity of patient care within and between laboratories vitrifying embryos by different methods.

Trial registration number: none.

P-132 Effect of l-carnitine supplementation during vitrification of mouse oocytes: a RNA-seqtranscriptome profiling and validation

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Study question: To investigate the protedctive effects of L-carnitine on cell damage of oocytes following cryopreservation-warming

Summary answer: Our data are consistent with L-carinitine enhancing the developmental potential of when used as a supplement during the course of oocyte vitrification in murine model.

What is known already: Supplementation of L-carnitine(LC) during in vitro muturation (IVM) is reported to improve cryotolerance of mammalian oocytes. However, potential side effects of these supplements on transcriptional regulation during cryopreservation are not well understood.

Study design, size, duration: Oocytes collected from C57 female mice (8 to 10-week-old), were divided into 4 groups: LMXB-1 (n = 30, fresh and non-vitrified), LMXB-2 (27 oocytes, vitrified), LMXB-3 (29 oocytes, vitrified +1 mg/ml LC) and LMXB-4 (29 oocytes, vitrification +2 mg/ml LC).

Participants/materials, setting, methods: RNA sequencing and in silico pathway analysis were used to identify differentially expressed genes (DEGs) that

involved in oocyte viability after vitrification. DEGs were also analyzed by constructing regulatory networks and protein-protein interaction (PPI) networks, transcription factor (TF)-miRNA-target, and drug-gene interaction analysis.

Main results and the role of chance: Following parthenogenetic activation, a reduction in apoptosis marker expression in the L-carnitine treated group that was not observed in control groups. Protective effects of LC were also observed in RT-PCR mRNA studies, ATP measurements, and morphology confirming beneficial effects of LC indicated bioinformatics analyses.

Limitations, reasons for caution: The research is only in murine model. Female preservation finially need to be used on human being. The study got the data that L-carnitine could enhance the oocyte's devolopmental potential following cryopreservation-warming.

Wider implications of the findings: Next step, more discarded human oocytes could go through the silimar research to see whether it could be the same results or not. If L-carnitine could enhance human oocyte's developmental potential following cryopreservation-warming, this research could be really beneficial to human being, especially women.

Trial registration number: HKDL 2017-389 from trial experimental ethical inspection Shanghai Ninth people's Hospital affiliated to Shanghai Jiao Tong University, School of medicine.

P-133 Impact of vitrification on human oocytes before and after in vitro maturation (IVM): a systematic review and meta -analysis

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Study question: What is the impact of vitrification on human oocytes before and after in vitro maturation?

Summary answer: Oocyte vitrification neither before nor after in vitro maturation (IVM) has not a significant effect on matutration rate, survival rate, fertilization rate and cleavage rate.

What is known already: There are controversies regarding IVM procedure, the time of storing frozen oocytes and maturation stage of vitrified oocytes and its impact on oocytes fertilization capability.

Study design, size, duration: A systematic review with meta-analysis was undertaken. Main search terms were those related key words. We searched Medline, Embase, Scopus and ISI web of science to detect English-language studies. The final search was performed on 27 January 2018.

Participants/materials, setting, methods: The original articles which studied ART outcomes after vitrification of MII or GV oocytes before or after IVM were included. Studies that compared the combination of vitrification and IVM with fresh oocytes were also included. Exclusion criteria were animal trials and the studies that performed cryopreservation using slow-freeze method. Oocyte maturation, survival, fertilization and cleavage rates were assessed. Bias and quality assessments were applied.

Main results and the role of chance: 1102 articles and fifteen studies were included for analysis. Five studies compared laboratory outcomes between oocytes that vitrified at the GV stage and those firstly matured in vitro, and then vitrified. MA showed that vitrification of oocytes at GV stage had a negative impact on maturation rate (RR = 0.78, CI: 0.59-1.04); cleavage rate (RR = 0.93, 95% CI: 0.61-1.42); but not on fertilization rate(RR = 1.01, 95% CI: 0.88-1.18) and survival rate(RR = 0.99, 95% CI: 0.95-1.04). Eight studies compared outcomes between the oocytes that vitrified at GV stage before maturation and oocytes which were matured in vitro without vitrification. MA indicated that oocyte vitrification at GV stage had a significant negative impact on maturation rate (RR = 0.77, 95% CI: 0.65-0.91) and cleavage rate (RR = 0.65, 95% CI: 0.54-0.78). Fertilization rate did not differ between vitrified oocytes at GV stage and directly in vitro matured oocytes without vitrification (RR = 0.99, 95% CI: 0.87-1.11). Only one study compared vitrified in-vivo matured oocytes versus vitrified in-vitro matured oocytes. According to the results, survival rate (RR = 0.94, 95% CI: 0.81-1.09), fertilization rate (RR = 0.83, 95% CI: 0.70-0.98) and cleavage rate (RR = 0.91, 95% CI: 0.82-1.02) were significantly lower in vitrified IVM oocytes. No evidence of publication bias was seen using asymmetry tests with regards to outcomes.

Limitations, reasons for caution: However exact inclusion criteria were used in the design of the systematic review, the included studies are heterogeneous in culture media and laboratory technique.

Wider implications of the findings: In general, oocyte vitrification decreases the consequent maturation rate by 23%. However, maturation rate, survival rate and fertilization rate as well as cleavage rates did not significantly differ between the oocytes vitrified before IVM versus oocytes vitrified after IVM.

Trial registration number: CRD42017054372

P-134 Clinical utility of decorin in follicular fluid as a biomarker of oocyte potential

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Study question: This study investigated whether decorin (DCN) is useful as a biomarker for outcomes of assisted reproductive technology (ART).

Summary answer: The concentration of DCN in follicular fluid (F-DCN) has a possibility to be a biomarker indicating the quality of oocytes collected from the corresponding follicle.

What is known already: In the ovary, previous studies have shown that DCN presents in the connective tissue, follicular thecal compartments, follicular fluid (FF) of ovulatory follicles, and corpus luteum, and plays roles in some important processes such as follicle growth, ovulation, and keeping corpus luteum by regulating growth factors. However the association between DCN and the outcomes of infertility treatment has not been discussed.

Study design, size, duration: A retrospective cohort study involving 88 patients treated with ART because of unexplained infertility was performed at Nagoya City University Hospital between April 2010 and March 2016. Serum, FF and oocytes were collected at ovum pick-up (OPU). FF and oocytes were aspirated and collected from the first punctured follicle of each ovary. Some FF were layered over a Ficoll-Paque gradient. The cell samples in the mononuclear cell layer were cryopreserved as the granulosa cells (GCs).

Participants/materials, setting, methods: F-DCN and the concentration of DCN in serum (S-DCN) was measured by ELISA. The relation between F-DCN and patient's age, Perfusion Index (PI) of the blood flow around the follicle at OPU, the total dose of gonadotropin administered, S-DCN, the concentration of IGF-1 in FF, fertilization, and quality of embryo were analyzed. In addition, immunostaining and western blotting were performed using the granulosa cells to identify existence and localization of DCN.

Main results and the role of chance: A total of 130 oocytes and the corresponding FF and serum samples from 88 patients. F-DCN was 39.26 ng/ml (median value); it was higher than that in serum. Correlations were not observed between F-DCN and patient age, the total dose of gonadotropin administered, IGF-1 concentration in FF, and PI of the blood flow around the follicle. However, F-DCN showed a weak negative correlation with S-DCN ($R = -0.189$; $P = 0.031$). F-DCN of the oocytes fertilized by intracytoplasmic sperm injection (ICSI) was significantly lower than that of oocytes that were not fertilized (33.24 ng/ml vs 40.18 ng/ ml; $P = 0.043$). When a cut-off level of 34.5 ng/ml was set according to the receiver-operating characteristic curve, the fertilization rate of the oocytes from the follicles in which F-DCN was lower than the cut-off level tended to be good compared to that of the oocytes with F-DCN higher than the cut-off level ($P = 0.052$). S-DCN of the good embryos was significantly higher than that of the poor embryos only for those oocytes fertilized by ICSI. DCN is less likely to be produced by GCs, because it was not detected in GCs by immunostaining and western blot analysis.

Limitations, reasons for caution: The sample size was relatively small, which made it difficult to analyze the association between DCN and pregnancy

or birth rate. As the reason for that, our study was limited to patients treated with ART only because of unexplained infertility and FF samples were collected from the first punctured follicles.

Wider implications of the findings: The fertilization rate of oocytes from follicles in which F-DCN was less than 34.5 ng/ml was high, indicating that it is possible to predict the potential of oocytes to be fertilized by examining F-DCN of the corresponding follicle during ICSI. Therefore, it is useful for planning embryo transfer and cryopreservation.

Trial registration number: This study is not registered.

P-135 Morphologic status and angulation of meiotic spindle in vitrification-warmed oocytes of donor bank: effects on the ICSI outcomes

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Study question: Do the morphology and angulation of meiotic spindle correlate with the fertilization outcomes after ICSI in vitrification-warmed oocytes of donor bank?

Summary answer: The correlation exists between the spindle morphology and fertilization outcomes, while it does not show between the spindle angulation and fertilization outcomes in vitrification-warmed oocytes.

What is known already: The meiotic spindle is crucial for the fertilization process, including completion of meiosis, second polar body extrusion, formation of pronuclei, and the following mitosis. Since the structure of spindle is dynamic depending on the timeline of oocyte, temperature, and physical stress, characteristics of spindle in the vitrification-warmed oocytes may show different phenomenon with that in the fresh oocytes.

Study design, size, duration: Between June and September of 2017, five hundred and twenty-five oocytes from donor bank were included in the analysis. A total of 37 egg donors were involved: mean age 24.7 ± 3.5 years; body mass index (BMI) 19.7 ± 1.8 kg/m^2; anti-mullerian hormone (AMH) value 7.0 ± 4.2 ng/mL.

Participants/materials, setting, methods: All the metaphase II (MII) oocytes were cryopreserved by vitrification (Cryotech®, Japan) at the bank. Once being matched to the appropriate recipients, the oocytes were warmed and cultured in standard conditions for at least 2.5 hours following with image taken for spindle (Oosight®, Hamilton Thorne). The spindle presence, morphology, and angle to polar body (PB) were measured. The correlation between the outcomes of ICSI and post-warmed spindle status were estimated.

Main results and the role of chance: The overall survival rate of vitrification-warmed oocytes was 92.4% (485/525), and two-pronuclei (2PN) rate was 75.1%. The morphology of post-warmed spindle was classified into normal, translucent, no-visible, and telophase; the 2PN rate in each group was as below (p-value was compared to the normal group): normal = 79.4%, translucent = 73.2% ($p = 0.30$), no-visible = 48.6% ($p<0.05$), and telophase = 37.5% ($p<0.05$). Of the 2PN rates in oocytes with different angulation, no correlation was found: 0–30° = 79.8%; 31-60° = 76.1%; 61-90° = 82.1%; 91-120° = 80.0%; >120° = 90.0%.

Limitations, reasons for caution: Individual biases in manipulation of spindle microscopy cannot be completely avoided.

Wider implications of the findings: Unlike the biological representativeness of spindle angulation in fresh oocytes, the correlation between the spindle angulation and fertilization outcome was not observed in vitrification-warmed oocytes. The displacement of spindle in vitrification-warmed oocytes could be caused by the physical effects during the freezing-thawing procedures.

Trial registration number: Not applicable.

P-136 Antioxidants in IVF and culture media significantly improve human embryo development: A prospective randomised multi-centre trial

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Study question: What is the effect of combined antioxidants Acetyl-L-Carnitine (ALC), N-Acetyl-L-Cysteine (NAC) and α-Lipoic Acid (ALA) on human embryo development, when present in IVF culture media?

Summary answer: Combined antioxidants present in G-Series media significantly increased the percentage of good quality embryos on day 3 and showed a trend to higher pregnancy rates.

What is known already: The combination of the antioxidants ALC, NAC and ALA in IVF media and/or culture media has a significant beneficial effect on mouse embryo development and viability.

Study design, size, duration: A sibling oocyte study, where oocytes were randomly distributed to the control or test group at the time of pick up. Day 3 embryo and day 5/6 blastocyst quality were assessed. Good embryo quality on day 3 was defined as 8 to 10-cells with even cells and low fragmentation; good quality blastocysts as equal or greater than 3BB. Clinical outcome was assessed on transfers of a single vitrified-warmed embryo on day 5.

Participants/materials, setting, methods: The study was conducted in 2 IVF centres in Japan. 115 patients were included in the study, generating a total of 1,350 MII oocytes. IVF/ICSI and embryo culture (G-1 and G-2 PLUS) were conducted in 5% oxygen in the presence or absence of antioxidants (10 μM ALC /10 μM NAC /5 μM ALA). Controls were oocytes and embryos that were not exposed to antioxidants in the medium.

Main results and the role of chance: The fertilization rate in the control and test group was 75% and 76% respectively. Of the resultant 2PN, 19.6% and 26.1% resulted in good quality embryos on day 3 ($P<0.05$). Good quality blastocyst rate was 25.4% vs 26.5% for the control and test group, respectively, and these results were not significant. 42 single embryo transfer cycles were performed in the control group and 45 in the test group. The ongoing clinical pregnancy rate, as determined by cycles with fetal heart beat was 52.4% vs 60.0%, however, the study was not powered for significance of clinical outcome data.

Limitations, reasons for caution: Transfers are mostly performed after vitrification/warming of embryos, which is why clinical outcome data are still not complete.

Wider implications of the findings: The presence of antioxidants during IVF and embryo culture imparts significant benefits on day 3 embryo quality. Hence, supplementation of antioxidants to IVF and culture media may improve the viability of human embryos in ART, plausibly through the reduction of oxidative stress.

Trial registration number: NCT02999958

P-137 Prolonged blastomere movement induced by the delay of pronuclear fading and first cytokinesis adversely affects pregnancy outcomes in human embryo

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Study question: Does the prolonged blastomere movement post first cytokinesis influence the pregnancy outcomes in human embryo?

Summary answer: The Prolongation of blastomere movement induced by the delay of pronuclear fading and first cytokinesis adversely affects pregnancy outcomes.

What is known already: Time-lapse monitoring systems allow consecutive observation of embryonic development and have been increasingly used for the selection of viable embryos. Morphokinetics during the first and second cytokinesis is an effective biomarker of developmental competence to high quality blastocysts in human. In a preliminary study using time-lapse imaging we characterized different patterns of blastomere movement between the first and second cytokinesis in human embryos. However, it is still unknown how

blastomere movement influences clinical pregnancy outcome and which factors influence the duration of blastomere movement.

Study design, size, duration: A total of 708 embryos from 708 patients (36.4 ± 0.2 years) were cultured in EmbryoScope+® time-lapse system and subjected to single fresh cleaved embryo transfer from April to July 2017 and retrospectively analyzed. Blastomere movement was observed between the first and second cytokinesis. First, the correlation between the duration of blastomere movement and clinical pregnancy was evaluated. Second, the association of the blastomere movement duration with patient characteristics and developmental speed until second cytokinesis was examined.

Participants/materials, setting, methods: Retrieved oocytes were inseminated either by conventional in vitro fertilization or intracytoplasmic sperm injection. Fertilized oocytes were cultured in time-lapse incubator and the time from insemination to pronuclear (PN) alignment (tPNa), PN fading (tPNf), first cytokinesis (t2), and second cytokinesis (t3) was annotated. Duration of blastomere movement (BMov) between t2 and t3 (dBMov) was monitored and the ratio of dBMov over the period of 2-cell stage (t3-t2) was calculated (dBMov/(t3-t2)).

Main results and the role of chance: The mean value of dBMov/(t3-t2) was 0.347 ± 0.008 (range: 0.033–1.000). Logistic regression analysis revealed that the increasing value of dBMov/(t3-t2) was statically associated with decreasing clinical pregnancy rate (Odds ratio: 0.193 [95% confidential interval: 0.081–0.480], P<0.01). The value of dBMov/(t3-t2) was not correlated with patient characteristics, including female age (P = 0.49), male age (P = 0.34), female body mass index (P = 0.68), number of previous cycles (P = 0.20), cause of infertility (P = 0.44), insemination methods (P = 0.73), total number of spermatozoa (P = 0.35), number of motile spermatozoa (P = 0.36), and number of abnormal spermatozoa (P = 0.19). No correlation between tPNa and the value of dBMov/(t3-t2) was found. On the other hand, the period of PN alignment (tPNf-tPNa) was positively associated with the value of dBMov/(t3-t2) (Spearman's rank correlation coefficient (SC): 0.097, P = 0.01). In addition, the period of PN fading (t2-tPNf) was significantly correlated with the dBMov/(t3-t2) value (SC: 0.178, P<0.01).

Limitations, reasons for caution: The limitation of this study was that the pregnancy outcomes after a single fresh cleaved embryo transfer were exclusively analyzed. Further studies are required to examine whether the blastomere movement affects the blastocyst development and subsequent pregnancy outcomes after blastocyst transfer.

Wider implications of the findings: The chance for a clinical pregnancy after single fresh cleaved embryo transfer is adversely affected by the prolonged blastomere movement which is associated with the delay of PN fading and first cytokinesis. These results support the possibility that blastomere movement can be used as a predictive parameter for pregnancy outcomes.

Trial registration number: None.

P-138 The proportion of monopronuclear zygote (1PN) after intracytoplasmic sperm injection (ICSI) is significantly higher in frozen-thawed oocytes than fresh oocytes

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Study question: Is there different distribution of pronuclear types between fresh and frozen-thawed oocytes after ICSI?

Summary answer: The proportion of monopronuclear zygote (1PN) in frozen-thawed oocytes is significantly higher than fresh oocytes, irrespective of recipient or infertile group.

What is known already: In in vitro fertilization (IVF), the first check point is to confirm the appearance of pronuclei, including shape and number, around 16-18 hours after insemination. The formation of two pronuclei is defined as a successful and normal fertilization; however, the other pronuclear types such as more or less than two pronuclei or uneven pronuclei are considered as abnormal fertilization. Nevertheless, blastocysts derived from abnormal fertilized oocytes have been referred to have potential to develop into normal chromosomal status and produce a healthy baby.

Study design, size, duration: A retrospective cohort study was conducted at Stork Fertility Center, between January 2017 to December 2017. Total 1155 cycles were included in the study and divided into oocytes recipient (N = 458) and infertility (N = 697) groups. Total 14615 mature oocytes (MII) were collected from recipient (n = 7603) and infertility (n = 7012) groups. The further analysis including pronuclear types and embryo development were evaluated only against the MII fertilized by ICSI.

Participants/materials, setting, methods: The recipient and infertility groups were subsequently categorized into two subgroups: fresh (N = 166 and 569, respectively) and frozen-thawed oocytes (N = 292 and 128, respectively) inseminated cycles. Moreover, all frozen-thawed oocytes were preserved by vitrification in this study. Total 6039 fresh and 5589 frozen-thawed oocytes were performed by ICSI, and the proportion of pronuclear types and following embryo development were observed.

Main results and the role of chance: In the recipient group, the mean age of the fresh and frozen-thawed subgroups were 23.86 and 24.42 years old, and 2031 and 4582 MII were performed ICSI respectively; the fertilization rate (FR), good embryo rate (GER) and good blastocyst rate (GBR) of the fresh and the frozen-thawed subgroups were 82.32% vs. 77.48%, 62.14% vs. 55.80%, and 63.10% vs. 54.33%. In the infertility group, the mean age of the fresh and frozen-thawed subgroups were 36.36 and 36.80 years old, and 4008 and 1007 MII were performed ICSI respectively; the FR, GER and GBR of the fresh and the frozen-thawed subgroups were 79.89% vs. 70.04%, 64.08% vs. 55.52%, and 57.87% vs. 42.63%. The different proportion of pronuclear types was found between fresh and frozen-thawed oocytes. The 1PN rate in frozen-thawed was significant higher than the fresh oocytes, which was showed in both recipient (10.83% vs. 5.61%, p<0.01) and infertility (12.1% vs. 5.39%, p<0.01) groups. This phenomenon might be a clue to explore the effect of frozen-thawed process and fertilization mechanism.

Limitations, reasons for caution: The difference between fresh and frozen-thawed oocytes such as spindle structure, protein expression profile, chromosomal constitution, mitochondria activity and cytoskeleton conformation should be distinguished to illuminate the causes of the different distribution of pronuclear formation. This phenomenon is restricted to ICSI since frozen-thawed oocytes could not be performed conventional IVF.

Wider implications of the findings: The exact factors of abnormal fertilization and the impact of frozen-thawed process have not been elucidated yet. This study might provide an additional aspect to investigate the crucial part of these issues and rescue the abnormal fertilized oocytes for further clinical applications.

Trial registration number: N/A.

P-139 Irregular oocyte swelling time in thawing solution (TS) highly correlates with the subsequent survival rate

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Study question: Whether irregular oocyte thawing time in thawing solution correlates with following oocyte survival rate?

Summary answer: Irregular oocyte swelling time in thawing solution (TS) highly correlates and dramatically increases the mortality after thawing.

What is known already: Vitrification is an optimal method employed for oocyte preservation to prevent intracellular and plasma membrane molecules such as enzymes, structural protein and mRNA damage from ice crystals. The critical process of oocyte vitrification is considered to exchange water and cryoprotectant between intracellular and extracellular environments. Membrane integrity is supposed to be one of the major reasons influencing water/cryoprotectant exchange rate and following survival rate after thawing.

Study design, size, duration: A retrospective cohort study throughout June 2017 to November 2017, total of 2144 oocytes in 162 cryopreservation/thawing cycles were included. The timing of swelling, flattening and recovering time

in thawing solution were recorded and analyzed to reveal the effects on the thawing outcomes.

Participants/materials, setting, methods: Every oocyte undergone thawing procedure were recorded with the individual timing of swelling, flattening and recovering (swelling combined with flattening). All the oocytes were classified according to the (1. thawing outcomes-survival or mortal to reveal the timing diversity; or (2. regular (>30 s) or irregular (≤30 s) swelling time in thawing solution (TS) to compare the mortality.

Main results and the role of chance: Based on the thawing outcome, firstly, oocytes were grouped into survival and mortal groups for comparing the swelling, flattening and recovering time of the two groups. The results indicated that the mean time of swelling (44.3 s vs. 28.9 s, $p<0.05$) and recovering (57.2 s vs. 39.9 s, $p<0.05$) in survival group was significantly higher than the mortal group. The primary disparity of recovering time were mainly derived from the swelling time, although the average flattening (12.9 s vs. 10.9 s, $p<0.05$) time of survival group was also significant higher than the mortal group. Secondly, in the contrary, thawed oocytes were classified according to swelling time (regular, >30 s or irregular, ≤30 s). Mortality of the regular group revealed significantly lower than the irregular group (2.97% vs. 26.9%, $p<0.05$). Taken together, the two aspects indicated that the swelling time is a critical indicator correlating to the mortality of oocyte thawing. These observations implicated that osmotic potential plays a critical role affecting the thawing survival rate. Furthermore, membrane permeability and surface molecular profile might influence cell resistant from osmotic potential, which accounts for the dramatically increasing mortality.

Limitations, reasons for caution: Vitrification strategy was adopted in all the frozen-thawed oocytes in this study. Vitrification Kit 101 (Cryotech) and Warming Kit 102 (Cryotech) were employed for performing frozen-thawed procedures. The phenomenon should be observed in other vitrification strategy but might fail to refer to other frozen-thawed methods such as slow freezing process.

Wider implications of the findings: The present study implies that swelling time in TS is correlated with oocyte mortality. The considerable increase of mortality in irregular group implies that membrane constitution such as inappropriate lipids/cholesterol ratio and suboptimal water/ion channels, might play a critical role in regulating the water/cryoprotectant exchange between intracellular and extracellular environments.

Trial registration number: N/A.

P-140 Revisiting a controversial topic: The vitrification-warming procedure does not induce zona pellucida hardening in human blastocysts

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Study question: Should the variation in zona pellucida (ZP) thickness during blastocoel expansion be considered an important factor of ZP hardness in vitrified-warmed blastocysts?

Summary answer: Unlike during the vitrification warming procedure, a reduction in ZP thickness during the physiological expansion of the blastocoel is a potential confounder of ZP hardness.

What is known already: Previous studies have demonstrated ZP hardening in cryopreserved blastocysts, resulting from improved clinical outcomes following assisted hatching and small experiments focused on the enzymatic digestibility of the zonae. However, to date, the regulatory pathways such as cortical reaction during oocyte ZP hardening remain unclear. Moreover, the main point of discussion regarding the multivariate associations of ZP hardening with respect to the vitrification-warming procedure and ZP thinning during blastocyst expansion was not addressed by these studies.

Study design, size, duration: This experimental study included fresh (n = 61) and vitrified-warmed (n = 36) blastocysts that were discarded during the treatment of patients. These blastocysts were harvested from IVF or ICSI cycles (n = 50) between March 2010 and May 2011.

Participants/materials, setting, methods: Two types of ZP hardness were estimated based on the response to enzymatic digestion and elastic behavior of ZP. Dissolution time was assessed by ZP disappearance in 0.1% protease solution. Elasticity was calculated from the maximum expansion rate in a sham-expansion model, which was micro-injection of Fluorinert™ fluid below ZP layer. The mean ZP hardness indexes were compared between fresh and vitrified-warmed blastocysts of each ZP thickness categories (>15, ≤15 to >7.5, and ≤7.5 μm).

Main results and the role of chance: Regarding the response of ZP to enzymatic digestion, the digestion time negatively correlated ($r = 0.67$, $p < 0.01$) with the reduction of ZP thickness during blastocyst expansion. The mean digestion time of vitrified-warmed ZP was similar to that of fresh ZP (405 vs. 390 s). The mean digestion time derived for the >15, ≤15 to >7.5, and ≤7.5 μm categories, i.e., 493, 375, and 334 s, respectively, were significantly ($p < 0.05$) decreased depending on the ZP thickness.Regarding the elastic behavior of ZP, the maximum expansion rate negatively correlated ($r = 0.92$, $p < 0.01$) with the reduction of ZP thickness during blastocyst expansion. The mean maximum expansion rate of vitrified-warmed ZP was similar to that of fresh ZP (24 vs. 28%). The mean maximum expansion rate derived for the >15, ≤15 to >7.5, and ≤7.5 μm categories, i.e., 40, 28, and 11%, respectively, were significantly ($p < 0.05$) decreased depending on the ZP thickness.

Limitations, reasons for caution: In this study, the results of molecular biology are absent.

Wider implications of the findings: The present study indicates that ZP softening might be induced by the reduction of ZP thickness during blastocoel expansion. Failure to evaluate the ZP thickness as a potential confounder might have led to biased results and conclusions, i.e., the demonstration that the vitrification-warming procedure induces ZP hardening in blastocysts.

Trial registration number: The authors declare no potential.

P-141 Is there a difference in embryo morphokinetics between ejaculated and non-ejaculated spermatozoa?

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Study question: Does sperm origin (ejaculated, epididymal, and testicular) affect pre-implantation embryo morphokinetics and morphodynamics?

Summary answer: Non-ejaculated spermatozoa negatively affect all developmental stages of preimplantation embryos.

What is known already: Spermatozoa undergo biochemical and physiological modifications during their transit in the epididymis. However, the influence of the testicular extracted spermatozoa on embryo developmental competence did not reach a firm consensus.

Study design, size, duration: A retrospective study was conducted at Azoury IVF clinic, from August 2013 until August 2017. It was performed on 86 couples, who have undergone only intracyoplasmic sperm injection (ICSI) cycles. The infertile couples were divided into four groups: normozoospermia group (n = 23 couples, n = 133 embryos); oligozoospermia group (n = 22 couples, n = 109 embryos); percutaneous epididymal sperm aspiration (P.E.S.A.) group (n = 21 couples, n = 119 embryos), and testicular biopsy group (n = 23 couples, n = 123 embryos).

Participants/materials, setting, methods: The couples where the females had 38 years old or less at the time of ICSI, were selected and divided into four study groups according to sperm quality and source. Only the data of the embryos with normal fertilization and cultured for 5 consecutive days without interruption was included. In addition, embryos that were subjected to embryo biopsy were excluded. Subsequently, embryo morphokinetics and morphodynamics were analysed through time-lapse microscopy (Embryoscope) using the Embryoviewer software.

Main results and the role of chance: During the pre-compaction stage, the embryos of the P.E.S.A and testicular biopsy groups showed a significant delay in the first embryonic cell cycle (ECC1) when compared to the embryos of the normozoospermia group ($p<0.05$ and $p<0.01$, respectively). In addition, the third embryonic cell cycle (ECC3) was significantly delayed in both non-ejaculated sperm groups compared to the normozoospermia group ($p<0.01$ and $p<0.001$, respectively). Interestingly, this delay in the development continued through the post-compaction stage. Particularly, the embryos of the testicular biopsy group had significant delay in the time of starting blastulation ($p<0.05$) and in the time to reach a full blastocyst ($p<0.001$) in comparison to those derived from the normozoospermia group. Regarding the morphodynamic parameters, a lower percentage of blastocysts showed blastocyst contraction in the P.E.S.A and testicular biopsy groups compared to the normozoospermia group ($p<0.05$ and $p<0.05$, respectively).

Limitations, reasons for caution: A very important limitation of this study is its retrospective nature itself. A randomized controlled trial (RCT) should be conducted to confirm the findings of this study.

Wider implications of the findings: The present study clearly shows a negative impact of non-ejaculated sperm on early embryo development. Hence, recommending the use of testicular sperm in case of repetitive IVF failure and high sperm DNA fragmentation should be taken with caution.

Trial registration number: Not applicable.

P-142 Characteristics of human single pronucleated zygotes derived from conventional in vitro fertilization, considering the genome composition, chromosomal distribution and morphological assessment

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Study question: Do human single pronucleated (1PN) zygotes derived from conventional in vitro fertilization (c-IVF) have developmental potential as normal fertilized zygotes leading to newborns?

Summary answer: Although approximately 80% of 1PN zygotes in c-IVF procedures were diploid, not all distributed their genome normally during the 1st cleavage.

What is known already: Cytogenetic and genetic composition based on histone modification analysis has revealed that many 1PN zygotes are diploid in c-IVF procedures. Some of them are able to develop to the blastocyst stage and result in normal deliveries in humans.

Study design, size, duration: We used 1PN zygotes obtained from February 2015 to September 2017 from c-IVF procedures (n = 60). Fresh or frozen/thawed zygotes were provided for cell biological and morphological analysis. Three normal two-pronuclear zygotes were also used as controls.

Participants/materials, setting, methods: Donated and ethically-approved zygotes derived from c-IVF were used in this study. 1PN zygotes were obtained more than 22 hours post insemination. We analyzed the genome composition by DNA and histone methylation status, chromosome distribution, pronucleus diameter, and the time-course from syngamy to the 1st cleavage. Immunofluorescence was performed using antibodies against 5mC, 5hmC, H3K9me3, alpha-tubulin and pericentrin. Live-imaging analysis was performed using TagGFP2-H2B mRNA and FusionRed-MAP4 mRNA.

Main results and the role of chance: Analysis of DNA methylation status by immunofluorescence for 5mC and 5hmC revealed that 80% of the 1PN zygotes (n = 10) showed a mixed 5mC/5hmC-positive genome in a single pronucleus, and analysis of histone methylation by immunofluorescence for H3K9me3 revealed that 78% of zygotes (n = 9) had a non-uniform H3K9me3-positive pronucleus or chromosomes. Analyses of spindle formation and chromosome segregation by immunofluorescence revealed that 45% of zygotes (n = 20) formed bipolar spindles at metaphase. At telophase, one zygote showed an unequal distribution of paternal and maternal genomes. In live-imaging analysis which visualized histone H2B (chromosomes) and MAP4 (microtubules), one zygote showed a tripolar spindle formation leading to abnormal chromosomal distribution during the 1st cleavage, while 56% of zygotes (n = 9) showed bipolar spindles and even chromosomal distribution. The average pronucleus diameter of zygotes which showed pronucleus disappearance and 1st cleavage was slightly bigger than the defectively-developed zygotes (n = 14, overlapped with live-imaging data). The time-course from pronucleus disappearance to 1st cleavage was almost the same as in normally-fertilized zygotes (n = 6, overlapped with live-imaging data).

Limitations, reasons for caution: This study was confined only to the period from zygote stage to 1st cleavage, so further developmental stages need to be analyzed.

Wider implications of the findings: Although we showed it is possible to select the diploid 1PN zygotes with developmental potency based on pronucleus diameter assessment, not all have the ability to distribute chromosomes normally during the 1st cleavage. Our findings may help in the selection of useable 1PN zygotes for clinical applications.

Trial registration number: Not applicable.

P-143 High-resolution nuclear magnetic resonance based profiling of spent human embryo culture media for assessment of in vitro embryo quality

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Study question: Over recent years, there has been much interest in the use of metabolomics to detect biomarkers of embryo viability, which might help embryologists to successfully increase implantation rates.

Summary answer: The aim of this study was to identify biomarkers from embryo metabolome that would allow us to predict and differentiate embryos of the best quality.

What is known already: The metabolic profile in the culture media used can be investigated using non-invasive metabolic techniques, such ^{1}H-NMR. This noninvasive quantification of embryonic metabolism can be a useful predictor of pregnancy outcome after embryo transfer, a potential supported by recent clinical studies that work with specific classes of metabolites such as glycolytic intermediates and amino acids (Singh, 2007).

Study design, size, duration: Multidisciplinary_study of several institutions which compass a highly analytical component, with random selection and assignment of the samples. The sample size (Perduzzi's formula [$n = 10 * (k + 1)$] and prevalence of poor quality embryos of 54%, $p < 0.005$) included 185 embryo cultures, assuming losses of 20%, being the final size of 222. The chronogram has combined embryonic selection tasks with NMR measurement periods with the aim of obtaining metabolic profiles and identifying biomarkers.

Participants/materials, setting, methods: The inseminated MII oocytes and were individualized in G1-Plus_medium (Vitrolife) left in culture during 48 hours (D+3). After embryo selection and transfer, embryo culture medium was collected and cryopreserved at -80 °C. The media for NMR measurements were prepared in a solution of NaCl in D_2O and were carried out on a Bruker_Avance_III_600 spectrophotometer equipped with a cryoprobe. The spectra were acquired using a ^{1}H CPMG sequence and processed through the TOPSPIN 3.2 software.

Main results and the role of chance: Schematically, the experimental procedure was carried out as follows: a) Collection of the samples, b) Analysis of the samples by NMR, c) Application of multivariate analysis techniques to the ^{1}H NMR data to identify the biomarkers that were associated with a better morphological kinetics and embryonic implantation. The Principal Component Analysis (PCA) model obtained so far allowed to discriminate between the embryo culture samples of the embryos that had an optimal development of those that did not. At the same time, the quantification of the metabolites that contribute most to the discrimination of the model was obtained, observing that the embryos with optimal development showed a higher consumption of metabolites such as pyruvate, glutamate, citrate, tryptophan (which means that they are metabolically more active). The worst embryonic development was

associated mostly to a lower consumption of pyruvate, the energy source, and of tryptophan, used in protein biosynthesis. The formate/acetate ratio significantly decreased in media samples belonging to embryos of type A (according to ASEBIR classification criteria), while it increased in embryo medium samples with worst development (types C and D).

Limitations, reasons for caution: Limitations: 1) rate of embryo fertilization; 2) the high operational cost associated with NMR measurements could be a limitation for routine prediction; and 3) the possibility that the tool is not sensitive enough to detect differences in metabolic embryos and does not achieve sufficient differentiation power is another weak point.

Wider implications of the findings: A large population size (> 200, average 36 years) from different geographical areas, as well as the use of mass spectrometry and separation methods in parallel to NMR spectroscopy, will try to give solidity to the study, although the preliminary results agreement with previous studies found in the bibliography.

Trial registration number: not applicable.

P-144 Does blastocyst re-expansion one hour post-warming predict clinical pregnancies?

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Study question: Is it possible to predict a clinical pregnancy based on early conventional warmed blastocyst re-expansion analysis?

Summary answer: We demonstrate statistically higher clinical pregnancy rates for blastocysts that displayed re-expansion in the first hour after warming, as opposed to those that did not.

What is known already: A few studies have demonstrated that post warming blastocyst expansion is a predictor of implantation. However, most studies focus on expansion at one time point several hours after warming with no information on the method of expansion evaluation. Moreover, the stage of blastocyst development is not always taken into account. Time lapse technology was very recently used in two studies and demonstrated that early onset of re-expansion and duration of re-expansion were predictors of pregnancy.

Study design, size, duration: A retrospective analysis of 264 single vitrified-warmed blastocyst transfers that took place between January 2014 and December 2016 were included in this study. Photographs of the warmed blastocysts were taken at 0 – 30 and 60 minutes post warming and were prospectively analyzed by two methods.

Participants/materials, setting, methods: Blastocyst post-warming behavior was evaluated by the means of morphological measurements taken by a computer program, as well as independently by three biologists based on photographs at 0-30 and 60 minutes post warming. Four main groups were established based on the ability of the blastocyst to compact and re-expand. Survival and clinical pregnancy rates were compared between groups for different stages of blastocyst development.

Main results and the role of chance: We observed different post-warming behavioral patterns depending on the stage of blastocyst development. Blastocysts (stage 3) showed a lesser amplitude of compaction/re-expansion by computer analysis and no significant difference was observed between expanding and non-expanding groups in terms of clinical pregnancy rates (23% versus 27% $p = 0.76$) despite increased survival for re-expanding blastocysts (93% versus 88% $p = 0.01$). For combined expanded and hatching blastocysts (stages 4 and 5) showing re-expansion within 30-60 minutes after warming, significantly higher survival (99% versus 79% $p<0.001$) and clinical pregnancy rates (33% versus 13% $p = 0.02$) were observed compared to embryos that did not show signs of re-expansion. No significant difference in clinical pregnancies was observed between embryos showing fast and slow re-expansion (35% versus 31%, $p = 0.064$). Nine percent of all vitrified-warmed blastocysts showed atypical behavior without any signs of compaction directly post warming, or during the 60-minute observation period. The overall clinical pregnancy rate for these embryos was 35%. Globally, the biologist's method confirmed the computer analyses though this method was better suited to detect a larger cavity without an overall increase of the size of the embryo.

Limitations, reasons for caution: Expanded and hatching blastocysts that did not re-expand post warming had reduced pregnancy rates. Nevertheless, the decision to discard them should be done with caution. Further investigation between slow and fast re-expansion groups need to be carried out, focusing on the method and timing of re-expansion analysis.

Wider implications of the findings: This study is the first to demonstrate different post-warming behavior depending on the stage of blastocyst development and to investigate various methods of blastocyst re-expansion analysis. Early decisions concerning the fate of the warmed embryos can be made without the need for sophisticated time-lapse technology.

Trial registration number: not applicable.

P-145 Correlation between euploid blastocysts expansion/morphology and clinical outcomes in cycles with single frozen embryo transfer

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Study question: How much the morphology of euploid blastocyst determines the clinical outcomes in frozen-thawed transfers after preimplantation genetic screening (PGS)?

Summary answer: The morphology of frozen-thawed euploid blastocyst does not affect the clinical outcomes in single embryo transfer (SET) cycles.

What is known already: Blastocysts are routinely evaluated with regard to the expansion of the blastocoel and the number of cells in the Inner Cell Mass (ICM) and in the Trophoectoderm (TE). However, the literature is not clear in euploid blastocysts evaluated by PGS, how much the morphology affects clinical outcomes. While some authors suggested that morphology was not associated with the chance of ongoing pregnancy, and 'poor" quality euploid blastocysts fared just as well as higher graded blastocysts, others believe that blastocyst morphology grading and particularly ICM is a useful predictor of ongoing pregnancy rate per biopsied euploid embryos.

Study design, size, duration: This is a retrospective cohort study including data from 270 SET of frozen-thawed euploid blastocysts performed between January 2016 and July 2017. Blastocysts were morphologically classified according with Gardner for expansion grade (EG), ICM and TE. Blastocysts were biopsied for PGS analysis by using NGS and then vitrified. Euploid blastocysts were warmed and single embryo transfers were placed after endometrial preparation.

Participants/materials, setting, methods: Blastocyst were classified as grade 3, 4, 5 or 6 for EG. The ICM and TE were classified as A, B and C, as grade A is best grade and C is poorer grade. Clinical pregnancy rate was analyzed according with each morphological classification of blastocysts and the multivariate analysis was performed to evaluate the association of EG, ICM, TE and the interaction among then in the clinical pregnancy rates, adjusted for confounders.

Main results and the role of chance: Patients included were 37.8 ± 3.6 years of age and the indications for PGS were implantation failure, recurrent miscarriage, advanced maternal age, severe male factor or patients' choice. The most of blastocysts were classified as EG grades 4 (40.4%) or 5 (47.5%), ICM grades A (43.3%) or B (40.4%) and TE grades A (32.9%) and B (38.8%). In the univariate analysis, we observed the clinical pregnancy rates yielded no statistically difference when blastocysts were classified by EG (3: 33.3%; 4: 46.6%; 5: 44.4%; 6: 35.7%, p value = 0,438), by ICM (A: 43.8%; B: 34.5%; C: 29.7%, p value = 0,258). When blastocysts were classified by TE morphology, the clinical pregnancy rate (A: 39.8%; B: 48.3%; C: 27.3%) was higher when TE=B (48.3%) compared to TE=C (27.3%; $p = 0.008$). The multivariate analysis did not show any association of EG (OR = 1.19; $p = 0.250$), TE (OR = 1.24; $p = 0.190$) or

ICM (OR = 1.35; p = 0.106) individually or the interaction of them (OR = 0.984; p = 0.223) with clinical pregnancy rate, adjusted to confounders.

Limitations, reasons for caution: This is a retrospective study including a relatively low number of samples and higher cohorts of cycles should be studied to confirm the outcomes. All patients included received SET; however not all cycles were elective SET, as some of them had only one euploid blastocyst available.

Wider implications of the findings: Our findings suggested that once the blastocyst is euploid after PGS evaluation, it morphology does not affect the clinical pregnancy rates. Based on that, we can speculate that high morphology grade and low morphology grade euploid blastocysts have same implantation potential and can be transferred arbitrarily.

Trial registration number: Not applicable.

P-146 ICSI procedure impairs the development of embryos to the blastocyst stage in patients with a slight degree of teratozoospermia: a prospective study

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Study question: Is intracytoplasmic sperm injection (ICSI) the wrong method of choice in infertile couples with a slight degree of teratozoospermia defined by the strict Kruger criteria?

Summary answer: Classical IVF insemination resulted in better embryo development than ICSI procedure in infertile couples with a slight degree of teratozoospermia.

What is known already: ICSI procedure enables the fertilization of oocytes with a low quality sperm or even, in extreme cases, with only a few spermatozoa available. Although, the overuse of ICSI is observed, since it is also performed in couples with normal sperm quality and female indications of infertility. It has already been shown that the overuse of ICSI does not improve or may even impair the outcome of IVF/ICSI cycles (Grimstad et al., 2016; Tannus et al., 2017) but it is still not clear at which percentage of normal spermatozoa ICSI is beneficial, if sperm concentration and motility are normal.

Study design, size, duration: Our prospective sibling-oocyte study included 27 couples who were treated in y. 2017 because of male infertility with a slight degree of teratozoospermia (7-14% of morphologically normal spermatozoa), defined by strict Kruger criteria, and with normal sperm concentration and motility. In each couple approx. half of oocytes were fertilized with ICSI (ICSI-group) and the remaining half of oocytes with classical IVF (IVF-group). The aim was to elucidate, which procedure results in a better clinical outcome.

Participants/materials, setting, methods: The female partners were aged 26-43 years and had at least 6 cumulus-oocyte complexes to express a normal ovarian reserve. Up to two optimal embryos were transferred into the uterus regardless the procedure. The IVF and ICSI-groups were compared in terms of fertilization rate, quality of day 3 and day 5 embryos, and pregnancy rate. To determine the differences between the two groups, a multivariate analysis was performed. Statistical significance was set at $P<0.05$.

Main results and the role of chance: The mean age of included patients was 35.6 ± 4.7 years for females and 36.0 ± 4.8 years for males. Mean numbers of oocytes included in each group were: 6.3 ± 3.6 for ICSI and 7.9 ± 6.1 for IVF- group. The fertilization (ICSI vs. IVF; 56.7% vs. 63.9%), cleavage (100% vs. 98.4%), and good quality day 3 embryo rates (55.0% vs. 63.9%) did not differ significantly between the two groups. Nevertheless, a significantly higher blastocyst (35.3% vs. 63.9%; P = 0.019) and good quality blastocyst rates (14.5% vs. 33.5%; P = 0.049) were observed in the IVF-group than in ICSI-group on day 5. The multivariate regression analysis showed that other factors such as female/male age and sperm quality parameters did not influence the difference between the two groups. The embryo transfer was performed in 24 cycles (without 2 cycles with 'freeze all" blastocyst freezing due to hyperstimulation). In 5 cycles embryos only from ICSI-group were transferred (number of ET embryos 1.0 ± 0) with 40% pregnancy rate and in 15 cycles embryos only from IVF-group were transferred (number of ET embryos 1.1 ± 0.3) with 66.7% pregnancy rate. None of these differences were significant.

Limitations, reasons for caution: The limitations of the study are in the relatively low number of included couples but the study is still in a progress. A further prospective study is needed. Achieved pregnancies need to be followed to evaluate the miscarriage and live birth rates according to the procedure (IVF, ICSI).

Wider implications of the findings: The results of this study show that the IVF procedure is optimal in couples with a slight degree of teratozoospermia along with normal sperm concentration and motility. In these couples the ICSI procedure may even impair the development of embryos to the blastocyst stage and a chance to get pregnant.

Trial registration number: not applicable.

P-147 Impact of embryo density on ICSI outcomes

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Study question: Does embryo density have an impact on embryo cleavage or development and does it enhance Intra-Cytoplasmic Sperm Injection (ICSI) outcomes?

Summary answer: The grouped cultured embryos with a density of 10 µl culture medium per embryo seems to promote embryo development and slightly improve ICSI outcomes.

What is known already: Among all culture methods, many authors agreed that group culture seemed to be beneficial to embryos through growth factors deposited in the droplet of culture medium. Though, not only growth factors but also toxic substances (such as ammonium and oxygen-derived free radicals) can be secreted by embryos in a group culture. Embryo density appears to be one of the most important settings in group culture to guarantee a better embryo development without exposing the embryos to an increased concentration of negative factors.

Study design, size, duration: It is a monocentric, prospective and randomized study, which lasted for one year, from June2015 to June2016.

Patients who had 2 or 3 fertilized oocytes were included in the study, which made a total number of 169 ICSI cycles.

Embryos were cultured either individually, or in a group of 2 or 3 embryos per droplet, which yielded to an embryo density of 30 µl, 15 µl, or 10 µl of culture medium per embryo for the three groups respectively.

Participants/materials, setting, methods: ICSI cycles were randomized, according to oocyte retrieval day, in three groups of different embryo density:

- Group A: Individual culture (1embryo per droplet: 30 µl of medium per embryo) (91 cycles)
- Group B: Group culture (2 embryos per droplet: 15 µl of medium per embryo) (43 cycles)
- Group C: Group culture (3 embryos per droplet: 10 µl of medium per embryo) (35 cycles)

Embryo transfer took place on day2 or day3, and sequential culture medium was used for the culture pursuit.

Main results and the role of chance: Group culture in an embryo density of 15 µl (GroupB) yielded to better cleavage rates than GroupA or C (96.% vs 95.2% vs 91% respectively, p = 0.08). Though, among the three groups, GroupC had the best embryo quality with a significant difference in the mean number of TOP quality embryos obtained (1.1 vs 0.73 vs 0.74 for GroupC, A and B respectively, **p = 0.023**).

Pregnancy rates were higher for GroupC compared with the two other groups (41.1% vs 33.3% vs 31.4% for GroupC, B and A respectively, p = 0.22). Implantation rates were also greater after transferring embryos cultured in a density of 10 µl than the two other groups (22% vs 21% vs 17.8% for GroupC, B and A respectively, p = 0.44). Nevertheless, those differences were not statistically significant.

Same findings were noticed for live birth rates from embryos of GroupC, which were greater than the two other groups with no statistical difference (18.6% vs 13% vs 10% for GroupC, A and B respectively, p = 0.29).

Our results suggest that embryo density of 10 µl seems to be suitable for obtaining a better embryo quality and for enhancing ICSI outcomes in terms of pregnancy, implantation and live birth rates after transfer of day2 or day 3 embryos.

Limitations, reasons for caution: Adopting day5 embryo transfer could provide more proofs of the benefits of culturing embryos with a 10 µl embryo density rather than other tested densities.

Wider implications of the findings: Culturing embryos in high density could generate some assessing and selection difficulties. That is why some authors brought the idea of culture systems, such as the Well-Of-the-Well system, in which embryos are cultured in a cone shape well to increase growth factor concentration while diluting negative ones.

Trial registration number:

P-148 Development of a chemically defined protein supplement for in vitro embryo culture

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Study question: Development of an effective, improved chemically defined protein supplement for IVF by identifying functional proteins found in the female reproductive tract and commercially serum-derived supplements.

Summary answer: A chemically defined protein supplement containing recombinant proteins identified in the female reproductive tract, HSA, and globulins, improved mouse and bovine blastocyst development in vitro.

What is known already: Commercially available protein supplements for embryo culture are used worldwide to effectively promote blastocyst development in IVF clinics. However, they generally consist of plasma derived human serum albumin (HSA) and globulin fraction IV, which lead to lot-to-lot variation. As a result, there is a need for the development of a chemically defined protein supplement that eliminates variability and delivers high quality blastocysts consistently. Recent studies have reported various biologic, functional proteins, including growth factors not only found in the female reproductive tract, which is the natural environment where embryos develop, but also in serum-derived protein supplements used in IVF labs.

Study design, size, duration: ELISAs were used to determine the presence and concentration of candidate factors in multiple lots of HSA and globulins. Mouse and bovine embryo assay (MEA/BEA) experiments were conducted to evaluate the effect of the identified factors on blastocyst development. ≥25 embryos were used per test group in both embryo assays. Embryos were cultured continuously in microdroplets overlaid with light mineral oil, and blastocyst rates were graded after 5 (mouse) or 7 (bovine) days of incubation.

Participants/materials, setting, methods: Frozen mouse zygotes from EmbryoTech were thawed and cultured in Continuous Single Culture Medium (CSCM®-NX) complemented with chemically defined protein supplement prototypes containing different defined protein compositions. All embryos were cultured in 5% CO_2 incubators at 37°C in 98% humidity. BEA experiments were performed at Applied Reproductive Technology, and all test samples provided were blinded to prevent biased evaluation and an internal control was always performed using specific bovine embryo culture media.

Main results and the role of chance: ELISA results demonstrated the presence of biological protein regulators, such as insulin in both the HSA and globulin/fraction IV, and concentrations were consistently higher in the fraction IV than in the HSA. These growth factor (GF) proteins, as previously reported, were also found in the oviduct and the endometrium. Test media prototypes were developed containing different combinations of these proteins. Even under more stressful and stringent embryo culture conditions of one embryo per droplet, MEA results showed that medium containing only insulin, performed equally well as control medium containing 10% Serum Substitute Supplement (HSA + globulin/fraction IV). In addition, test medium containing insulin and selected factors consistently improved blastocyst rate (≥80%) when compared to the control. Additional studies were done using the more sensitive BEA system, and these experiments also demonstrated that presence of defined protein supplement improved blastocyst rate as well as rate of fertilization, in vitro. Replacement of serum-derived components with recombinant proteins in IVF culture supplements not only eliminated inconsistencies and demonstrated improved performance, but it also removes the negative factors in human biological samples that may present toxic effects to the cultured embryos.

Limitations, reasons for caution: Present study of the newly developed, chemically defined protein supplement does not include human embryo development data. Human studies are in progress for full evaluation of the GF benefits to human embryo growth.

Wider implications of the findings: GF were shown to exist in the female reproductive tract as well as commercially available supplements that IVF laboratories have used for years. Therefore, GF in the chemically defined protein supplement will bring consistency in blastocyst development and can be considered safe as GF were present in previously accepted products.

Trial registration number: Not Applicable.

P-149 Early rescue ICSI has good clinical results - Record of 14 years at our clinic

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Study question: Long-term clinical performance of early rescue ICSI.

Summary answer: Early rescue ICSI can result in clinically valuable outcomes, producing normal rates of fertilization, blastocyst formation, pregnancy, and production.

What is known already: Although the outcome of early rescue ICSI has been reported in the past, there have been no reports that describe experiences over a prolonged period (14 years in this presentation).

Study design, size, duration: We performed a retrospective analysis of 5171 oocytes in which early rescue ICSI was performed, among 29,776 oocytes taken from 10,750 cases undergoing IVF in 2004-2017. The fertilization rate, blastocyst rate, pregnancy rate, and production rate were examined. As a control, we used 30,114 oocytes taken from 12,815 cases who underwent conventional ICSI during the same period. The stimulation methods used in this study were natural cycle, letrozole cycle, or clomiphene cycle.

Participants/materials, setting, methods: Cumulus cells were removed 5 hours after insemination, and oocytes were observed.

(1) After stripping, release of the second polar body was observed.
(2) Fertilization cone or cytoplasmic flare was observed.
(3) Although not applicable to 1-2, spindle body was not observed in the egg by observation with the spindle visualization system (PolScope).

Observations were carried out until 7 hours after insemination, and early rescue ICSI was performed when none of 1-3 was applicable.

Main results and the role of chance: The normal fertilization rate of early rescue ICSI was 81.8% (4232/5171), and the 3PN rate (which indicates the possibility of multisperm fertilization) was 4.8% (249/5171). The good blastocyst rate was 36.2% (1140/3149), the pregnancy rate was 56.3% (429/762), and the production rate was 67.9% (248/365).

The normal fertilization rate of conventional ICSI conducted during the same period was 80.9% (24344/30097) and the 3PN rate was 3.2% (972/30097). The good blastocyst rate was 33.0% (6879/20839), the pregnancy rate was 48.9% (2489/5088), and the production rate was 66.6% (1426/2141).

There was no significant difference in normal fertilization rate. The 3PN rate, the good blastocyst rate and the pregnancy rate were significantly higher in the early rescue ICSI group ($P < 0.05$). There was no significant difference in production rate.

Good blastocysts were defined as those with a diameter of 160 µm or more and a recognizable ICM. All embryo transfers were performed using the freeze-thaw single embryo transfer protocol, and production rates were based on embryo transfers up to 2016.

Limitations, reasons for caution: In Japan, some patients do not like ICSI (due to concerns over health of the resulting children), and there are institutions that perform IVF as much as possible and only carry out early rescue ICSI where fertilization fails.

Wider implications of the findings: The clinical outcome of embryos fertilized by early rescue ICSI is equal to or higher than that of conventional ICSI and is a useful technique for patients who do not like ICSI. Because the 3PN rate was higher than conventional ICSI, more effective multispermic fertilization prevention measures are necessary.

Trial registration number: Not applicable.

P-150 Clinical value of embryos with three or more blastomeres at the first division (direct cleavage embryos) but with nuclei in only two blastomeres

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Study question: Does the clinical outcome differ between direct cleavage embryos with two and three or more nucleus-bearing blastomeres at the first division?

Summary answer: Direct cleavage embryos with two nucleus-bearing blastomeres had a significantly higher rate of blastocyst formation than embryos with three or more nucleus-bearing blastomeres.

What is known already: There are many reports that the rate of development to the blastocyst stage is markedly decreased in direct cleavage embryos, and that these embryos have an increased risk of aneuploidy during development into blastocysts. However, few reports have investigated whether the number of blastomeres with nuclei in direct cleavage embryos affects their developmental outcomes.

Study design, size, duration: We performed a retrospective analysis of embryos that were cultured in the period 2013 to 2015 and that had been recorded using a time lapse monitoring system (EmbryoScope, Vitlolife).

In total, 1135 embryos with three or more blastomeres at the first division were included in the study. Cells with a diameter of 40 μm or more were defined as blastomeres.

Participants/materials, setting, methods: The first division of embryos cultured within the target period was observed with a time lapse monitoring system. Embryos with a nucleus in only two blastomeres (two nucleus group) and embryos with a nucleus in three or more blastomeres (three or more nucleus group) were classified among 1135 direct cleavage embryos. The rates of good blastocyst formation (a diameter of 160 μm or more and a recognizable ICM), pregnancy, and production were compared.

Main results and the role of chance: The rates of formation of good blastocysts were 26.0% (159/611) for the two nucleus group and 5.2% (27/524) for the three or more nucleus group; the rate in the two nucleus group was significantly higher ($P < 0.05$). After freezing, thawing and transplantation by single embryo transplantation, a pregnancy rate of 46.9% (38/81) was achieved in the two nucleus group and of 40.0% (6/15) in the three or more nucleus group; production rates were 65.8% (25/38) and 83.3% (5/6), respectively, for the two groups, which was not significantly different.

Limitations, reasons for caution: In Japan, PGS has not been approved, and a method for confirming euploidy of the embryos on the basis of morphology and developmental kinetics during culture is required.

Wider implications of the findings: The two nucleus group is presumed to have a normal chromosome distribution (blastomeres without a nucleus may be fragments). Although an abnormal distribution of chromosomes is expected in embryos with three or more nuclei, these embryos can produce successful pregnancies. The reasons for this will be studied in the future.

Trial registration number: Not applicable.

P-151 Clinical outcomes of metaphase II oocytes with the presence of refractile bodies in patients undergoing intracytoplasmic sperm injection treatment

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Study question: Does the presence of refractile bodies (RF) negatively affect fertilization, embryo development and implantation rates following intracytoplasmic sperm injection (ICSI)?

Summary answer: Oocytes with RFs showed lower embryo development and implantation rates than those without RFs, however there were no significant negative effects on fertilization rates.

What is known already: RFs are a one of the main morphological abnormalities that can be observed in the cytoplasm of human oocytes. RFs consist of a mixture of lipids and dense granular materials, having a yellow autofluorescence which is consistent with the typical autofluorescence of lipofuscin. Lower fertilization rates and lower embryo development rates have been consistently reported among oocytes with RFs when compared with those without RFs. However, the implantation potential of embryos derived from oocytes with RFs still remains unclear.

Study design, size, duration: To have the background of patients comparable and know whether RFs have negative impact on clinical outcomes, RF positive cycles which had both RF positive (RF(+)) and negative (RF(-)) oocytes were subjected into this study. A total of 316 ICSI cycles performed between January 2013 and June 2016 were retrospectively analyzed regarding fertilization and embryo development rates and 438 single frozen embryo transfers performed prior to January 2017 were analyzed to compare the implantation rates.

Participants/materials, setting, methods: MII oocytes with the presence of RFs ≥5 μm in diameter were recorded as RF(+). Retrieved oocytes were morphologically classified into RF(+) and RF(-) oocytes at the time of ICSI. A comparison of the fertilization and the blastocyst formation rates on Day 5 was made between the two groups. Implantation rates were assessed in frozen-thawed single embryo transfer cycles. Statistical significance was determined by Chi-Square test.

Main results and the role of chance: A total of 3,085 oocytes were retrieved. Of these, 648 (21.0%) were RF(+). Fertilization rates in RF(+) and RF(-) were 76.5% and 77.2% respectively and no significant differences were detected (Odds Ratio (OR) = 0.96; 95% CI: 0.79-1.18, $P = 0.730$). The blastocyst formation rates on Day 5 in RF(+) and RF(-) were 45.8% and 52.2% respectively and were significantly lower in RF(+) oocytes than those in RF(-) oocytes (OR = 0.77, 95% CI: 0.63-0.94, $P = 0.011$). Implantation rates in RF(+) and RF(-) were 24.2% and 42.2% respectively and were also significantly lower in RF(+) oocytes than those in RF(-) oocytes (OR = 0.44, 95% CI: 0.26-0.73, $P = 0.001$). Furthermore, the implantation rates in RF(+) oocytes when high quality blastocysts were transferred was 28.6%, which was also significantly lower than that of 46.1% in RF(-) oocytes (OR = 0.47, 95% CI: 0.26-0.86, $P = 0.011$). However, no significant differences were found regarding miscarriage rates (16.7% vs. 30.1%) (OR = 0.47; 95% CI: 0.15-1.44, $P = 0.158$).

Limitations, reasons for caution: As the presence of RFs was only recorded at the time of ICSI, the presence of RFs in immature oocytes could not be analyzed. Since this study only focused on the differences between RF(+) and RF(-) oocytes from RF(+) cycles, comparisons with RF(-) cycles have yet to be done.

Wider implications of the findings: Our results suggest that oocytes with the presence of RFs have a lower potential to develop into blastocysts and even when they develop into high quality blastocysts, the chances of implantation are reduced. Further study is required to elucidate the causes of RF formation and thus improve implantation rates.

Trial registration number: Not applicable.

P-152 A higher incidence of smooth endoplasmic reticulum clusters in patients treated with a regimen of aromatase inhibitors

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Study question: Can a regimen of aromatase inhibitor (AI) reduce the occurrence of smooth endoplasmic reticulum clusters (sERCs) in oocytes?

Summary answer: Contrary to expectations, the occurrence of sERCs was significantly higher in oocytes from patients treated with AI than in those treated with clomiphene citrate (CC).

What is known already: sERC is one of the dysmorphic phenotypes found in human oocytes and significantly reduces pregnancy rates. A comparatively higher number of abnormities in live-births appear to be associated with the presence of sERCs in oocytes, although healthy babies can be born from embryos derived from sERC(+) oocytes. sERC formation is related to the higher levels of serum E2 detected in sERC(+) cycles. It has recently been reported that embryos derived from sERC(+) oocytes are prone to a higher frequency of aberrant spindle formation and mitotic cleavage. The levels of serum estradiol are known to be lower in patients with AI.

Study design, size, duration: This was a retrospective cohort study of patients whose AMH levels were ≦1.0 ng/ml, involving a total of 598 cycles between July 2014 and December 2016. The choice of ovarian stimulation protocol was dependent on serum AMH levels and age. Patients treated with AI and CC were in the same classification and the choice of AI or CC was according to the patients' preferences, therefore the two regimens, AI and CC are comparable.

Participants/materials, setting, methods: All of the participants were infertile women who were being treated by ICSI. When there was at least one sERC positive oocyte among the retrieved oocytes the cycle was defined as a sERC(+) cycle. The rates of sERC occurrence were calculated for each regimen of ovarian stimulation. The AI and CC regimens were compared regarding the sERC(+) rates and the serum estradiol and progesterone levels on the date of hCG administration.

Main results and the role of chance: The average age and AMH(ng/ml) level were 41.4 ± 3.5 and 0.38 ± 0.3 respectively for patients treated with AI and 41.3 ± 3.5 and 0.41 ± 0.29 for those with CC. The two regimens were comparable regarding age and AMH level (Age: OR 1.01, 95% CI 0.96-1.06, P = 0.79; AMH: OR 0.75, 95% CI 0.41-1.36, P = 0.35). The occurrence of sERCs was found to be 13.2% (23/174) in oocytes from patients treated with AI which was significantly higher than that of 3.8% (16/424) in oocytes from those treated with CC (OR 3.88, 95% CI 2.00-7.55, P<0.0001). Among the patients treated with AI, serum estradiol(pg/ml) and progesterone(ng/ml) levels on the date of hCG were 549.1 ± 388.2 and 1.20 ± 0.72 in sERC (+) and 366.0 ± 276.0 and 0.75 ± 0.55 in sERC(-) cycles. Both the estradiol and the progesterone levels were significantly higher in sERC(+) than in sERC(-) cycles (E2: OR 1.18, 95% CI 1.01-1.39, P = 0.033; P4: OR 3.10, 95% CI 1.57-6.37, P = 0.001). With regard to the CC cycles, the serum estradiol and progesterone levels on the date of hCG were 1305.4 ± 443.1 and 0.83 ± 0.51 in sERC(+) and 994.8 ± 491.6 and 0.71 ± 0.46 in sERC(-) cycles. Conversely, no significant differences were detected (E2: OR 1.12, 95% CI 0.97-1.27, P = 0.12; P4: OR 1.64, 95% CI 0.58-3.83, P = 0.32).

Limitations, reasons for caution: The comparison was limited to two ovarian stimulation protocols. Other stimulation protocols, such as long, short, and antagonist protocols, could not be compared with AI in this study, as the patients' backgrounds (age and levels of AMH) were all different.

Wider implications of the findings: As AI did not reduce the occurrence of sERCs, the elevation of estradiol may not be the cause of sERC occurrence, but a consequence. Considering the higher levels of progesterone in sERC(+) cycles, the negative effects of premature luteinization on oocytes, which frequently occur with AI protocol, require further study.

Trial registration number: Not applicable.

P-153 Male Factor is the most important factor influencing the frequency of unequal direct cleavage events as visualized by time-lapse during early embryo development

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Study question: To study Irregular Direct Cleave (DC) events under differing Infertility causes, IVF protocols and age groups to identify sub-groups with increased prevalence.

Summary answer: Irregular direct cleavage events were observed with a consistent regularity in all groups studied. Only male factor patients showed significant increase in occurrence.

What is known already: Irregular divisions during initial cleavage stage of embryo development generate low viability embryos which either arrest at cleavage stage or have elevated aneuploidies or mosaicism. The events are very specific, appear to occur at random, but with a consistent regularity, are not an artefact of stimulation, and the underlying mechanism is not clearly understood.

Study design, size, duration: A retrospective study over the period of 2012-2017 with all embryos incubated in Embryoscope and all annotations performed by the same person, and irregular direct cleavages defined as DC1, DC2 & DC3 (referring to direct cleave 1st cycle, 2nd cycle and 3rd cycle). Embryos were incubated for 3-5 days in Sage-1-step media. All embryos were sub characterised into Stimulated IVF cycle, Natural cycle, PGS (RIF, RM, Altered Karyotype), Age, & male factor (Ejaculated, TESE, Ionophore).

Participants/materials, setting, methods: A total of 7052 embryos were included. Patients included, (a) IVF with ICSI (2998 embryos); (b) PGD-AS: Implantation Failures RIF (n = 706), Recurrent Miscarriages (RM) (n = 894), & Translocations (n = 448); (c) Male: Ejaculated (n = 786), TESE (n = 278) & ionophore-enhanced fertilisation (n = 204); & (d) Natural Cycle IVF (n = 738).

Only 1-3 cell cleavages were included, all ambiguous cleavages excluded.

Setting, private IVF facility.

Main results and the role of chance: Irregular cleavages DC1, DC2 & DC3 occurred with regular frequency across all groups studied irrespective of age, aetiology, or if embryos were generated with ovarian stimulation or natural cycle. The overall proportion of embryos with irregular direct cleavage up to 8 cell stages were 1692 / 7052 (23.9%), with a distribution: DC1, 955/7052 (13,5%), DC2, 700 / 7052 (9,9%), & DC3, 37 / 7032 (0,5%). The proportion of embryos showing multiple direct cleavages DC1 + DC2, DC2 + DC2, DC1/2 + DC3 were only 40 / 7032 (0,5%).

Male factor patients showed a significant increase (p>0,01) in direct divisions DC1 with respect to all the other groups studied, and there was increasing frequency of DC1 with decrease in sperm quality (+ ionophore (37/204, 18,1%) > TESE (47/278, 16,9%) > Ejaculate (122/786, 15,5%). There were no significant differences in DC2 or DC3 for any of the groups studied.

All the DC1 embryos arrested at cell stages of development. DC2 embryos developed to blastocyst but with much lower frequency than non-DC embryos.

Limitations, reasons for caution: Great care was taken to standardise all timings and annotations to include any comparable irregular direct cleavage events and avoid pseudo-cleavage events. Natural cycles are primarily non-elective and include older patients (>40 years) and poor responders. DFI was not used as criteria for male factor.

Wider implications of the findings: Decoupling of visible events of time lapse analysis is an important task in trying to understand the underlying cause or mechanism. The observation that sperm quality can increase DC1 but not DC2/3 implies independent mechanisms. Multinucleation may play more important role in DC2/3.

Trial registration number: None.

P-154 Effectiveness of ≥170-µm diameter criterion for blastocyst cryopreservation

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Study question: Does setting a ≥170-µm diameter criterion for blastocyst cryopreservation influence clinical outcomes following implantation of frozen-thawed embryos?

Summary answer: Pregnancy rates following frozen-thawed embryo transfer were constant, irrespective of blastocyst diameter.

What is known already: Frozen blastocyst transfer is known to yield higher pregnancy rates than fresh blastocyst transfer due to embryo–endometrium synchronization.

We previously froze good-quality blastocysts at stage 3, and then thawed them on the day before transfer. Blastocysts were developed to stage 4 on the

following day and then implanted, and we reported a resultant improvement in post-freeze-thaw pregnancy rate over thawing on the day of transfer.

Based on this, we changed our cryopreservation criterion to stage 4 (blastocyst diameter ≥170 μm) in 2017.

Study design, size, duration: We targeted 547 cycles subject to single frozen-thawed blastocyst transfer between January and October 2017 for analysis in this study. Only good-quality blastocysts with diameter ≥170 μm at days 5 and 6 were eligible for analysis. Blastocyst diameter was measured from images taken immediately prior to cryopreservation, using an EmbryoScope system.

Participants/materials, setting, methods: Blastocysts were classified by diameter into Categories A (170 to 179 μm), B (180 to 189 μm), C (190 to 199 μm), and D (≥ 200). We compared pregnancy rates between the four blastocyst categories after 5- and 6-day incubation periods.

Main results and the role of chance: Pregnancy rates achieved with transferred blastocysts after 5-day incubation showed no significant differences between the 4 diameter categories, at 41.8% (64/153) for Category A, 39.5% (45/114) for Category B, 47.6% (30/63) for Category C, and 41.6% (32/77) for Category D.

Pregnancy rates achieved with transferred blastocysts after 6-day incubation showed no significant differences between the 4 diameter categories, at 29.5% (13/44) in Category A, 21.4% (9/42) in Category B, 45.5% (10/22) in Category C, and 46.9% (15/32) in Category D; however, the rates in Categories C and D tended to be high.

Limitations, reasons for caution: This study is limited to its retrospective design and small sample size.

Wider implications of the findings: Pregnancy rates showed no significant difference between transferred blastocyst categories irrespective of incubation periods; therefore, we considered that the cryopreservation criterion setting investigated in this study did not influence pregnancy outcome. Accordingly, we suggest that a criterion of diameter ≥170 μm is effective for blastocyst cryopreservation.

Trial registration number: Not applicable.

P-155 SCF in follicular fluid and granulosa cells is positively associated with oocyte developmental potential in humans

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Study question: To explore whether the levels of SCF in follicular fluid (FF) and GCs can be used as a potential marker for predicting oocyte developmental potential.

Summary answer: The levels of SCF in FF and GCs might be considered as a new marker for predicting oocyte developmental potential.

What is known already: Stem cell factor (SCF), which is derived from granulosa cells (GCs), plays a key role in the process of follicular development and oocyte maturation.

Study design, size, duration: Follicular fluid and GC samples from 150 female patients undergoing intracytoplasmic sperm injection were collected in this study.

Participants/materials, setting, methods: The SCF concentrations in FFs and SCF messenger RNA (mRNA) in GCs were evaluated by using enzyme-linked immunosorbent assay and real-time polymerase chain reaction, respectively.

Main results and the role of chance: The results showed that the levels of SCF protein and mRNA were significantly associated with oocyte maturation, normal fertilization, cleavage, and embryo quality. Moreover, the levels of SCF protein and mRNA in pregnancy group were also higher than those in the non-pregnancy group. The cutoff value of SCF in FF for predicting high-quality embryo was 1.346, with a sensitivity of 57.8% and a specificity of 72.4%, and the cutoff value of SCF in GCs for predicting high-quality embryo was 6.650, with a sensitivity of 64.4% and a specificity of 78.1%.

Limitations, reasons for caution: The number of samples is the limitation in this present. Further study need to comfired this results.

Wider implications of the findings: Our results showed a positive and statistically significant relationship between SCF level and oocyte maturation, normal fertilization, cleavage, embryo quality, and clinical pregnancy.

Trial registration number: not applicable.

P-156 A new meta-analysis about an unclear topic in ART: Fresh embryo versus freeze-all embryo transfer strategies

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Study question: Can cryopreservation of all embryos with subsequent embryo transfer (Freeze-all) promote improvements in the clinical ART outcomes compared with fresh embryo transfer (Fresh-ET)?

Summary answer: Freeze-all does not improve clinical outcomes when the mean number of oocytes retrieved is <15. It seems be useful when >15 oocytes are collected.

What is known already: Freeze-all has been hypothesized as the preferred way to avoid the potential deleterious effects of controlled ovarian stimulation (COS) on endometrial receptivity in fresh cycles of IVF/ICSI. It has been suggested that the supra-physiologic hormonal levels during COS promote a suboptimal uterine environment, impairing clinical outcomes in ART cycles. There are few randomized controlled trials demonstrating higher pregnancy rates with frozen-embryo transfer than with Fresh-ET. A previous meta-analysis has shown more favourable clinical outcomes with Freeze-all than with Fresh-ET. However, with the randomized trials published recently, a new meta-analysis is essential to guide the scientific community on this issue in reproductive medicine.

Study design, size, duration: A systematic review based on electronic searches of databases(PubMed, EMBASE, Web of Science, SCOPUS, and Cochrane Central Register of Controlled Trials; key-words:Freeze-all; Fresh-embryo; pregnancy rates; oocytes-retrieved) up to January 2018 was conducted to identify randomized controlled trials(RCTs) comparing the ART outcomes of fresh versus elective frozen-embryo transfer. The primary outcome was live birth rate (per woman randomized). Secondary outcomes included ongoing pregnancy rate(per woman randomized), clinical pregnancy rate(per patient randomized) and miscarriage rate(from clinical pregnancies).

Participants/materials, setting, methods: Six RCTs were included as targets for data extraction and meta-analysis. Most of the trials did not allow the clear identification of the study subjects(e.g,PCOS). Data were combined for meta-analysis using the StatsDirect statistical software. Dichotomous data are expressed as Relative Risk (RR) with a 95% confidence interval (CI). The amount of heterogeneity was evaluated using Cochran's Q and I^2. Study data were combined using a random-effects model. P-values <0.05 were considered statistically significant.

Main results and the role of chance: The results of this meta-analysis are divided into three parts, based on the mean number of oocytes retrieved.

Part I: mean number of oocytes retrieved >12 and < 21

1. Live birth rates(4-trials): Freeze-all: 46.9%(1081/2305) versus Fresh-ET:43.9%(1020/2321) with no statistical significant differences(RR = 1.13; 95%CI = 0.97-1.32; $P = 0.12$). Heterogeneity:$I^2 = 77.4\%$; CochranQ = 13.2, $P = 0.04$.
2. Ongoing pregnancy rates(6-trials): Freeze-all:49.7%(1209/2435) versus Fresh-ET:48.0%(1177/2450), with no significant differences(RR = 1.11; 95%CI = 0.98-1.25; $P = 0.1$). Heterogeneity: $I^2 = 67\%$; CochranQ = 15.1, $P = 0.01$.
3. Clinical pregnancy rates(5-trials): Freeze-all:54.5%(1278/2344) versus Fresh-ET: 53.7%(1269/2362), with no significant differences(RR = 1.04; 95%CI = 0.96-1.14; $P = 0.35$). Heterogeneity: $I^2 = 47.5\%$; CochranQ = 7.62, $P = 0.10$.
4. Miscarriage rates(5-trials): Freeze-all: 10.3%(132/1278) versus Fresh-ET: 12.1%(154/1269), with no significant differences(RR = 0.85; 95%CI =

0.65-1.12; $P = 0.25$). Heterogeneity:$I^2 = 23.8\%$; CochranQ = 5.25, $P = 0.26$.

Part II: mean number of oocytes-retrieved was >12 and <15

1. Live birth rates(3-trials): Freeze-all: 46.2%(1025/2214) versus Fresh-ET: 44.1%(985/2233), with no significant differences(RR = 1.07; 95%CI = 0.93-1.22; $P = 0.36$). Heterogeneity: $I^2 = 72.2\%$; CochranQ = 7.2, P = 0.03.
2. Ongoing pregnancy rates(4-trials): Freeze-all: 48.8%(1114/2284) versus Fresh-ET: 48.1%(1107/2300), with no significant differences(RR = 1.04; 95%CI = 0.93-1.16; $P = 0.47$). Heterogeneity: $I^2 = 59.3\%$; CochranQ = 7.30, $P = 0.07$.
3. Clinical pregnancy rates(4-trials): Freeze-all: 54.2%(1239/2284) versus Fresh-ET: 53.7%(1235/2300) with no significant differences(RR = 1.03; 95%CI = 0.94-1.13; $P = 0.50$). Heterogeneity: $I^2 = 53.8\%$; CochranQ = 6.5, $P = 0.09$.
4. Miscarriage rates(4-trials) Freeze-all: 10.3%(128/1239) versus Fresh-ET: 12.1%(149/1235), with no significant differences(RR = 0.87; 95%CI = 0.63-1.20; P = 0.39). Heterogeneity: $I^2 = 41.8\%$; CochranQ = 5.15, $P = 0.16$.

Part III: mean number was ≥15 and <21

1. Ongoing pregnancy rates(2-trials): Freeze-all: 62.9%(95/151) versus Fresh-ET: 46.7%(70/150), with statistical significant differences(RR = 1.33; 95%CI = 1.01-1.76; $P = 0.04$). Heterogeneity: CochranQ = 1.75, $P = 0.18$.

Limitations, reasons for caution: Most of the trials included did not define the population studied as PCOS/non-PCOS, so we cannot rule out the presence of ovulatory/anovulatory patients in the same study. Therefore, the mean number of oocytes retrieved was considered in the present meta-analysis. Some studies had high heterogeneity, thus high risk of bias.

Wider implications of the findings: Freeze-all could be favourable when a high number of oocyte is collected (≥15), suggesting an association between higher COS and impairment of endometrial receptivity. However, when the mean number of oocytes collected is <15, Freeze-all does not appear to be advantageous. RCTs on this topic are required.

Trial registration number: Not applicable.

P-157 Failure of cytokinesis and its higher incidence during ICSI procedures and in oocytes containing smooth endoplasmic reticulum clusters

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Study question: Can the failure of cytokinesis be attributed to ICSI procedures and oocytes which contained smooth endoplasmic reticulum clusters (sERCs)?

Summary answer: Embryos fertilized by the ICSI have a higher incidence of meiotic failure and oocytes containing sERCs have a higher incidence of meiotic and mitotic failure.

What is known already: sERCs are one of the dysmorphic phenotypes that have been reported in human oocytes. Significantly reduced pregnancy rates and a comparatively higher number of abnormities in live births appear to be associated with the presence of sERCs in oocytes. However, some reports have shown that healthy babies can be born, without reduced pregnancy rates, from oocytes observed to contain sERCs. In ESHRE 2017 we reported a higher incidence of cleavage failure among embryos derived from sERC(+) oocytes in contrast to their sERC(-) siblings. However, the incidence of cleavage failure among embryos in sERC negative cycles has been under investigation.

Study design, size, duration: This was a retrospective observational cohort study, involving a total of 1,415 oocytes, evaluated between January 2011 and December 2015. Oocytes were obtained from 43 sERC(+) cycles, which had at least one sERC(+) oocyte among the retrieved oocytes, and 143 sERC(-) cycles. During the course of this study, time-lapse recording was restricted to patients aged between 30 and 40 years of age who yielded 4 or more oocytes.

Participants/materials, setting, methods: The presence of sERCs was evaluated by careful observation of 7 focal planes using an EmbryoScope®Time-lapse system while studying the dynamic changes within oocytes and embryos. Statistical analysis was carried out to explore the nature of the abnormalities. Logistic regression analysis was carried out to explore the independent variables for meiotic and mitotic cleavage failure. Independent variables included fertilization procedure (ICSI or IVF), cycle occurrence of sERCs and oocyte-based occurrence of sERCs.

Main results and the role of chance: Among 1,415 oocytes, sERCs were found in 5.7% of the oocytes (81/1415) using a time-lapse observation system. The occurrence of mitotic cleavage failure after the IVF procedure among sERC(+) oocytes in sERC(+) cycles, sERC(-) oocytes in sERC(+) cycles, and sERC(-) oocytes in sERC(-) cycles was 22.9% (8/35), 8.7% (10/115) and 7.7% (31/404) respectively. By comparison, the occurrence of mitotic failure after the ICSI procedure across the same categories was 33.3% (7/21), 20.4% (11/54) and 5.5% (15/274) respectively. The occurrence of meiotic cleavage failure after the IVF procedure among these categories was 8.9% (4/45), 0.7% (1/141) and 0.8% (4/506) respectively, while the occurrence of meiotic cleavage failure after the ICSI procedure across these categories was 4.0% (1/25), 3.0% (2/67) and 2.9% (10/340) respectively. The incidence of mitotic cleavage failure in oocytes with sERCs was found to be significantly higher than that in oocytes without sERCs (OR = 2.560, 95% CI: 1.210-5.410, P = 0.014). The incidence of meiotic cleavage failure during the second polar body extrusion in oocytes with sERCs was also significantly higher than that in oocytes without sERCs (OR = 5.140, 95% CI: 1.190-22.200, P = 0.028). Furthermore, ICSI was found to have a greater frequency of meiotic failure than IVF (OR = 0.413, 95% CI: 0.174-0.981, P = 0.045).

Limitations, reasons for caution: The mechanism of the frequent failure of cytokinesis in embryos derived from sERC(+) oocytes is still unknown. This requires further investigation, as the appearance of sERCs could be a consequence rather than a cause of cleavage failure.

Wider implications of the findings: Our current data, showing that aberrant cleavage frequently occurs in sERC(+) oocytes during meiosis and mitosis and in ICSI cases during meiosis, suggests that abnormal embryo development could be related to disturbed Ca^{2+} distribution patterns. Regarding cytokinetic failure, an embryonic cell could become tetraploid, which may induce abnormal chromosomal configurations.

Trial registration number: not applicable.

P-158 Annual variation in ICSI cycle outcome in temperate zones is related to weather-temperature not calendar - seasons nor day-light duration as proved by regression analysis

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Study question: If the annual variation of ICSI outcome in our geolocality is related to meteorological year seasons (winter –spring-summer –autumn) or to weather temperature?

Summary answer: Weather temperature ≥ the 50th -centile, not calendar season in our geolocation independently predicts better IR and CPR in ICSI as shown by regression analysis.

What is known already: Whereas many studies reported significant seasonal variation in the outcome of IVF/ICSI cycles few studies denied the presence of such seasonality. The mechanisms underlying periodicity of human in vitro conception however are still unclear. While many authors attributed the annual variation in ICSI outcome to daylight length, others linked it to temperature changes. Peak natural fertility times vary from one latitude and climate to another and geographical discrepancies reflect differences in the mix of daylight

and temperature that most closely approximates ideal conditions for human conception. Are the same mechanisms apply to in vitro human conception?

Study design, size, duration: We retrospectively analyzed 3586 fresh first completed ICSI cycles performed in our center, geolocation -N 31.044183, E 31.378584300000057,by the same team of clinicians and embryologists over 6 years (2011-2016). We included all female ages and all ICSI indications. The cycles were assigned to one of 4 calendar- seasons (winter-spring-summer-autumn) then 2 temperature- centiles (November-_April) < & (May-October)≥ 50th centile: 20 °C) depending on date of ovum pick-up (OPU).

Participants/materials, setting, methods: We compared ages, infertility duration, eggs retrieved (ER), dose/ER, fertilization rate (FR), number of embryos-transferred (NET), blastocyst –ratios (RBL), clinical pregnancy rate (CPR) implantation rate (IR) in 4 calendar and 2 temperature seasons. Binomial logistic (BNLR) and multiple regression (MR) analysis were used to compare the predictive values of *age, NET, RBL, calendar-season, temperature-centile, day-light duration-centile*) for, IR, CPR.

Main results and the role of chance: No statistically significant differences were found between cycle features nor cycle outcome in the 4 calendar seasons: winter (888- ET), spring (843- ET),summer (840-ET), autumn (887 -ET) respectively (IR: 13.8, 13.1, 14.1, 13.2) (p = 0.68)**#**, CPR: 41.5, 40.6, 42.3, 40.5 (p = 0.86) **#**.Comparing the 2 temperature- centiles (November-April **temp < 20° C, 1747 ET) &** (May-October **temp ≥ 20 ° C – 1718 ET**) respectively): age **29.38 ± 5.6, 29.83 ± 5.6 (p = 0.02) *, Infertility-Duration 5.69 ± 4.26, 5.51 ± 3.975 (0.268) *, ER 11.16 ± 6.3, 11.02 ± 6.4 (p = 0.52) *, Amps/ER 3.92 ± 4.09, 3.94 ± 4.81(p = 0.92) *, FR 76.61 ± 22.02, 76.63.52 ± 22.0 (0.97) * mean NET 2.73 ± 0.83, 2.72 ± 0.85 (p = 0.9) *, RBL 36.2%, 38.9% (p = 0.11), total number of transferred embryos 4749, 4683, Total number of Sacs 601, 679, IR 12.7%, 14.5% (P = 0.0098) #, number of clinical pregnancies 690, 753, CPR 39.8% ,43.8% (P = 0.0026) #. (* T-test, # χ 2-test) Using MR for IR & BNLR for pregnant outcome employing the 6 variables significantly predicted both IR and CPR (p < .000) but, day- light duration, calendar-season did not significantly add to prediction (p>0.05). Absolute RR = 4 and Relative RR = 9.%, Number-Needed -to -Treat = 25**

Limitations, reasons for caution: The large sample size flagged minor differences in age (mean-29) and infertility duration (mean-5) as significant which is difficult to defend. The assignment to one calendar season or temperature centile by date of OPU does not exclude effects of other seasons when ET is done in subsequent season.

Wider implications of the findings: Patients in our geolocation should be counseled about the relatively better outcome in warmer weather compared to cold weather.This recommendation is only valid in locations which share us the weather temperature centiles.

Trial registration number: enter 'not applicable.

P-159 Re-biopsy combined with double vitrification during preimplantation-genetic-testing cycles: comprehensive description of blastocysts rescued after failing the first round of diagnosis

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Study question: Which are the technical and clinical results of re-biopsied blastocysts that failed the first round of qPCR-based aneuploidy testing on trophectoderm biopsies?

Summary answer: The risk of inconclusive diagnoses is <3%.Re-biopsied blastocysts after double vitrification show similar survival, euploidy and implantation rates as blastocysts diagnosed at the first analysis.

What is known already: The implementation of multicellular trophectoderm biopsy approach has significantly reduced the risk for amplification failure and inconsistent results during preimplantation-genetic-testing cycles. Yet, to date few reports defined the rate of re-biopsy procedures required, as well as their related technical and clinical results. On average, 2-6% of the blastocysts were reported to result in an inconclusive diagnosis after comprehensive-chromosome-testing (CCT), of which 1-2% due to DNA amplification failure. The technical and clinical outcomes seem comparable to blastocysts with a conclusive result after the first biopsy.

Study design, size, duration: 8991 blastocyst biopsy procedures were conducted between April 2013 and September 2017 at 7 IVF centres in Italy and analysed by qPCR at a single genetic lab. 206 blastocysts were successfully re-biopsied after warming and re-expansion, and then re-vitrified. 41 frozen single-embryo-transfers (SETs) of blastocysts diagnosed euploid after trophectoderm re-biopsy were performed to date. Logistic regression analyses were performed to investigate the parameters associated with an inconclusive diagnosis.

Participants/materials, setting, methods: Only preimplantation-genetic-testing-for-aneuploidies (PGT-A) cycles with a freeze-all approach were included. Vitrification was performed within 30 min from trophectoderm biopsy on collapsed blastocysts. qPCR-based CCT method was adopted. Amplification failure or nonconcurrent molecular data resulted in an inconclusive diagnosis. Only frozen euploid SETs were performed ≈2 hr after warming. Clinical pregnancy, miscarriage (<20weeks) and ongoing pregnancy rates were monitored.

Main results and the role of chance: 8991 blastocysts were included (3244 PGT-A cycles conducted from 2687 couples, mean maternal age: 38.5 ± 3.9, 25-45). 2.6% of the trophectoderm biopsies resulted in an inconclusive diagnosis (n = 228/8991, 95%CI:2.2%-2.9%), of which 2% (n = 176/8991) due to amplification failure and 0.6% (n = 52/8991) due to nonconcurrent results. The only parameters significantly associated with the risk of obtaining an inconclusive diagnosis were the IVF centre (OR = 1.13, 95%CI:1.1-1.2 from the one performing the highest to the one performing the lowest number of procedures; p<0.01) and the day-of-biopsy (OR = 0.55, 95%CI:0.43-0.69 from day 5 to day7; p<0.01).

213 blastocysts were warmed to be re-analysed. The survival rate after warming was 98.1% (n = 209/213) and the survival rate after re-biopsy was 98.6% (n = 206/209). All the re-biopsied blastocysts resulted in a conclusive diagnosis, among them the euploidy rate was 51.9% (n = 107/206) (The euploidy rate of blastocysts biopsied only once was 45.7% (n = 4000/4778)).

41 blastocysts diagnosed euploid after re-biopsy have been warmed to date. All of them survived (100%, n = 41/41) and were transferred. 51.2% clinical pregnancy (n = 21/41), 9.5% miscarriage (n = 2/21) and 46.3% ongoing pregnancy (n = 19/41) rates were reported.

Limitations, reasons for caution: Only targeted-qPCR-based CCT method was adopted in this retrospective analysis. A more powered report of the clinical (and possibly obstetrical and perinatal) outcomes after frozen euploid SET of re-biopsied blastocysts requires a larger sample size. Ideally, these clinical data should be clustered also per blastocysts' quality and day of full-blastulation.

Wider implications of the findings: Trophectoderm biopsy approach is confirmed technically-consistent and clinically-secure. The residual risk for inconclusive diagnoses (2.6%) seems related to day-of-biopsy (possibly the number/volume of retrieved cells) and IVF centres' expertise. Yet, it is worth re-biopsying undiagnosed blastocysts since the survival, euploidy and ongoing implantation rates seem comparable to the control.

Trial registration number: None.

P-160 Blastocyst culture using G-TL single-step versus Origio sequential medium: a semi-randomized approach

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Study question: Does human blastocyst formation and clinical outcome after IVF differ when using a single-step medium or sequential culture media?

Summary answer: Significant differences were observed for fertilization and embryo development in favour of the sequential medium. However, clinical pregnancy rate was not different.

What is known already: Embryo culture conditions represent a key part of IVF success. The development of culture media is based on two distinct approaches. 1) "Back to nature" in which the embryos are exposed to stage-specific media, designed to reflect the physiological changes in nutrient concentrations in the fallopian tube and the uterus (sequential media). 2) "Let the embryo choose" in which the embryos select the nutrients they require from a medium that provides them all at once (single media). At present, insufficient evidence exists to prefer sequential or single-step media for the culture of human embryos to the blastocyst stage.

Study design, size, duration: A preliminary sibling oocyte study (50 ICSI cycles) did not reveal differences in fertilization and embryo quality between the single-step medium (G-TL, Vitrolife) and the sequential media (Origio Sequential, Origio). In order to focus on the clinical outcome, a semi-randomized controlled trial including unselected ICSI cycles was performed between July 2016 and December 2016. In total, 2012 ICSI cycles were assigned to G-TL single-step medium (n = 1007) or Origio sequential media (n = 1005) on alternate days.

Participants/materials, setting, methods: In the G-TL single-medium group, injected oocytes were individually cultured in the same 25 µl droplet until day 5/6. In the cycles with Origio sequential media, injected oocytes were individually cultured in 25 µl cleavage medium and replaced to 25 µl blastocyst medium on day 3 until day 5/6. Fertilization, embryo development, embryo utilization rate (embryos transferred and vitrified per fertilized oocyte) and clinical pregnancies (with FHB) per intended transfer were compared between the two media.

Main results and the role of chance: Mean female age was 35.1 (±4.9 SD) in the G-TL single-step medium group and 35.4 (±5.1 SD) years in the Origio sequential media group. On average, 7.0 (±0.2 SD) and 7.1 (±0.2 SD) mature oocytes were available for ICSI. Fertilization rates in G-TL single-step medium (67.5%) and Origio sequential media (71.9%) were significantly different ($p = 0.0006$). Embryo development (top and good quality embryos) was significantly in favour of Origio sequential media, both on day 3 (74.8% versus 69.8%, $p = 0.0008$) and on day 5 (47.6% versus 40.5%, $p = 0.0005$). Likewise, the embryo utilization rate in Origio sequential media (44.8%) was significantly higher than in G-TL single-step medium (40.1%, $p = 0.0013$). Nevertheless, no significant difference was observed in the clinical outcome between the two media. In some cycles, freeze all policy was applied (236 cycles in the G-TL single-step group and 256 cycles in the Origio sequential group). Clinical pregnancy rate with FHB per intended transfer was 25.4% in G-TL single-step medium and 24.5% in Origio sequential media ($p = 0.708$).

Limitations, reasons for caution: In the present comparison between a single-step and a sequential medium clinical pregnancy rate was used as endpoint. However, in view of the significant differences in embryo development and embryo utilization rate, it might be worthwhile to look at cumulative live births.

Wider implications of the findings: The present study contributes to evaluate the performance of single-step versus sequential media in terms of embryo development and clinical outcome. In a smaller sibling oocyte study the present findings remained unnoticed. Differences in embryo development between media might also depend on specific laboratory conditions.

Trial registration number: not applicable.

P-161 Correlation among pAKT, pERK1/2 and DNA fragmentation index in human cumulus cells to determine oocyte competence

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Study question: The aim was to correlate specific biological aspects of cumulus cells isolated from individual cumulus-oocyte complexes (COCs) with the clinical outcome of the related embryos.

Summary answer: In case of positive clinical outcome, pERK1/2 nuclear localization in cumulus cells was correlated positively with pAKT expression levels and negatively with the DFI value.

What is known already: It is widely acknowledged that the activated pERK1/2 and pAKT factors play a key role in supporting the survival pathway, exerting an anti-apoptotic effect; it is also known that pERK1/2 translocates into the nucleus to interact with transcription factors and the DNA itself to promote cell viability and proliferation. Previous results demonstrated that in the cumulus cells of the oocytes able to produce blastocysts, the pAKT/DFI ratio was higher than in cumulus cells of embryos arrested during the *in vitro* culture. In addition, a significant direct correlation was found between pAKT and pERK1/2, suggesting a cooperative action as survival factors.

Study design, size, duration: The study had the duration of 24 months and involved 26 patients after informed consent. For each follicle containing a mature oocyte, the levels of pAKT, the nuclear localization of pERK1/2 and the DNA fragmentation index (DFI) in cumulus cells of single COCs were evaluated and put in correlation with the positive or negative clinical outcome of the related embryos according to the ability to reach blastocyst stage and the results assessed for statistical significance.

Participants/materials, setting, methods: Normo-responder patients of ART procedures were selected. The cumulus cells were harvested after hyaluronidase treatment of the individual COCs and centrifugation. The oocytes were transferred to the culture medium until used for ICSI. The DFI was evaluated *via* TUNEL assay. The levels of pAKT and the nuclear localization of pERK1/2 were assessed via immunofluorescence microscopy and densitometric analysis of the images using the appropriate software. The statistical significance was evaluated *via* the non-parametric Kruskall-Wallis test.

Main results and the role of chance: Out of 126 MII oocytes, 91 were fertilized and the derived embryos had the following evolution: 53 were transferred "in utero", 8 were arrested during "in vitro" culture and 30 were cryopreserved. In case of the positive clinical outcome, we found that in cumulus cells of the corresponding COCs the nuclear localization of pERK1/2 showed a significant inverse correlation with the DFI value, which is an apoptosis hallmark, ($r_s = -0.39$, $p = 0.007$), and a significant direct correlation with the intracellular accumulation of pAKT ($r_s = 0.477$, $p < 0.001$). These results were not obtained with cumulus cells of COCs related to negative clinical outcome of the arrested embryos. In addition, all the cumulus cells examined showed a significant inverse correlation between the intracellular accumulation of pAKT and the DFI value ($r_s = -0.37$, $p < 0.001$), confirming its role as a survival factor also in this cell type. Therefore, our results suggest that high levels of nuclear pERK1/2 accumulation coupled with an increase of pAKT and a low DFI value in the cumulus cells of the corresponding COCs might be considered as a marker of oocyte competence leading to the development of embryos of presumed good quality.

Limitations, reasons for caution: The present results are preliminary and further investigation is needed to improve their significance level and to gain further insights into the involvement of the biological aspects under study in the acquisition of oocyte competence.

Wider implications of the findings: Our results suggest that the nuclear localization of pERK1/2 coupled to an enhanced intracellular accumulation of pAKT may play an anti-apoptotic role and increase cell viability thereby providing a novel marker tool to facilitate the choice of the best oocyte to be fertilized and submitted to an ICSI cycle.

Trial registration number: The trial is an observational study and no registration is needed.

P-162 Degree of blastocoel re-expansion is the single most determinant factor for enhanced live birth rates in vitrified-warmed single blastocyst transfer cycles

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Study question: To evaluate the independent effects of Inner-cell-mass (ICM), trophectoderm (TE), and degree of blastocoel re-expansion on live-birth rate in vitrified/ warmed single blastocyst transfer cycles.

Summary answer: Re-expansion of blastocoel with degree of 3-4 is imperious to ICM and TE cell gradation for better live-birth rates in vitrified-warmed single blastocyst transfer cycles.

What is known already: The relevance of blastocoel expansion for clinical outcomes in blastocyst transfer cycles has been documented. However recent studies have presented contradictory results regarding significance of ICM gradation, TE gradation and degree of blastocoel expansion/re-expansion for increased live-birth rates in fresh as well as vitrified-warmed blastocyst transfer cycles. There is no consensus yet on which of these is the most significant predictor for live-birth. Moreover, few studies have reported combined results from fresh/frozen and single/double transfer cycles. Therefore, we aimed at verifying the relevance of each of these three parameters in predicting live-birth rates exclusively in vitrified-warmed cycles involving single-blastocyst transfer.

Study design, size, duration: Retrospective study of vitrified-warmed cycles involving women (n = 216) undergoing elective / non elective single blastocyst transfer from February 2014 to November 2016 at our private ART centre. Oocyte donation, Embryo-donation, assisted hatching and preimplantation genetic diagnosis cycles were excluded. Ethics Committee of the centre approved this retrospective study. All blastocysts were graded as per Gardner and Schoolcraft method of classification as 1-6 for degree of expansion and grades A/B/C for ICM and TE.

Participants/materials, setting, methods: Natural-cycle endometrial preparation was done with hormone supplementation followed by luteal-phase support with micronized progesterone. Endometrial response (thickness and homogeneity) was noted by ultrasound. Elective/non-elective single blastocyst-transfer was done 3 hours after warming of vitrified blastocyst. Warmed blastocyst was compared with pre-vitrified blastocyst for degree of expansion, gradation of ICM/TE. β-hCG level measured on day8 of transfer indicated pregnancy. Positive cardiac activity at sixth week confirmed clinical pregnancy. Live-birth was the primary outcome measure.

Main results and the role of chance: Women were classified into Live-birth (LB; n = 74) and non-pregnant (NP; n = 142) groups. Age, BMI, infertility period as well as number of oocytes retrieved, rate of formation of good quality cleavage-stage and blastocyst-stage embryos and survival rates post-warming did not differ significantly between the two groups. Same brand of embryo-transfer catheter and same brand and volume of media was used for transfer. However, the degree of re-expansion was significantly higher in LB group than in NP group (Mean ± SD: 3.6 ± 1.0 v 3.2 ± 0.94, P = 0.0068). The Fisher-Exact test odds ratio for achieving a live-birth with 3-4 degree of re-expansion was 2.78 (p = 0.0016) whereas the odds ratio was much lower (0.36) with any other degree of re-expansion. Although ICM grade was higher/better in Live-birth group than in non-pregnant group, the difference remained statistically non-significant (Odds ratio 1.25, p = 0.82). No significant difference was observed in the TE grades between the two study groups (p = 0.17). A notable difference was also observed in the endometrial echopattern (p = 0.03) although the endometrial thickness remained comparable between the two groups. Thus, post-warming degree of re-expansion is the single most promising predictive factor for live birth rates in such cycles.

Limitations, reasons for caution: Although the results are promising, this is a retrospective study with a relatively small sample size. A multicentric/randomized trial with much larger sample size is essential to generate a strong power for degree of re-expansion to be predictive of live-birth.

Wider implications of the findings: A good experimental design catering exclusively to vitrified-warmed cycles involving single blastocyst transfer is a strong point of this study. Therefore, even a relatively small sample size leaves no ambiguity regarding the significance of selection of best blastocyst on the basis of degree of re-expansion in predicting live birth rates.

Trial registration number: Not Applicable.

P-163 Morphokinetic analysis of euploid blastocysts: searching for non-invasive criteria of embryo implantation additional to chromosomal assessment

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Study question: Can we define any morphokinetic parameter able to increase our predictive power upon implantation potential of euploid blastocysts cultured in time-lapse?

Summary answer: Blastomeres' symmetry at 4 cell-stage and blastocyst morphological quality after full-expansion were significantly correlated with the implantation of euploid blastocysts.

What is known already: To date, the assessment of a normal chromosomal constitution through comprehensive-chromosome-testing techniques represents a powerful predictive parameter upon blastocyst's reproductive potential. However, ≈50% of euploid blastocysts fail to implant.

The study of preimplantation embryonic development, via morphokinetic parameters in time-lapse, do not provide to date solid evidence of correlation with the clinical outcomes after IVF. Although, the additional predictive value of morphokinetic in preimplantation-genetic-testing (PGT-A) cycles conducted in time-lapse is yet to be assessed.

Study design, size, duration: Retrospective exploratory study. All transferred euploid blastocysts obtained after consecutive PGT-A cycles conducted in a time-lapse system between March 2014-March 2017 were included (n = 320). All morphokinetic parameters of embryo evaluation were compared among implanted and not implanted euploid blastocysts. Logistic regression analyses and receiver operating curve (ROC) analysis were conducted. Power calculations for significant differences were performed with the software G*Power v3.1.

Participants/materials, setting, methods: 130 implanted and 190 not implanted euploid blastocysts were included and confronted for the following timings: tPB2(2ndPolar-Body extrusion), tPNf (pronuclei formation), t2,t3,t4,t5, t8 (2-,3-,4-,5- and 8-cell division), cc1(t3-t2), cc2(t4-t3), cc3(t5-t4), tM(morula), tSB(starting-blastulation), tB(full-expansion). Furthermore, blastomeres' symmetry and fragmentation at t4, and blastocyst's quality at tB were evaluated and blindly-confirmed by two independent operators. The results were corrected through univariable and multivariable logistic regression analyses for all putative confounders including maternal age.

Main results and the role of chance: Significant differences were reported between implanted and not implanted euploid blastocysts for cc2 (1.3 ± 2.3 h,0-12.6 h versus 2.5 h±4.3 h,0-26.5 h, p<0.01), cc3 (12.4 ± 5.2 h,0.3-45.5 h versus 10.7 ± 6.5 h,0-43.4 h, p = 0.02) and tM (88.7 ± 10.5 h,66.9-121.7 h versus 91.4 ± 10.0 h,61.3-123.1 h, p = 0.03). Furthermore, low-fragmentation (<10%; n = 111/243, 45.7%) at t4 resulted in higher ongoing implantation rates after euploid blastocyst transfer than high-fragmentation (≥10%; n = 9/77,24.7%; p<0.01 and power = 92%). Similarly, even blastomeres' symmetry (n = 114/241, 47.3%) at t4 resulted into a better reproductive outcome than uneven one (n = 16/79,20.3%; p<0.01 and power>99%). At last, blastocysts' quality at tB was also correlated with a positive ongoing implantation (excellent: n = 86/184,46.7%; good: n = 21/49,42.9%; average: n = 17/51,33.3% and poor: n = 6/36,16.7%; p<0.01 and power = 95%). The univariable logistic regressions confirmed these data. Conversely, the multivariable logistic model outlined only blastomeres' symmetry (even/uneven, OR = 0.36, 95%CI:0.19-0.67; p<0.01) and blastocyst's quality (excellent/good/average/poor, OR = 0.77, 95%CI:0.61-0.98; p = 0.04) as predictive of implantation success/failure. The ROC curve analysis defined an area under the curve (AUC) for this model of 0.65, 95%CI:0.60-0.71, p<0.01.

Limitations, reasons for caution: The main limitations of this study are its retrospective nature and the subjectivity of blastomeres' symmetry definition at t4. Possibly, these data should be confirmed through a computational/mathematical assessment. Moreover, the extension of the sample size may allow the evaluation also of anomalous patterns of development, such as direct/reverse cleavage.

Wider implications of the findings: A model including blastomeres' symmetry at 4cell-stage and blastocyst's quality at full-blastulation may represent a valuable selection tool to predict euploid blastocysts' reproductive competence for any PGT-A cycle, regardless the adoption of a time-lapse system. Nonetheless, these data should be confirmed from a prospective study.

Trial registration number: None.

P-164 Does embryonic culture environment affect ploidy rates

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Study question: Does culture condition affect the chances of detection of an euploid embryo in a benchtop incubator (BT) compared to Time lapse monitoring incubator (TLM)?

Summary answer: This study demonstrates that uninterrupted controlled culture environment (TLM) has an increased probability of detection of an euploid embryo in a PGT-A cycle.

What is known already: Most aneuploidies are meiotic in origin and maternal age related. Aneuploidies do not always impair embryo development and blastocyst formation can be achieved. Studies suggest several factors may adversely affect the incidence of chromosomal anomalies. Additionally, environment is known to affect outcome through epigenetic modulation. A recent study concluded that the incidence of mosaicism and euploid rates differed amongst labs, thought to be due to screening methods, culture conditions and biopsy stage and unknown indication for PGT-A. Our study explores the potential impact of culture environment on ploidy, evaluated by comparing two different incubator types in the same laboratory.

Study design, size, duration: Retrospective single centre cohort study, a total of 732 blastocysts were biopsied on D5/6 between May 2017 to December 2017. The genetic testing was performed using Next Generation Sequencing Technique (NGS) at a single reference centre (coopergenomics-UK). The euploid rate in embryos cultured in BT incubator, interrupted single-step medium (n = 349 blastocysts) vs those cultured in a TLM incubator, uninterrupted single-step medium (n = 383 blastocysts) were compared.

Participants/materials, setting, methods: Patients undergoing PGT-A cycle with at least two failed previous IVF attempts, advanced maternal age, recurrent miscarriage, or previous aneuploidy pregnancy. Study was conducted at a single centre IVF unit CRGH London. The average female age was 38.1 ± 4.3. Post ICSI, oocytes were either cultured in a BT incubator or TLM incubator. Trophectoderm biopsy was performed on D5/6 removing between 6-8 cells. The ploidy rates were subsequently compared between groups.

Main results and the role of chance: Age was normally distributed and comparable (p = 0.229) in both incubators. Fertilisation rate in the TLM was significantly higher (77 ± 17.3 vs 71 ± 14.9, p = 0.047) when compared to the BT Incubator. The blastulation rate was significantly higher in the TLM group versus BT group (64.8 ± 36.5 vs 63.4 ± 36.1, p = 0.000) respectively. The Euploid rate obtained from both groups showed a higher proportion of euploid embryos in the TLM incubator 25.5% (98/383) compared to those cultured in the BT incubator 20.6% (72/349) (p = 0.04).

Limitations, reasons for caution: Further PGT-A cycles resulting in increased number of biopsied blastocysts to be evaluated in a different centre to observe this findings in both culture environment.

Wider implications of the findings: First single centre study to directly compare euploid rates in two different culture conditions. Studying the algorithm patterns in euploid embryos could provide the means for non-invasive PGT-A screening. Further evaluate how culture conditions at a cellular level could affect epigenetic mechanism in embryo/s resulting in a higher aneuploid rate.

Trial registration number: not applicable.

P-165 The effects of 2-APB on the intracellular calcium concentration and ultra structure damage of vitrification oocyte

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Study question: Can the change of calcium concentration of oocyte induced by cryoprotectant be regulated to reduce the damages of ultra structures?

Summary answer: IP3 receptor inhibitor 2-APB can effectively inhibit the increase of calcium concentration of oocytes and relieve the damages induced by freezing.

What is known already: Vitrification can effectively preserve human oocytes with high survival rate about 90-95%,Which was accompanied by compromised developmental potential and ultra structure damage of oocyte induced by cryoprotectants and low temperature. The increase of intracellular calcium concentration of oocyte induced by cryoprotectants was supposed mediating the injury happening in oocyte cryopreseving process. 2-APB which was known as IP3 receptor inhibitor can effectively inhibit calcium releasing from intracellular calcium store of endoplasmic reticulum for many different non excitatory cells.

Study design, size, duration: More than 500 cumulus-oocyte-complexes (COCs) were collected from 15 mice. The total of 478 MII oocytes with first polar body and normal morphology was used in subsequent calcium detection, vitrification and ultra structure analysis. The oocytes derived from the same mouse were allocated into 3 different groups at random: control, croprotectant without or with 2-APB. 60 oocytes were used for calcium labeling and other oocytes were vitrified and thawed with different treatment for subsequent analysis.

Participants/materials, setting, methods: A Total of 15 mice and 478 oocytes was included in present study. The oocytes were divided into 3 groups at random: control, croprotectant without or with 2-APB, in which the oocytes were treated with culture medium, cryoprotectants, and cryoprotectants with 0.1umol/l 2-APB respectively, following with analysis of intracellular calcium concentration by laser scan confocal microscope, ultra structures of freeze-thawing oocytes by transmission electron microscope.

Main results and the role of chance: Exploring the calcium transients demonstrated that cryoprotectant(n = 15) induced instantaneous increasing of Ca^{2+} in oocyte, and the concentration at steady state was also higher than that of fresh oocyte in control group(n = 15)(123.59 ± 4.73 vs.94.75 ± 3.54) (P<0.05); However the 2-APB treatment (n = 15) can effectively stabilize calcium level, the calcium transient peak and steady-state levels were lower than that cryoprotectants induced, and the concentration of calcium at steady-state was similar with that of the control group(92.76 ± 4 .22 vs.94.75 ± 3.54) (P>0.05). Undergoing freezing,the calcium concentration of 2-APB group was lower than that without 2-APB group(122.21 ± 4.33 vs.139.70 ± 6.00) (P<0.05). Examination by transmission electron microscope indicated that microvilli shortened and deficiency, cortical granule reduced in the cortex area in cryoprotectant, mitochondrial swelled, intermediate filament disassembled, edge of lipid drop blured, and a large number of vacuoles in matrix formed in vitrification group without 2-APB comparing with that in control group. Treatment with 2-APB relieved the injuries of the microvilli, cortical granule, mitochondria and lipid drops etc, which was partly resulted by vitrification. But the damages were still severer than that of control group.

Limitations, reasons for caution: More studies are needed to evaluate the developmental potential of oocytes treated by IP3 receptor inhibitor.

Wider implications of the findings: Our findings suggest a possibility to relieve the oocyte injuries induced by cryoprotectant and low temperature for oocytes which undergo cryopreservation.

Trial registration number: n/a.

P-166 High pressure freezing: A potential method for improving oocyte cryopreservation

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Study question: Do oocytes frozen under high pressure survive and exhibit better ultrastructural preservation than those frozen by standard vitrification methods?

Summary answer: Oocytes frozen under high pressure have improved ultrastructural preservation and survive freezing if cryoprotectants are used.

What is known already: Current vitrification methods are able to preserve oocyte viability but cause molecular and structural changes to both the nuclear and cytoplasmic compartments, which may compromise quality. Cortical granule release can occur during the cryopreservation process causing premature hardening of the zona pellucida (ZP) and necessitating the use of ICSI. High pressure freezing (HPF) is known to provide superior ultrastructural preservation, and potentially improved molecular preservation of cells. Some cell types survive this process. However, it is not known whether high pressure freezing can be used to preserve viability of oocytes or embryos.

Study design, size, duration: Grade 1 or 2 immature cumulus-oocyte complexes (COC) were collected from adult sheep ovaries. Groups of 16 to 24 COC or in vitro matured oocytes (MO) underwent HPF or vitrification, with or without cryoprotectants (CP) and were stored in liquid nitrogen for a minimum of one week. Three frozen oocytes from each group were examined by transmission electron microscopy following freeze substitution, embedding and sectioning. Thawed oocytes were matured, fertilised and cultured.

Participants/materials, setting, methods: COC and MO were frozen and thawed either without CP in H199 with 10% FBS or with CP as follows. Oocytes were equilibrated in base medium (H199 with 20% FBS; BM) with 7.5% each DMSO and ethylene glycol (5 to 15 min), then frozen within 60 sec in BM with 0.5 M sucrose and 15% each DMSO and ethylene glycol. Oocytes were thawed in BM with 1 M sucrose (1 min) then 0.5 M sucrose (3 min) and BM (5 min).

Main results and the role of chance: None of the HPF oocytes, without CP, survived thawing. However, the ultrastructure of these oocytes, following freeze substitution, was superior to those HPF or vitrified with CP, and conventionally fixed oocytes. 44% of the HPF and 21% of the vitrified COC, and 50% of the HPF and 43% of the vitrified MO underwent cleavage following IVF, though numbers were too low to perform meaningful statistics. None developed to morula stage. Oocytes vitrified with CP all had irregularly structured ZP containing pores of variable size. Oocytes frozen with HPF and CP had superior ultrastructural preservation of the ZP, with four out of the 6 oocytes examined having ZP with a uniform structure and no pores.

Limitations, reasons for caution: This was a pilot study to assess the potential of HPF for oocyte cryopreservation and low numbers of oocytes were examined. Polyspermy was not assessed in the fertilised oocytes.

Wider implications of the findings: High pressure freezing appears to provide improved ultrastructural preservation, particularly of the ZP, which may avoid the need for ICSI. The use of ICSI bypasses natural sperm selection processes and may also be associated with an increase in epigenetic defects in the resulting embryo.

Trial registration number: Not applicable.

P-167 Let-7b microRNA modulates attachment of trophoblastic cells and integrin β3 expression of endometrial cells

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Study question: Does let-7b microRNA (miRNA) modulate attachment of trophoblastic cells and expression of integrin (ITG) family in endometrial cells?

Summary answer: Transfection of let-7b miRNA inhibitor decreases the attachment of trophoblastic spheroids, whereas transfection of let-7b miRNA mimic increases the expression of ITG-B3 in endometrial cells.

What is known already: Several miRNAs are known to be involved in regulating the implantation process, there is incomplete understanding of how let-7 family controls implantation. Expression of let-7b miRNA was significantly increased in outgrowth and peri-implantation embryos compared to pre-implantation embryos. ITG family expression levels in uterine biopsy samples suggested as a biomarker for evaluating uterine receptivity and determining the optimal time for embryo transfer. The ITG is an important molecule involved in attachment and invasion of trophoblasts and endometrial cells.

Study design, size, duration: Our previous studies have focused on the expression and function of let-7 family in blastocysts, outgrowth embryos and endometrial cells for successful implantation. This study was performed to evaluate the regulatory function of let-7b miRNA in human trophoblastic JAr cells and endometrial Ishikawa and ECC-1 cells.

Participants/materials, setting, methods: The expression profile of let-7b was clarified in a series of developing mouse embryos, and the target gene was confirmed by bioinformatics. Inhibitor or mimic of let-7b miRNA was transfected into human trophoblastic and endometrial cells, respectively. To investigate the effects of the let-7b on attachment and implantation process, transfected trophoblastic spheroids were co-cultured on endometrial cells. Expression patterns of integrin family after transfection of miRNAs were evaluated by immunoblot analysis in endometrial cells.

Main results and the role of chance: Expression of let-7b miRNA was significantly increased in outgrowth embryos on 7.5 days post coitum compared to pre-implantation embryos. When let-7b inhibitors were transfected into trophoblastic JAr cells, attachment rate of trophoblast spheroids onto endometrial cells was significantly decreased in both Ishikawa and ECC-1 cells. We found that ITG-B3 could be directly targeted by let-7b miRNA by in silico analysis. There were no significant differences of ITG-B4 and ITG-A5 expression after transfection of let-7b inhibitor and mimic transfection in endometrial cells. However, expression of ITGB3 was upregulated when mimic let-7b miRNA was transfected into Ishikawa endometrial cells.

Limitations, reasons for caution: Functional approaches were carried out by analyzing transient expressions of transfected microRNA modulators using *in vitro* model. The consequence of the let-7b miRNA on implantation process still remains to be determined in physiological *in vivo* model. Other target genes of let-7b in embryo implantation should be further elucidated.

Wider implications of the findings: Our findings indicate that the let-7b miRNA plays an important role in embryo implantation as a regulator of ITG-B3 expression. These results could provide a valuable understanding of the regulation involved in the miRNA-mediated implantation between trophoblastic and endometrial cells. It could subsequently improve outcome in human ART program.

Trial registration number: Not applicable.

P-168 Reproductive outcomes after vitrification of oocytes generated from human chorionic gonadotropin (hCG)-primed in vitro maturation cycles

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Study question: What is the effect of cryopreservation of oocytes that matured in vitro (vIVM) on reproductive outcomes compared to fresh in vitro maturation (fIVM) cycles?

Summary answer: Lower fertilization, embryo cleavage and good embryo rates were found in vIVM cycles. Live birth rates were significantly lower in vIVM compared to fIVM.

What is known already: Although two successful live births and one ongoing pregnancy from cancer survivors were reported after cryopreservation of IVM embryos obtained, there is no report of a live birth from the cryopreservation

of oocytes produced by IVM of immature oocytes for cancer patients. In previous studies, lower reproductive potentials were found in vIVM cycles compared to oocyte vitrification in IVF cycles.

Study design, size, duration: This is a retrospective cohort study conducted at a university hospital affiliated IVF unit. Fifty-six cycles of vIVM and 263 fIVM cycles in women diagnosed with polycystic ovarian syndrome ovaries (PCOS) were included in the analysis. All women underwent a human chorionic gonadotropins (hCG) primed IVM cycle.

Participants/materials, setting, methods: The study group included PCOS patients who failed ovulation induction with intrauterine insemination and were offered IVM cycle followed by oocyte vitrification and warming. The embryological aspects and clinical outcomes of vitrification/warming were compared to controls undergoing fresh IVM cycles during the same period. The main outcome measure was the live birth rate.

Main results and the role of chance: From 1070 oocytes that were collected (56 patients) a total of 68 MII and 1,002 GV oocytes were retrieved; 645 oocytes (64.4%) matured in vitro and underwent vitrification/warming. In the fIVM group, 485 MII and 4,296 GV oocytes were retrieved; 2,630 (61.2%) matured to MII. Survival rate after warming was 59.8% (416/713). Fertilization and embryo cleavage rates per oocyte were significantly lower in the vIVM. Clinical pregnancy (10.7% vs. 36.1%) and live birth rates (8.9% vs. 25.9%) per cycle were significantly lower in the vIVM group than in the fIVM group ($P = 0.005$ and $P<0.001$, respectively). Five healthy singleton babies were born in the vIVM group, and 80 babies including 18 twins and 1 triplet were born following fIVM cycle. Furthermore, the association between live birth and vitrification, age, number of in vitro matured oocytes, AFC and number of embryo transferred was analyzed using a stepwise logistic regression analysis. Vitrification of IVM oocytes was associated with a lower likelihood of a live birth (adjusted odds ratio, 0.3 95% confidence interval 0.1 to 0.7).

In our fertility preservation program, 6 cancer survivors underwent a transfer of embryos from vitrified IVM oocytes that resulted in 2 chemical pregnancies.

Limitations, reasons for caution: The retrospective nature of this study.

Wider implications of the findings: Our results suggest that although vitrification of IVM oocytes is an alternative for women who cannot pursue IVF oocyte vitrification for fertility preservation, the reproductive potential of these oocytes is impaired.

Trial registration number: NA.

P-169 A new approach to reduce thermal and oxidative stress during vitrification procedure

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Study question: Determine whether suspended graphene can be used instead of sucrose to reach higher thermal conductivity while maintaining the efficiency of vitrification solution.

Summary answer: Graphene can be proposed as a conductive cryoprotectant, leading to a more moderate expression of apoptosis, oxidative and thermal stress-related genes.

What is known already: The most effective characteristic of vitrification is high cooling/warming rate, which helps to reduce ice crystal formation and cellular damage. In recent years, the ultra-rapid cooling rate has been achieved using small volumes of vitrification solution along with various cryodevices. However, the heat conductivity of vitrification solutions as an effective parameter on the cooling/warming rate has received less attention.

Study design, size, duration: To determine the optimal concentration of graphene, 100 blastocysts in each group were vitrified using different graphene-based solutions (vitrification solution: 0.5, 1, 1.5, 3, 7 M; thawing solution: 2, 3, 4 M; dilution solution: 1, 1.5, 2 M), evaluated in terms of survival and hatching rate. The optimal solution was then compared with sucrose-based one in order to analyze all the aspects described below.

Participants/materials, setting, methods: In vivo produced mouse blastocysts were vitrified using Cryotop as described by Kuwayama (Kuwayama et al., 2005), with slight modifications. After 12 h, vitrified blastocysts were compared with fresh blastocysts in terms of survival, hatching and implantation rate, cellular peroxide levels and expression level of genes related to thermotolerance (*Hspa1a*) oxidative stress (*Sod1*, *Sod2*) and apoptosis (*Trp53*, *Bax*, *Bcl2*).

Main results and the role of chance: The optimum concentrations of 1.5, 2 and 4 M were first selected for vitrification, thawing and dilution solutions, respectively. According to our results, natural honey as a cryoprotectant could be as efficient as sucrose in terms of survival, hatching and implantation rate. The real-time RT-PCR analysis also revealed a reduction in the expression of *Hspa1a, Sod1*, *Sod2* and *Trp53* transcripts in the graphene group compared to the sucrose group; the abundance of *Bax* and *Bcl2*, however, did not change. Furthermore, there was a lower cellular peroxide level in the graphene-based group compared with sucrose-based one.

Limitations, reasons for caution: Further studies are necessary to evaluate development-related genes expression and live birth rate in blastocysts vitrified using graphene-based solution to assess long-term safety outcomes.

Wider implications of the findings: Our findings highlight the importance of heat conductivity of vitrification solutions, demonstrating that graphene can act as a cryoprotectant as well as a heat transfer agent.

Trial registration number: None.

P-170 Effects of resveratrol, GM-CSF, and DCA on the development of embryos in aged mice

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Study question: To determine whether supplementation of culture media with resveratrol, GM-CSF, and DCA influence embryo development and pregnancy rates in aged mice.

Summary answer: Resveratrol significantly increased pregnancy and implantation rates even though there were no differences in fertilization and development rates.

What is known already: Major implications of older patients are low fertilization rate, poor embryonic development, and increased rate of chromosomal aberrations, which leads to an increased miscarriage and aneuploidy. It is associated with several molecular mechanisms contributing to ovarian aging such as alterations in gene expression and mitochondrial dysfunction. There were some reports that resveratrol has a positive effect on embryo development through improving mitochondria function. Moreover, addition of dichloroacetic acid (DCA) to culture media improved blastocyst development and mitochondrial output in embryos produced from aged mice. Granulocyte-macrophage colony-stimulating factor (GM-CSF) had significant effect on ongoing implantation rate in women with previous miscarriage.

Study design, size, duration: BD F1 female mice at 58-62 weeks of age were used for this study. MII oocytes obtained by super-ovulating mice were fertilized and cultured in MRC media with or without resveratrol (0.5 uM), GM-CSF (2 ng/ml), and DCA (1.0 mM). Blastocysts from each group were transferred into 2.5 days post-coitum (dpc) pseudo-pregnancy ICR mice using non-surgical embryo-transfer device.

Participants/materials, setting, methods: Fertilization and embryo development rates were monitored. To assess pregnancy and implantation rates, expanded or hatching blastocysts from each groups were transferred into pseudo-pregnant ICR mice. The pregnancy rate was measured as the number of pregnant mice as a proportion of the total transfer mice. Implantation rates were calculated as the number of live pups as a proportion of the total number of embryos transferred.

Main results and the role of chance: A total of 673 MII oocytes were fertilized in vitro (control: 186; Resveratrol: 177; GM-CSF: 158; DCA: 152). There were no statistically differences in fertilization rates among groups (control: 94.6%; Resveratrol: 97.2%; GM-CSF: 96.2%; DCA: 97.4%, respectively). The addition of resveratrol, GM-CSF, and DCA (92.4%, 92.1%, and 95.9%,

respectively) tended to increase the blastocyst rate, but only DCA group was significantly higher than control group (86.9%, *P<0.05*). After blastocyst transfer to recipient females, there was no significant increase in pregnancy and implantation rates when embryos were fertilized and cultured in the presence of GM-CSF (50.0% and 22.3%) and DCA (53.8% and 16.7%) as compare to the control (52.6% and 19.2%). However, supplement of resveratrol significantly increased pregnancy and implantation rates (75.0% and 33.8%) compared to the other three groups (P<0.05).

Limitations, reasons for caution: In this study, only development and pregnancy rates were measured in order to examine the effect of each factor in aged mice. Further investigations on the mechanism are needed to confirm the effect of each factor.

Wider implications of the findings: Resveratrol added to the media enhanced pregnancy and implantation rates, although there was no difference in fertilization and blastocyst rates. It suggests that the addition of resveratrol to embryo culture media for patient with advanced maternal age may potentially increase IVF outcomes.

Trial registration number: None.

P-171 Effect of resveratrol supplement during in vitro culture on the mitochondrial membrane potential and histone acetylation of embryos from aged mice

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Study question: Does resveratrol supplementation of culture media affect mitochondrial membrane potential and histone acetylation of embryos from aged mice?

Summary answer: The addition of resveratrol to embryo culture media improves mitochondrial membrane potential and inhibits abnormal histone acetylation of embryos from aged mice.

What is known already: Advanced maternal age affects the quality of oocytes and blastocysts. Reproductive aging pathologies are frequently associated with mitochondrial dysfunction and gene expression of histone deacetylase, which may be involved in the process of epigenetic modification. There were some reports that resveratrol has the ability to prevent age-related diseases by improving mitochondria function. In addition, resveratrol activates sirtuin 1 (SIRT1), an NAD^+-dependent histone-deacetylase, which is able to remove the acetyl group from histone H4 at lysine 12 (H4K12) in somatic cell. It was reported that level of H4K12 acetylation in oocytes increased during the aging process in human and mouse.

Study design, size, duration: BD F1 female mice at 58-62 weeks of age were used for this study. As a control, 6-8 weeks old female mice were used for young group. MII oocytes obtained by super-ovulating mice were fertilized and cultured in MRC media with or without resveratrol (0.5 uM). Their embryonic development rates were monitored. On the other hand, mitochondrial membrane potential and level of histone acetylation were examined at different developmental stages (2-cell, 4-cell, morula, and blastocyst).

Participants/materials, setting, methods: Mitochondrial membrane potential throughout the pre-implantation embryo development was analyzed by 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) staining. The acetylation pattern of H4K12 was examined by immunofluorescence staining using an anti-acetylated H4K12 antibody.

Main results and the role of chance: Fertilization rates were no statistically differences among three groups (Young: 98.1%, Old: 94.6%, Old-res: 97.2%, respectively). However, blastocyst rate in young group (99.4%) was significantly higher than that in old group (86.9%) (P<0.05). The addition of resveratrol (Old-res group) increased the blastocyst rate (92.4%), but there was no statistical differences compared to the other two groups. The average mitochondrial membrane potential in young group was higher than that in old groups, but there was a statistical significant difference at 2-cell and 4-cell stages (P<0.05). Resveratrol supplement increased the level of mitochondrial membrane potential throughout the stages of pre-implantation embryo in aged mice. These increases were significantly greater than either young or old groups (P<0.05). The acetylation levels of H4K12 were examined and compared among three groups. Immunocytochemistry with specific antibody against AcH4K12 showed that the fluorescence signal was statistically higher in old group than in young group throughout the pre-implantation embryo development. The acetylation levels of H4K12 tended to decrease with the addition of resveratrol and it was statistically significant only at the 4-cell stage.

Limitations, reasons for caution: In the preliminary study, 0.5 uM resveratrol enhanced embryo development and mitochondrial membrane potential of embryos from aged mice. Therefore, 0.5 uM resveratrol was used in this experiment. However, the dose of resveratrol should be determined carefully because the appropriate dosage reported by researchers were varied.

Wider implications of the findings: Poor development of embryo in aged mice was associated with mitochondrial dysfunction and abnormal histone acetylation. In addition, resveratrol added to the media may result in an improvement of mitochondria membrane potential and stabilization of histone acetylation. Addition of resveratrol to culture media for aged women may increase IVF outcomes.

Trial registration number: None.

P-172 Embryo culture medium based on the composition of human oviductal fluid contributes to improving the viability of mouse embryos

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Study question: Does a culture medium reflecting the inorganic salts, carbohydrates, organic acids, and amino acid concentrations of human oviductal fluid improve the viability of mouse embryos?

Summary answer: Embryo culture media based on human oviductal fluid composition with modified potassium and phosphate concentrations improve the hatching rate and cell numbers of mouse blastocysts.

What is known already: Commercially available HTF medium based on six components of human oviductal fluid and sequential media based on three components of human oviductal/uterine fluid have been used clinically in human assisted reproductive technology. However, information on the composition of female reproductive tract fluids is far from complete because of the limited volumes available for analysis. There is no medium with almost all of the components (more than 30) similar to human oviductal fluid.

Study design, size, duration: We developed a high-sensitivity multicomponent analysis system and analyzed human oviduct fluid. In a prospective study, mouse embryo culture media based on this analysis were compared with conventional culture medium. In vitro fertilized 1-cell mouse embryos were cultured in the media and observed every day until day 4. Blastocysts were analyzed to assess their viability. In total, 780 fertilized oocytes and 725 blastocysts were examined.

Participants/materials, setting, methods: Human oviductal fluid samples aspirated laparoscopically from 28 women aged 26–39 years were analyzed to determine the concentrations of 31 components by liquid chromatography with tandem quadrupole mass spectrometry and ion chromatography. Zygotes from ICR strain mice were cultured in control medium, $KSOM^{AA}$, or test media for 4 days to assess blastocyst formation and hatching rates. Blastocysts were analyzed by TUNEL staining to measure total cell numbers and the percentages of apoptotic cells.

Main results and the role of chance: Because the apoptosis rate of blastomeres cultured in media based on human oviductal fluid was higher than in $KSOM^{AA}$ medium (3.8% vs 5.2%), the potassium and/or phosphate levels in the new media were reduced from the levels found in human oviductal fluid levels to levels in conventional media (potassium, from 15.3 to 5.5 mmol/L; phosphate, from 2.5 to 0.3 mmol/L). As a result, using a potassium-modified medium or a potassium- and phosphate-modified medium, the hatching rate was improved (from 73.1% to 86.5% and 88.5%, respectively; $p < 0.05$) and the numbers of blastomeres increased (from 80.2 ± 1.5 to 95.8 ± 2.0 and 90.2 ± 2.0; $p < 0.01$) compared with the $KSOM^{AA}$ medium. In the phosphate-

modified medium, the numbers of blastomeres increased (from 80.2 ± 1.5 to 90.7 ± 2.2, p < 0.01) compared with the KSOM^{AA} medium.

Limitations, reasons for caution: Oviductal fluid samples were collected from ampullae after ovulation from patients suspected of infertility. The analytical values might differ if the donor and collection times or sites differ. This was a study using mouse embryos and their performance in such defined culture media might differ from human embryos.

Wider implications of the findings: We analyzed more than 30 components of human oviductal fluid for the first time and clarified that it was effective in helping to formulate new embryo culture media. We believe that these results have the potential to lead to the development of an ideal culture environment for human embryos.

Trial registration number: Not applicable.

P-173 Are morphology of first polar body related to embryo quality and euploidy rate in couples?

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Study question: What is the parameter of an oocyte that indicates the embryo quality and euploidy rate?

Summary answer: Morphology of first polar body has a relationship with the euploidy rate especially in under 38 years old maternal group.

What is known already: Previous studies have reported that morphology of first polar body (1st PB) affects oocyte viability and embryo quality. Furthermore, it has been reported that morphology of 1st PB is correlated with chromosome abnormality of embryos.

Study design, size, duration: This retrospective study was carried out to investigate the difference of euploidy rate and embryo quality according to morphology types of 1st PB in couples with advanced maternal age. Morphological classifying of 1st PB was evaluated in 676 MII oocytes of 101 PGS cycles using array-CGH. During the period from January 2016 to November 2017, this study was performed in couples suffering from recurrent implantation failure or repeated spontaneous abortion over 2 times.

Participants/materials, setting, methods: Observation of 1stPB was performed using an inverted microscope during Intracytoplasmic Sperm Injection (ICSI). Morphology of 1st PB is classified into 4 different groups: Type 1: ovoid 1st PB with smooth surface; Type 2: ovoid 1st PB with rough surface; Type 3: fragmented 1st PB; Type 4: large 1st PB. All embryos were biopsied at day 3 and day 5, and then analyzed by array-CGH.

Main results and the role of chance: The highest day2 and day3 good quality embryo (GQE) rate is type1 among four types (70.6 % and 58.1 %), with no statistically significant difference. However, the euploidy rate of type1 was observed significant difference with among each type2 and type4 (33.1% vs. 23.3%, $p<0.05$ and 33.1% vs.3.8%, $p<0.05$). Groups were divided to under 38 and over 38 years old, type1 and type2 of under 38 years of age group have higher euploidy rate than over 38 years old group with statistically significant difference (40.5% vs. 22.8%, $p<0.05$ and 29.2% vs. 16.2%, $p = 0.05$).

Limitations, reasons for caution: This study has numerical differences with each type which needs to be further validated. More studies should be performed to demonstrate these significant differences.

Wider implications of the findings: We found a relationship between the morphology of 1st PB and the euploidy rate in PGS cycles. Study suggests that the morphology of 1st PB can be one of the indicators of embryo euploid.

Trial registration number: N/S.

P-174 Comparison of clinical pregnancy and live birth rates among fresh day 5 and frozen-thawed day 5, day 6 blastocyst transfer

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Study question: Is there a significant difference in outcome of IVF programmes with fresh day 5(d5ET), frozen day 5(d5FET) and frozen day 6(d6FET) blastocyst transfer?

Summary answer: According to our data based on 9671 fresh and frozen blastocyst transfers the highest pregnancy and live birth rates are in IVF programmes with d5FET.

What is known already: Blastocyst cryopreservation in IVF cycles is used to accumulate embryos in poor-responder patients, allows time to perform genetic testing, provides the opportunity to postpone a transfer away from the non-physiologic endometrium, which occurs during a stimulation cycle, and may improve pregnancy rate (PR) and fetal outcome. Some studies show higher ongoing pregnancy and live birth rate (LBR) following d5FET compared with d6FET.

Study design, size, duration: We performed retrospective comparative study of PR and live birth rate/transfer among 3 groups of ICSI-ET cycles: 3254 with d5ET (average woman age 34 ± 4,5 years), 3969 with d5FET (average woman age 34 ± 4,0 years) and 2448 with d6FET (average woman age 34 ± 4,7 years). All patients underwent IVF and ET from 2009 to 2016 at AltraVita IVF clinic, Moscow. Cycles with a single ET of a good and high quality blastocyst were included in the study.

Participants/materials, setting, methods: Vitrification method by Cryotech and Kitazato, Japan, was used for vitrification/thawing of blastocysts. Embryos were evaluated before transfer and were assigned grades according to Gardner blastocyst grading scale. For our investigation analysis cases with blastocyst transfer grades (1-4) AA, AB, BA, BB were chosen. Ongoing pregnancy after ET was confirmed by one fetal sac and heart beat. PR and LBR were statistically analyzed using Chi-square test.

Main results and the role of chance: Outcome results in the group of d5FETwere 36.6 % PR and 26.3 % LBR, which is significantly higher compared to 26,5 % PR (P<0.01; OD 1.64, 95% CI, 1.49-1.82) and 18.5 % LBR (P<0.01, OD 1.12, 95% CI, 1.05-1.37) in the group with d5ET and 27,5 % PR (P<0.01, OD 1.53, 95% CI, 1.37-1.70) and 18.1 % LBR (P<0.01, OD1.2, 95% CI, 1.06-1.36) in the group with frozen d6FET. There were no significant differences in the pregnancy rate 26,5% versus 27,5 % (OD 0.95, 95% CI, 0.84-1.07) and LBR 18.5% versus 18.1% (OD 1.01; 95% CI, 0.88-1.18) between groups with fresh d5ET and frozen d6FET, respectively.

Limitations, reasons for caution: ICSI-ET cycles groups contain data on all types of stimulation protocols without exceptions.

Wider implications of the findings: We've analyzed a high number of ET cycles in our study. The pregnancy and life birth potential of good and high quality frozen-thawed day 5 blastocysts is higher than fresh day 5 blastocysts and frozen-thawed day 6 blastocysts. D5FET may be considered as a preferable strategy during the IVF treatment.

Trial registration number: N/A.

P-175 Moving towards individualized embryo transfer-Guidelines for number of embryos to be transferred based on key performance indicators

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Study question: Can key performance indicators (KPIs) help in formulating guidelines for number of embryos to be transferred?

Summary answer: Single embryo transfer (SET) should be considered in donor oocyte cycles and in women < 30 years if cleavage rate (CR) is above 95%

What is known already: KPIs in the Assisted Reproduction Techniques (ART) laboratory act as early warning signs. The Vienna Consensus Statement of July, 2017 of the European Society of Human Reproduction and Embryology (ESHRE) defines competency (COMP) and benchmark (BENCH) levels of various KPIs. Globally, there is a recommendation to shift to SET to avoid

complications of multiple pregnancies. However, it is a common practice at most of the centres in several countries including in India to transfer two embryos in routine and three in poor prognosis cases to increase the pregnancy rate.

Study design, size, duration: This study reported an analysis of 3-year data of 699 in-vitro fertilization (IVF) cycles with fresh embryo transfer at a tertiary IVF centre. KPIs analysed included: metaphase II (MII), fertilization (FR), CR and embryo quality rates (EQR), which were compared with COMP and BENCH. Outcomes compared included clinical pregnancy rate (CPR) and implantation rate (IR). Statistical analysis was done with one way ANOVA and Chi Square test methods for comparison using SPSS-21.

Participants/materials, setting, methods: The IVF cycles were divided in four groups based on age: Group I, ≤ 30 years; group II, age 31-35 years; group III, age > 35 years; and group IV, donor oocyte irrespective of recipient's age. Each group was subdivided into two sub-groups based on CR: A with CR < 95% (COMP level) and B with CR > 95 %. The CPR, IR and multiple pregnancy rates were compared amongst groups and within sub groups.

Main results and the role of chance: The mean CPR and IR rates respectively: Group I were 36.92% & 22.31%; group II, 33.05% & 18.53%; group III; 21.57 % & 11.44 %,and group IV; 43.79% & 27.02%. The CPR and IR were the highest in donor oocyte group, decreased as the age increased. Women above 35 years' age performed worst. These differences were statistically significant amongst various groups. In sub groups, CPR and IR were higher with cleavage rate > 95 % than CR < 95%, but the difference was statistically insignificant. Multiple pregnancy rate was the 11.83 % in group IV, followed by group I with 9.23 %, group II 7.73% and in group III 3.92%. Thus, a clear recommendation for SET can therefore be made for Group-IV. Similarly in group I with CR >95%, a policy of SET can be preferred over double embryos transfer (DET). Group II with age 31-35 years should be given DET due to a lower IR. In group III, all cases should be transferred three embryos to improve CPR.

Limitations, reasons for caution: KPI like MII, FR and EQR, being above BENCH were not considered. Any dip in an individual case will affect decision. The other variables which may affect the IVF outcome like endometrium, day of ET, previous failures were not taken into consideration. Moreover patient's choice has also to be considered.

Wider implications of the findings: Mathematical modelling based on clinical and laboratory KPIs can help individualize the number of embryos to be transferred.

Trial registration number: Since it was a retrospective analysis of data, trial registration number was not sought.

P-176 Enhancing blastocyst quality by priming spermatozoa with cumulus mass fragments for human ICSI cases

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Study question: Does priming spermatozoa with cumulus mass before ICSI enhance developmental speed and quality of blastocysts?

Summary answer: ICSI with cumulus primed sperm not only increases developmental speed but also enhance the quality of blastocysts.

What is known already: By using big data set from US Society of Assisted Reproductive Technology (SART), it has found that application of ICSI for non-male factor cases significantly decrease pregnancy outcomes. In mouse model, ICSI with acrosome intact sperm has severe negative impact upon egg survival. Induce acrosomal reaction before ICSI can prevent this negative impact.

Study design, size, duration: The study is a cross sectional -control vs. treatment design. Totally 1447 eggs from 97 ICSI cases during September 1 - December 23, 2017 included in the study.

Participants/materials, setting, methods: The study population was with mean age 33.5 and median age at 33 in a fertility clinic. All ICSI cases with at least 6 oocytes and 5 million motile sperm/sample included in the study. In each case, half number of mature eggs assigned as control (ICSI with swim up sperm; Standard ICSI) and the other half number of mature oocytes ICSIed with autologous cumulus primed sperm (Primed ICSI). Generalized Estimation Equation used for statistical analysis.

Main results and the role of chance: For convenience, Standard ICSI is brief as Standard. Primed ICSI is brief as Primed. For fertilization, Standard has 482 fertilized out of 713 ICSIed eggs (67%) vs. Primed ICSI 546/764 71%)(not significant); Day 5 blastocyst quality > = 3BB, Standard 61/482 (12.6%) vs Primed 102/546 (18.6%) p<0.01); Total good quality blastocyst (> = 3BB) rate: Standard 254/482 (52.6%) vs. Primed 324/546 (59.3%) p<0.05; Biopsiable rate (at exapnded blastocyst stage): Standard 193/254 (75.9%) vs. Primed 246/324 (75.9%) not significant; euploid rate: Standard 80/193 (41.4%) vs. Primed 103/246 (41.8%) not significant.

Limitations, reasons for caution: The statistical power is limited due to small sample size.

Wider implications of the findings: There are reasons for worldwide elevation of ICSI utilization. To alleviate the negative impact of ICSI, the couple's autologous cumulus mass fragments show promising improvement. If confirmed by big data power, the cumulus priming procedure may will be the standard ICSI protocol worldwide.

Trial registration number: not applicable.

P-177 Blastocyst diameter is an important parameter for predicting live birth in frozen single blastocyst transfer cycles

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Study question: Which morphological parameter is most important to select the best blastocyst for transfer?

Summary answer: The blastocyst diameter on Day 5, rather than inner cell mass (ICM) or trophectoderm (TE) grade, is the most significant predictor of live birth.

What is known already: To assess the blastocyst, three morphological parameters have routinely been used: degree of blastocoele expansion, size and compactness of the ICM and number of TE. Although blastocysts with the highest scores for all three parameters achieve high implantation rates (IRs), there is controversy over which parameter is the strongest predictor of live birth. In addition, even for the same expansion grade, the range of embryo diameters is wide.

Study design, size, duration: This study is a retrospective analysis of 1107 vitrified/warmed Day 5 single embryo transfers of good morphology blastocysts (≥3BB) between 2012 and 2015. Exclusion criteria included patients with ≥38 years of age, repeated implantation failure (≥6 embryo transfer) and hatching/hatched blastocyst. Blastocysts were divided by their diameter, ICM and TE grade. Implantation and live birth rates (LBRs) were compared.

Participants/materials, setting, methods: The blastocysts were observed at 114-116 hours after insemination. The blastocyst diameter was measured between the outside borders of the TE. Blastocysts were classified according to their diameters (<140μm, 140-160μm, 160-180μm, ≥180 μm), ICM (A, B) and TE (A, B). Adjusted odds ratios (AOR) were calculated by multivariate logistic regression.

Main results and the role of chance: The mean patient age was 32.4 ± 3.4 years. Univariate regression analysis showed blastocyst diameter and TE grade to be correlated with IR and LBR. The IRs for blastocyst diameter groups (<140μm, 140-160μm, 160-180μm, ≥180μm) were 37.1%, 53.9%, 59.1% and 61.3% respectively; while the LBRs were 28.2%, 38.6%, 46.5% and 48.6%, respectively (P<0.05). The IRs for TE grade (A, B) were 56.9% and 50.0%, respectively (P<0.05); while the LBRs were 44.4% and 37.3%, respectively (P<0.05). However, there was no significant association between ICM grade and LBR. LBRs were 42.8% and 37.2% for ICM grades A and B, respectively.

Furthermore, multivariate logistic regression analysis showed that only blastocyst diameter was significantly associated with live birth (<140μm versus 140-160μm: AOR 1.56 95%CI 1.08-2.27; <140μm versus 160-180μm: AOR 2.12 95%CI 1.47-3.06; <140μm versus ≥180μm: AOR 2.26 95%CI 1.50-3.43).

Limitations, reasons for caution: Only high-quality embryos used in frozen transfer were considered in this study. In addition, hatching/hatched embryos were excluded because an accurate diameter was not measureable.

Wider implications of the findings: Our data show a strong correlation between blastocyst diameter on Day 5 and probability of live birth. More individualized timing of embryo transfer in frozen embryo cycles may be needed to improve implantation rates, especially for blastocysts that are <140µm.

Trial registration number: Not applicable.

P-178 What stage of in vitro embryo development is affected by oxygen tension? A randomized clinical trial (RCT)

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Study question: Is embryo development influenced by oxygen tension applied during culture? If yes, are both cleavage and blastocyst stages vulnerable to oxidative stress (OS)?

Summary answer: Hypoxia during embryo culture improves embryo quality and development ability. However, late pre-implantation embryo development seems to be less vulnerable to oxidative stress.

What is known already: In mammals, uterine environment is at low oxygen concentration (2-8% O_2). Thus, human embryo culture under 2-8% O_2 is recommended by ESHRE revised guidelines for good practices in IVF labs. Indeed, hypoxia (5% O_2) seems to improve embryo quality at cleavage and blastocyst stages, presumably by reducing damages of OS. Nevertheless, recent meta-analyses concluded with a very low evidence to a superiority of hypoxia on IVF/ICSI outcomes. Furthermore, a study on mouse embryos suggested a negative impact of OS only at cleavage stage. This hypothesis has still never been investigated in humans.

Study design, size, duration: From 01/2016 to 12/2017, 721 IVF/ICSI cycles were included in this RCT. At Day-0 (D0), cycles were randomized using a 1:2 allocation ratio: group "20%-O_2" (n = 241); group "5%-O_2" (n = 480). Extended culture (EC) was performed when ≥5 D2-good-quality-embryos were available (n = 83 in subgroup A ("20%-O_2")). In subgroup "5%- O_2", 175 EC cycles were randomized again at D3 (using 1:1 ratio): group B: 5%-O_2 until D6 (n = 88); or C: switch to 20%-O_2 from D3 to D6 (n = 87).

Participants/materials, setting, methods: Inclusion criteria were: intracouple IVF/ICSI using fresh/frozen ejaculate; female age <40 years; absence of hydrosalpinx; ≥8 cumulus-oocyte complexes retrieved. Oocytes were fertilized and cultured in similar benchtop incubators, under 5 or 20% O_2. Fertilization rate, cleavage-stage quality (D2-top-quality-embryo (D2-TQE): 4 blastomeres, <20% cytoplasmic fragmentation), blastocyst quality when necessary (D5-top-quality-blastocyst (D5-TQB): ≥B4AA/AB/BA (Gardner and Schoolcraft's classification)) and implantation rate (IR) were compared between groups "20%" and "5%" (=cleavage-stage analysis), or A(20%), B(5%) and C(5%-to-20%) (=EC analysis).

Main results and the role of chance: In cleavage-stage analysis, all characteristics were similar between groups "20%" and "5%" O_2. A total of 6 304 embryos were analyzed. A significantly higher number of early-cleaved embryos was obtained under 5%-O_2 (3.3 *vs.* 2.4 under 20%; p = 0.0009). A trend towards higher D2-TQE rate was achieved under 5%-O_2 (40.8% *vs.* 37.0%; p = 0.067). Then, overall IR was comparable whatever the culture condition applied from D0 to D3 ("5% O_2": 29.7% *vs.* "20% O_2": 27.8%). Considering EC analysis, both demographic and clinical parameters of the cycles were also comparable. Blastulation rates were similar in groups A, B and C (68.1%, 70.4% and 71.6%, respectively). Regarding blastocyst quality, embryo culture under 20%-O_2 from D0 to D6 (group A) resulted in significantly lower D5-TQB rates/blastocyst (15.2%), than in both groups B (23.4%; p = 0.0029) and C (20.1%, p = 0.04). Furthermore, blastocyst quality was statistically equivalent between groups B and C. Finally, blastocysts IR were similar in groups A, B and C (43.4% *vs.* 39.4% *vs.* 42.7%, respectively).

Limitations, reasons for caution: Embryos were not analyzed using morphokinetic parameters, which could have made the interpretation less subjective.

Wider implications of the findings: If confirmed, these results would encourage to systematically culture embryos under hypoxia during only early development stages, since OS might be detrimental exclusively before embryonic genome activation.

Trial registration number: ID-RCB: 2015-A02019-40

P-179 Functionally active extracellular vesicles are released and taken up by zona-intact bovine embryos produced in vitro

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Study question: Are extracellular vesicles (EVs), as putative autocrine factors, released into the culture medium by bovine embryos and are they able to cross the zona pellucida?

Summary answer: Extracellular vesicles were isolated from EV-free media conditioned by bovine embryos and were, after fluorescent labeling, taken up and internalized by zona-intact embryos.

What is known already: Group culture of bovine embryos in medium containing bovine serum albumin (BSA) is currently the most successful method to produce embryos in vitro. Autocrine factors play a key role in group culture, with EVs being possibly important autocrine mediators. Moreover, EVs are present in various body fluids including serum, suggesting that BSA may support bovine embryo development by enriching medium with extracellular vesicles. Theoretically, EVs (50-300 nm) can pass through the pores of the bovine zona pellucida (mean diameter 200 nm). We hypothesize that EVs are released and taken up by embryonic cells, serving as positive mediators in group culture.

Study design, size, duration: In vitro maturation and fertilization was performed using routine methods. For in vitro culture, presumed zygotes were allocated to Synthetic Oviduct Fluid (SOF) containing insulin, transferrin and selenium (ITS) and supplemented with 0.4% BSA. To assess the presence of embryo secreted-EVs, both standard and ultracentrifuged (EV-depleted; centrifuged at 100,000 g for 18 h at 4°C) media were tested. At day 8, blastocysts were recorded and quality was evaluated by differential staining.

Participants/materials, setting, methods: One ml of embryo-conditioned medium was pooled at day 8 and subsequently subjected to Optiprep® density gradient ultracentrifugation and size exclusion chromatography to isolate EVs. Identification and characterization of EVs was performed by nanoparticle tracking analysis, transmission electron microscopy and western blotting (CD9, TSG101,CD63). Next, EVs were pre-labeled by PKH67 dye and subsequently co-incubated with day 6 embryos for 48 hours to assess EV passage through the zona pellucida. The uptake was analyzed by confocal microscopy.

Main results and the role of chance: Blastocyst development and hatching rates were not significantly different comparing standard and EV-depleted embryo culture medium (40 ± 3.43 % vs 38.88 ± 1.48 %; 25.20 ± 1.16 % vs 24.0 ± 0.83%; normal medium vs EVs free medium; P>0.05). In contrast, a significant difference between culture media was observed in total cell number of expanded and hatched blastocysts (151.0 ± 1.95 vs 126.8 ± 1.74; 209.6 ± 1.31 vs 162.12 ± 2.29; normal medium vs EV-depleted medium; P<0.05). Moreover, apoptotic cell ratio was significantly higher in expanded blastocysts cultured in EV-depleted compared to standard medium (8.7 ± 0.35 vs 6.1 ± 0.50; P<0.05). Based on this data, EV-depleted culture medium had an significant adverse effect on embryo quality. Moreover, embryos cultured in EV-depleted medium secreted EVs in the culture medium. At day 8, EVs sizing from 25 to 250 nm were isolated, which indicates the secretion of EVs through the pores of the zona pellucida. By nanoparticle tracking analysis, the concentration of EVs extracted from 1 ml of conditioned medium was 1.36×10^8 particles/ml. Western blotting analysis demonstrated the presence of CD9, TSG101, CD63 markers in embryo-derived EVs. Co-incubation of PKH67 prelabeled EVs and embryos demonstrated the uptake of embryo-derived EVs by zona-intact embryos. We conclude that functionally active EVs can cross the zona pellucida.

Limitations, reasons for caution: The major limitation in the current study was the collection of high volumes of embryo conditioned medium, as it requires 400-500 in vitro produced embryos to obtain 1 ml conditioned

medium. To counteract this limitation we were obliged to make use of pooled embryo conditioned medium.

Wider implications of the findings: Our data demonstrated that EVs are secreted by in vitro cultured bovine embryos in the medium, cross the zona pellucida and can be taken up by embryos. These facts indicate the potential role of EVs as mediators in embryo development, also with possible implications for human embryo culture.

Trial registration number: Not Applicable.

P-180 A role of E-cadherin-Wnt/β-catenin-lin28/let7 pathway in epithelial-mesenchymal transition (EMT) during extravillous trophoblast differentiation

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Study question: How dose E-cadherin change in embryo trans-activate canonical Wnt/β-catenin signaling pathway and its downstream target lin28(a/b)/let7 affect extra-villous trophoblast EMT during normal implantation.

Summary answer: E-cadherin bound β-catenin release feeds into Wnt signaling pathway and promote trophectoderm EMT through up-regulation lin28a/b as well as suppression mir-let-7.

What is known already: Wnt/β-catenin signaling is one of the several pathways that involve in EMT, E-cadherin associated with β-catenin on the cellular membrane to prevent β-catenin participate in signaling pathway. Our own data showed that up-regulation of lin28a is required for the activation of mouse blastocysts and mir-let 7 was up-regulated in dormant embryo but lower expressed in implantation competent embryo in our microarray analysis.

Study design, size, duration: The expression of EMT related marker, lin28a,b, mir-let-7 family was detected in Wnt/β-catenin activated and inhibited human trophectoderm cell line, HTR-8/SVneo and Jeg-3. The function of E-cadherin disassociation with β-catenin in activate Wnt pathway was confirmed. The in vivo and in vitro effect of Wnt/β-catenin-lin28/let7 on embryo EMT was examined.

Participants/materials, setting, methods: Human trophectoderm cell line HTR-8/SVneo and Jeg-3 was used for in vitro assay. Pregnancy was produced by natural mating between female and male ICR mice, and C57BL6/J mir-let-7g overexpression transgenic mouse for in vivo assay. The expression of active β-catenin, E-cadherin, EMT marker, lin28a,b, mir-let-7 was detected in both cell line and mouse embryo in Wnt induced EMT by QPCR, Western blot, and immunostaining.

Main results and the role of chance: E-cadherin dissociation from adhesion junction (AJ) with β-catenin cause β-catenin gain the ability to be translocated into nucleus and activate Wnt signaling in Jeg-3 cell. Wnt/β-catenin activation trans-activate lin28a/b in Jeg-3 and HTR-8/SVneo in E-cadherin dissociated cell. Lin28 functionally regulates the expression of mir-let-7 family in Jeg-3 and HTR-8/SVneo. E-cadherin dissociation from AJ, Wnt/β-catenin activation, Lin28 overexpression and mir-let-7 knockdown cause Jeg-3 and HTR-8/SVneo cell EMT in vitro and also increase the invasion and migration rate. In the embryo in vitro co-culture process, from 24 h attachment to 48 h invasion, E-cadherin was down-regulated in invasive trophectoderm but active-β-catenin was increased, mesenchymal related markers were upregulated. In vivo, the implantation rate and embryo EMT of let-7g overexpressed mouse was decreased.

Limitations, reasons for caution: E-cadherin dissociation from AJ with β-catenin cause β-catenin and E-cadherin both translocated into nucleus, and Wnt activation, how the specific regulatory mechanism of E-cadherin affect Wnt activation still do not know.

Wider implications of the findings: Our findings indicate that trophectoderm cell experience Wnt/β-catenin pathway activation as E-cadherin dissociation from AJ, and supression its down-stream target mir-let-7 by lin28, thus promote EMT. This information contribute to a better understanding of the mechanisms that the miRNA-mediated regulation of embryo implantation, and subsequently improve treatments for infertility.

Trial registration number: no.

P-181 Embryo cryopreservation is associated with significantly higher birth weight compared with sibling cohort fresh embryo transfer: a bicenter cohort

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Study question: To determine if the freeze-thaw procedure itself is involved in birth weight difference between singletons born after fresh embryo transfer and those born after frozen embryo transfer.

Summary answer: Birthweight after frozen embryo transfer (slow freezing or vitrification) was significantly higher (215.6 g) than after fresh embryo transfer (3487.4 g vs 3271.8 g, p = 0.0001).

What is known already: It is well established that the frozen embryo transfer (FET) is associated with a change of birth weight in comparison with fresh embryo replacement even after taking into consideration relevant confounding factors. However, no study has compared the birth weight of consecutive siblings conceived using the same oocyte/embryo cohort but different transfer procedures (fresh vs frozen embryos).

Study design, size, duration: Retrospective bicenter cohort study. The cohort included 145 sibling pairs where the older sibling was born after fresh embryo transfer and the second after embryos frozen-thawed replacement from the same in vitro fertilization (IVF) cycle.

Participants/materials, setting, methods: The cohort included all IVF cycles where fresh embryo transfer that resulted in a singleton live birth (fresh group n = 145) was followed by FET that led to a singleton live birth (FET group n = 145). Twin pregnancies and stillbirths were excluded. The embryos were frozen either by slow freezing or after vitrification. Birth weight were adjusted for gestational age and the mean adjusted birth weight in the two groups were compared with the Wilcoxon test.

Main results and the role of chance: The mean adjusted birth weight of the FET group was significantly higher (by 215.6 g) than that of the fresh group (3487.4 g vs 3271.8 g respectively, p = 0.0001). These result were also significant for transfer at the blastocyst stage (3513.5 g vs 3290 g, p = 0.0074) but not at the cleaved stage (3448.7 g vs 3258 g, p = 0.1074). Concerning the number of embryos transferred, when one embryo was transferred, the birth weight was, indeed higher after FET than after fresh embryo transfer (3494.2 g vs 3296.9 g, p = 0.0044) but the results were not significant when two embryos were transferred (3452.8 g vs 3186.7 g, p = 0.3123). A significantly higher risk of being large for gestational age (LGA) > 90ème percentile or >97ème percentile after FET versus fresh embryo transfer was revealed: RR, 1.13 (95% CI, 1.03-1.23) and RR, 1.1 (1.02-1.19) respectively. As both embryos (fresh and frozen) were from the same IVF cycle to avoid confounding factors and the only difference between groups was the absence/presence of the cryopreservation step, our results strongly suggest that the cryopreservation procedure affects the birth weight of sibling embryo cohort.

Limitations, reasons for caution: The main limitation of this study, is the retrospective design.

Wider implications of the findings: ART techniques, such as cryopreservation procedure, alter birth weight, possibly through epigenetic modifications in FET (freezing/thawing procedure, cryoprotectants).

Trial registration number: Not applicable.

P-182 Clinical validation of a non-invasive embryo selection algorithm combining time-lapse morphokinetics and the oxidative status of spent embryo culture media

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Study question: Determine if novel embryo selection technique based on spent´s embryo culture media oxidative profile combined with time-lapse morphokinetic analysis could predict implantation potential.

Summary answer: Implanting transferred embryos showed a more extensive oxidative metabolism which, when combined with morphokinetic data, led to the development of a predictive algorithm.

What is known already: Despite IVF's (*in vitro* fertilisation) widening application and technological progress, it remains associated with two main weaknesses that derive partly from our inability to adequately assess embryo quality: low implantation rates and high multiple pregnancy rates. Novel non-invasive strategies based on spent culture media analysis provide additional valuable data to current morphology and morphokinetic analysis performed by time-lapse technology. In particular, the assessment of the embryo's oxidative profile with the Thermochemiluminescence (TCL) Analyzer™ (Carmel Diagnostics, Israel) suggests a new approach in determining embryo's quality or viability and subsequent implantation potential.

Study design, size, duration: A retrospective cohort study was performed with a total of 505 spent embryo culture media, including 205 single-embryo transfers (SET) with known implantation, from 390 IVF cycles. Embryos were cultured and monitored in independent-well slides in the time-lapse system incubator Embryoscope® (Vitrolife) and were subsequently transferred at blastocyst stage. Implantation potential and embryo quality at day 5 (Transferred+Vitrified vs. Discarded embryos) were considered, in terms of oxidation, to find a predictive profile of pregnancy success.

Participants/materials, setting, methods: Oxidative status of 15 µl/embryo of Blastocyst medium (Cook) samples were assessed by the Thermochemiluminescence (TCL) Analyzer™, based on the heat-induced oxidation of biological fluids, leading to the production of light energy counted as photons emitted per second (**cps**). TCL parameters recorded were **cps** amplitude after 55 seconds (**H1**), 155 seconds (**H2**) and 255 (**H3**), in a 300-second period. Oxidative data was normalized with a smoothing algorithm (**sm**) and analyzed by the statistical test ANOVA.

Main results and the role of chance: Regarding day 5 embryo quality, transferred and vitrified embryos showed significantly higher values (sig. <0.05) for the oxidative parameters **H1sm**, **H2sm** and **H3sm**. In addition, out of 205 transferred embryos, 54.1% succeeded at implantation showing again higher significant values (sig. <0.05) in the oxidative parameters. This therefore implies high quality embryos have a more extensive oxidative metabolism exerting an oxidative load on their surrounding media. A combined assessment algorithm, including morphology, morphokinetics and the embryo's culture media oxidative status was subsequently developed as a predictive clinical tool of embryo selection, prior to transfer. Motato et al. (2016) morphokinetic model based on blastocyst expansion (**tEB**; optimal range ≤ 112.9 hours) and timing of transition from 5-blastomere embryo until 8-blastomere embryo (**t8–t5**; optimal range ≤ 5.67) was combined with TCL parameter **H2sm** (optimal range ≤ 92.96). A hierarchical classification was generated with six embryo categories (A - F) according to their implantation potential (76.5–29.2%).

Limitations, reasons for caution: The present retrospective study and its developed selection algorithm require an additional prospective validation for its routine clinical use. Oxidative status database will increase while using TCL to pursue a more accurate **H2sm** optimal range.

Wider implications of the findings: The fair correlation between TCL oxidative results, embryo quality and implantation potential proves its application as a clinical biomarker. Its combination with morphokinetic data aims for the improvement of our current selection criteria. A more accurate selection of the best embryo, especially in good-quality embryo cohorts, would determine IVF success.

Trial registration number:

P-183 Detection of multinucleated trophectoderm cells by time-lapse microscopy: Implications for PGT-A biopsy results

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Study question: Is the nuclear status of trophectoderm (TE) cells uniform? If multinuclear TE (MNT) cells are used for PGT-A analysis, will they impact the aneuploid results?

Summary answer: MNT cells can be identified in human blastocysts. Biopsy and analysis of these cells using current PGT-A techniques may yield invalid results.

What is known already: Detection of triploidy and polyploidy using current chromosomal testing techniques (NGS or PCR) is limited. Bovine embryos contain polyploidy cells in the TE. So far, multinuclear cells in the human TE of preimplantation-stage blastocysts have not been reported. In contrast, polyploid multinuclear cells have been identified in the human trophoblast and syncytiotrophoblast. Day 11 human embryos contain single nucleated TE cells in cells adjacent to the epiblast compared to those in peripheral TE, which contain multinucleated cells. The chromosomal status of single multinucleated blastomeres on day 3 indicated polyploidy and/or aneuploidy. The chromosomal status of MNT cells is unknown.

Study design, size, duration: This is an observational study of IVF embryos cultured under time-lapse microscopy (TLM) incubation until the blastocyst stage.

Participants/materials, setting, methods: Embryos were cultured and monitored in a TLM incubator (EmbryoScope, Vitrolife, Sweden). At the blastocyst stage, the embryos were carefully monitored for the presence of binucleated and/or multinucleated cells in the TE. If MNT cells were detected, a retrospective analysis of TLM videos was performed to identify their origin.

Main results and the role of chance: Multinuclear blastomeres were positively identified with high specificity during the first three embryo cleavages. In addition, we observed the existence of MN cells in the TE during blastocyst development. This novel observation can be easily identified by TLM in fully expanded blastocysts where TE cells are stretched out. We observed MNT cells at various stages of blastocyst development, usually as single cells, an observation which appeared to be independent of the quality of the blastocyst or density of TE cells. We observed that MNT cells can be formed de novo during TE development by the fusion of two or more single nucleated TE cells. Interestingly, these MNT cells can subsequently cleave to two daughter cells with single nuclei. The chromosomal analysis may be altered if MNT cells or their progeny are used during TE biopsy. The chromosomal status of a single MNT cell and its descendant cells is unknown. The occurrence of MNT cells can be a normal event during blastocyst development, and it may indicate the plasticity of TE formation. It is possible that the quantity, quality, and nuclear status of TE cells could have an impact on the accuracy of PGT-A.

Limitations, reasons for caution: This is an observational study. The chromosomal status of individual MNT cells cannot be performed.

Wider implications of the findings: During TE biopsy, it is imperative to assess the nuclear status of the TE. MNT cells should not be used for biopsy, as they might cause an incorrect diagnosis of the embryo.

Trial registration number: N/A.

P-184 Does the transfer of a poor quality embryo together with a good quality embryo affect the In Vitro Fertilization (IVF) outcome?

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Study question: Whether a poor quality embryo has a negative effect on a good quality embryo when transferred along with a good quality embryo.

Summary answer: A poor quality embryo does not negatively affect a good quality embryo, when transferred together in a double embryo transfer.

What is known already: Embryo quality is one of the main predictors of success in IVF cycles. Many studies have shown a strong association between embryo morphology, implantation, and clinical pregnancy rates. In theory, the poor quality embryo has potential for a successful pregnancy, and may lead to higher spontaneous abortions and overall decreased clinical pregnancies and live birth rates. IVF cycles which result in only one good quality embryo, and a second poor quality embryo present a dilemma when the decision involves transferring two embryos.

Study design, size, duration: This single-center, retrospective cohort study involved patient who had only one good quality embryo in fresh cleavage stage embryo transfer using non-donor oocytes at the Reproductive and Genetic Hospital of CITIC Xiangya in the years 2010–2016. The analysis involved 409 of 418 enrolled cycles in patients who were included in the study group and 409 control cycles in patients who were identified by propensity matching from a cohort of 1339 cycles.

Participants/materials, setting, methods: We enrolled patients who had only one good quality embryo in the cleavage stage(D3), and involving double embryo transfers(DET) with one good quality embryo and one poor quality embryo(study group) or single good quality embryo transfers(SET)(control group). Propensity matching was used to identify a propensity matched control group from a cohort of 1339 cycles based on age, BMI, basical FSH, endometrial thickness on the day of hCG, number of oocytes retrieved, and quality embryo.

Main results and the role of chance: 818 cycles were included in the study. 409 cycles in women who had DET with one good quality embryo and one poor quality embryo [study group (G + P)], and 409 cycles in women who had SET with one good quality embryo [control group (G)]. There was no difference in patient baseline and cycle characteristics between the two groups. The clinical pregnancy rate (39.9% vs. 34.2%, P > 0.05), implantation rate (21.3% vs. 17.5%, P > 0.05), and live birth rate (28.6% vs. 26.2%, P > 0.05) were no difference between study and control groups respectively. The early abortion rate (17.8% vs. 17.1%, P > 0.05) and ectopic pregnancy rate (0.6% vs. 1.4%, P > 0.05) were no difference between study and control groups respectively. The twin pregnancy rate of the study group (G + P) (6.7%) was slightly higher than that of the control group (G) (2.1%) (P = 0.096), without reaching statistical significance.

Limitations, reasons for caution: The extrapolation of the results is limited by the retrospective nature of the study.

Wider implications of the findings: A poor quality embryo does not negatively affect a good quality embryo, when transferred together in a double embryo transfer.

Trial registration number: No.

P-185 Double biopsy at the blastocyst stage by retrieving blastocoelic fluid and trophectoderm cells does not affect embryo viability

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Study question: Does the procedure of double biopsy affect embryo viability?

Summary answer: The simultaneous biopsy of blastocoelic fluid (BF) and trophectoderm (TE) cells does not decrease embryo viability, whereas double biopsy at previous stages negatively affects implantation.

What is known already: The current trend in Preimplantation Genetic Testing (PGT) programs is to perform biopsy of TE cells. This gives several advantages including the high probability of having informative results when compared to cases in which genetic analysis is done on polar bodies (PBs) or blastomeres. Consequently, the need to perform re-biopsy is not a common event following TE biopsy, but it can be necessary when biopsy is done at previous stages.

Study design, size, duration: This retrospective study includes 450 PGT cycles from September 2009 to September 2017. According to the clinical indication, biopsy was performed on PBs (n = 131 cycles), blastomeres (n = 227 cycles) or TE cells (n = 80 cycles). In cases of non-informative results, re-biopsy was performed. Only single embryo transfer were included in the study to evaluate the effect of double biopsy on ongoing pregnancy rate (>16 weeks).

Participants/materials, setting, methods: Indications to PGT were monogenic diseases (PGT-M, n = 93) or aneuploidy (PGT-A, n = 357). Following biopsy, Whole Genomic Amplification (WGA) was immediately performed and, after positive amplification, embryos were cryopreserved at the blastocyst stage to complete the genetic analysis. In case of negative amplification, re-biopsy was performed at the cleavage stage after PB biopsy and after blastomere biopsy. For blastocysts, the BF was used as back-up sample.

Main results and the role of chance: PGT-M and PGT-A cycles were equally distributed in the three biopsy groups (PBs, blastomeres, TE cells). Re-biopsy was performed in 14.5% of PB cycles, 9.8% of blastomeres cycles and 5% of TE cycles. The ongoing pregnancy rate in PB group was 14.2% after re-biopsy and 29.5% after single biopsy, while after blastomere biopsy it was 14.2% and 25% respectively. When pooling these two groups, the difference between re-biopsy and controls was significantly different (14.3% vs. 27%, P<0.05). For blastocysts, the ongoing pregnancy rate was 50% after biopsy of both TE and BF compared with 39% after biopsy of TE only. A significant difference was found when relating the ongoing pregnancy rate after re-biopsy that was significantly lower when done at the cleavage stage and the double biopsy at the blastocyst stage (P<0.001). Finally, a difference was also found when comparing the controls of single biopsies done at earlier stages (PB and cleavage stage) with blastocyst biopsy (P<0.025) confirming the clinical advantage related to blastocyst transfer.

Limitations, reasons for caution: The limited number of cases in the three biopsy groups is probably softening differences that are suggested by the observed trend.

Wider implications of the findings: Biopsy at the blastocyst stage is the preferable option either alone or combined with removal and storage of the BF. The last approach could provide a back-up sample to be used in case of non-informative genetic results. In addition, artificial blastocyst collapsing could possibly improve the results after vitrification.

Trial registration number: Not applicable.

P-186 High oxygen tension during in vitro maturation of human oocytes improves developmental competence

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Study question: Is it advantageous to culture cumulus-oocyte complexes (COCs) under high oxygen tension (20%) during in vitro maturation?

Summary answer: Culturing COCs under high oxygen tension (20%) during in vitro maturation increased the rate of blastocyst development.

What is known already: Previous studies have investigated whether oxygen tension during in vitro culture of oocytes affects clinical outcome. However, there are few reports on the optimum oxygen level for in vitro maturation. It has been suggested that oxygen concentration during in vitro maturation (IVM) affects murine blastocyst cell numbers and fetal development. It is thought that oxygen tension influences reactive oxygen species levels in oocytes matured in vitro and it is also known to influence the normal fertilization rates of bovine oocytes in IVF. However the influence of oxygen tension on embryo developmental competence after human oocytes maturation is unknown.

Study design, size, duration: The study was performed from March to December 2017. We collected immature COCs from IVF patients undergoing letrozole stimulation and divided them into two groups. 188 COCs from 59 patients were cultured with 5% O2 and 133 COCs from 47 patients were cultured with 20% O2.

Participants/materials, setting, methods: COCs were cultured in TCM199 supplemented with FSH and EGF in 5% O2 or 20% O2. ICSI was performed on these oocytes which where then cultured in a one step medium for six days in 5% O2, 6% CO2 and 89% N2. We measured oocytes maturation rate, normal fertilization rate and blastocyst formation rate.

Main results and the role of chance: Oocytes maturation rate in 5% O2 was 55.3% and in 20% O2 was 53.4% (P > 0.05). There was no significant difference in normal fertilization rate between the two groups (5% O2 = 73.8% vs. 20% O2 = 78.6%). The blastocyst development rate following oocyte maturation in the 20% O2 group (47.3%) was significantly higher than that in the 5% O2 group (25.2%, P < 0.05).

Limitations, reasons for caution: This study is limited to immature oocytes obtained from patients undergoing IVF.

Wider implications of the findings: This is the first study to demonstrate the effectiveness of high oxygen tension during IVM in human oocytes. Our results show that oocytes cultured in 20% O2 during IVM exhibit enhanced developmental competence.

Trial registration number: none.

P-188 Artificial oocyte activation improves pre- and post implantation development of ICSI embryos irrespectively of sperm origin

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Study question: Is the effect of artificial oocyte activation (AOA) on embryonic development and pregnancy outcome associated with sperm origin?

Summary answer: Regardless of sperm origin, AOA effectively improved fertilization and pre- and post-implantation development by increasing the quantity and the quality of blastocysts available for transfer.

What is known already: AOA has been a therapeutic option for patients with a history of ICSI fertilization failure.

In earlier studies, it has been shown that AOA is effective in improving not only the fertilization rate, but also the pregnancy rate in cases where impaired fertilization was observed in preceding cycles.

However, whether sperm origin has any impact on the development of AOA embryos is not well understood.

Study design, size, duration: In order to examine the effect of AOA on late embryonic development, we compared ICSI outcome between non-AOA and AOA cycles in patients with various fertilization rates and different sperm origin, ejaculated (EJ) and testicular spermatozoa (TESE).

Between October 2013 and November 2017, total of 153 infertile couples with EJ and 48 with TESE ICSI were involved in the study.

All patients gave written consent, and institutional review board approval was granted.

Participants/materials, setting, methods: Consenting patients were grouped according to the sperm origin as well as fertilization rates in initial non-AOA cycles.

For AOA, ICSI inseminated oocytes were exposed to 10 μM calcium ionophore (A23187) for 15 minutes.

Developed embryos were all vitrified, and warmed-replaced in subsequent embryo transfer cycles, while no fresh embryo transfers were elected.

The rates of fertilization, blastulation, good blastocyst, clinical pregnancy, and on-going pregnancy were compared between non-AOA and AOA cycles.

Main results and the role of chance: The couples initially underwent 341 non-AOA cycles (280 with EJ and 61 with TESE), followed by 427 AOA cycles (328 EJ and 99 TESE, respectively). Both EJ and TESE groups were further divided into three subgroups according to initial non-AOA fertilization rates, i.e., low (<30%), sub-optimal (30-60%), and satisfactory (>60%). Semen parameters were comparable among the three groups. In both EJ and TESE groups, the rates of fertilization after AOA were significantly improved from non-AOA cycles in all (P<0.001), but satisfactory group as expected. We defined good blastocyst rate as the proportion of blastocyst with good quality per embryo reached the blastocyst stage. Interestingly, AOA treatment remarkably increased blastulation and good blastocyst rates in sub-optimal group with EJ sperm (38.4 vs 62.0 and 14.0 vs 43.3%, P<0.01), and satisfactory group with TESE sperm (22.2 vs 40.0%, N.S. and 12.5 vs 62.5%, P<0.05). More importantly, overall clinical pregnancy rates and on-going pregnancy rates after AOA were obviously improved regardless of sperm origin (15.8 vs 31.5 and 12.1 vs 27.0% with EJ, 16.6 vs 52.3 and 11.4 vs 38.8% with TESE, respectively, P<0.01).

Limitations, reasons for caution: We included only cycles with motile sperm. Further investigations are needed to assess the effect of AOA in cases with severely compromised sperm quality. Long-term follow-up of children born after AOA with calcium ionophore has not been well studied yet.

Wider implications of the findings: AOA was effective for improving fertilization as well as late embryonic development irrespective of sperm maturity. Considering the beneficial effect observed in TESE cases with satisfactory fertilization, our findings indicate potential use of AOA even at first attempts once the safety is warranted.

Trial registration number: None.

P-189 Should we vitrify the early blastocyst? Retrospective study on 851 warming cycles with single embryo transfer

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Study question: To optimize the outcome of frozen-thawed cycles with single embryo transfer (SET), survival rate after thawing and ongoing pregnancy rates were compared between early and non collapsed full blastocysts.

Summary answer: Survival rate after thawing is not affected by the blastocyst expansion while early blastocyst transfers (B1-B2, Gardner classification) yield significantly less clinical pregnancies.

What is known already: Extended embryo culture to blastocyst stage allows better embryo selection and higher implantation rates. Since a couple of years SET policy is widely used to reduce the risk of multiple pregnancies and freeze all strategy is routinely performed in order to transfer embryos in a more physiological environment and to overcome ovarian hyperstimulation consequences. Studies have shown the superiotity of vitrification of blastocysts compared to cleavage stage.

Lately extensive research was done around the best vitrification/thawing protocol and techniques: closed vs open system, media, shrinkage prior to vitrification, blastocyst stage at vitrification, automation.

Study design, size, duration: Observational retrospective study on 851 frozen cycles with SET during 2016-2017. Blastocysts were divided in four groups based on Gardner's classification: 1 early (B1-B2, 251 embryos), 2 full (B3, 233 embryos), 3 expanded (B4, 301 embryos) and 4 hatching/hatched (B5-B6, 66 embryos). All blastocysts were transferred on monitored natural cycles under ultrasound control 6/7 days after LH surge. Survival rate after thawing (SR) and ongoing pregnancy rate (OPR) were compared between the groups.

Participants/materials, setting, methods: Study was performed in a private IVF center.

Blastocysts were scored at day 5 according to the classification of Gardner. No shrinkage was done before vitrification of expanded blastocysts.

Vitrification/thawing were performed using closed high security straws (CryoBioSystem) in combination with Irvine Scientific® Freeze/Thaw kit.

After thawing the blastocysts were cultured for two hours (Sage 1-Step®, Origio) in order to assess survival and reexpansion. Damaged and non-reexpanded blastocysts were discarded.

The transfer catheters were loaded with EmbryoGlue® (Vitrolife).

Main results and the role of chance: Patients mean age and infertility indications were comparable between the four groups (p>0.05). Ovarian stimulation protocols at retrieval (antagonist with recombinant FSH) were the same for all the patients.

Survival rates were similar between 4 groups (p>0.05): B1-B2 97.2%, B3 97.8%, B4 98.3%, B5-B6 96.9%.

OPR per transfer in B1-B2 (20.5%) was significantly lower compared individually to each group: B3 (32.0%, p = 0.029), B4 (38.2%, p = 0.001), B5-B6 (35.9%, p = 0.049). Furthermore, the OPR per transfer of full-expanded-hatched blastocysts were significantly higher than early blastocysts (37.8% vs 20.5%, p = 0.0008).

Limitations, reasons for caution: The study was undertaken to evaluate D5 blastocysts at vitrification. D6 blastocysts were not included. Blastocysts obtained at D6 must be evaluated in order to decide if one extra day of culture may benefit to early blastocysts before undergoing vitrification.

Wider implications of the findings: Our two years experience of blastocyst SET in frozen cycles shows that OPR are significantly lower with early blastocysts even if SR are comparable to late blastocysts. Therefore, our vitrification policy has been moved to one extra day of culture for blastocysts that are not expanded on D5.

Trial registration number: Not applicable.

P-190 Increased scrutiny of NGS profiles / ranking does not improve eSET implantation of euploid blastocysts: A randomized comparative trial

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Study question: Can NextGeneration sequencing (NGS) profile ranking of euploid embryos better predict eSET implantation potential of top quality blastocysts in contrast to morphological grading alone?

Summary answer: When blastocysts are both top quality and euploid, the lack of noise on a NGS profile adds no benefit for eSET compared to embryo grading.

What is known already: Blastocyst morphology assessment is the primary determinant for embryo transfer selection. Preimplantation genetic screening (PGS) now provides patients the ability to know the ploidy status of embryos before being transferred, which increases the implantation potential of eSETs. NGS provides every embryo a unique genetic profile which gives geneticists more information on genetic "noise" and low-level mosaicism that remains inconsistent between samples, patients, technicians and labs. Transferring mosaic embryos of differing profile levels has resulted in viable pregnancies, further questioning the clinical relevance of mosaic profiles. Furthermore, the risks to the offspring of a mosaic embryos is relatively unknown.

Study design, size, duration: Prospective randomized multicenter trial, initiated April 2016 and ended December 31, 2017. A total of 61 patients qualified for randomization by producing ≥ two top quality euploid day 5 or day 6 blastocysts. Upon enrollment, eSET selection was randomized between two pre-determined, double blinded ranking groups: Group 1 - embryologists ranked euploid blastocysts based on morphologic grade; and Group 2 - geneticists ranked transfer selection based on their interpretation of the NGS profiles.

Participants/materials, setting, methods: The study was performed at two locations (Clinic A: Newport Beach, CA; and Clinic B: Austin, TX) involving 6 physicians and differing embryology lab management and protocols. A single genetics lab performed NGS analysis using MiSeq (Illumina), with genetic noise/rank evaluated by a geneticist's interpretation. All embryos were cultured through day 6, and trophectoderm biopsy and vitrification performed according to individual lab procedures. Implantation was measured by a visual sac and all pregnancies documented.

Main results and the role of chance: Eighty patients agreed to participate in this IRB approved study, however 19 (23.8%) failed to achieve inclusion criteria. Fifty-seven eSET's have been performed to date. Group 1 (Morphology) achieved a 75.9% implantation and 62.1% ongoing pregnancy rate. Group 2 (Genetics) produced a 78.6% implantation and a 71.4 % ongoing pregnancy rate. There was no statistical difference ($p>0.05$) between either group for implantation, spontaneous abortion and/or ongoing pregnancy rate. Between clinics, clinic A accomplished a combined 96.7% implantation rate and 87.1% ongoing pregnancy, while clinic B had a 53.8% and 42.3% implantation and pregnancy rate, respectively. Clinic A exhibited no trends ($p<0.1$) between morphology and genetics, whereas clinic B revealed a preference for Group 2 (Genetics) for ongoing pregnancy. The latter finding that genetic profile ranking is associated with reduced miscarriage rates, warrants further investigation with a larger sample population. There were no significant differences between labs regarding fertilization rates, blastocyst yields or euploid rates. Patient age was not different between clinics, with a mean of 34 years old for qualifying participants. Clinic A had a single physician perform all stimulation and transfers, whereas Clinic B involved a rotation of 5 physicians for treatment.

Limitations, reasons for caution: Although sample size was low, it is important to note that patient selection was not biased, with study qualification requiring two top quality euploid blastocysts, not achievable for all IVF patients. Levels of mosaicism under 30% were classified as euploid and further scrutiny of extremely low levels was not performed.

Wider implications of the findings: Morphological grading has long been a standard for improving IVF success rates. With the adoption of NGS testing, more scrutiny of the PGS results has placed further importance on the role of the evaluating geneticist. This study indicates to the contrary, the embryologist is still vital for eSET embryo selection.

Trial registration number: None.

P-191 Development of a Mouse Embryo Assay with enhanced sensitivity for detection of peroxides in mineral oil samples

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Study question: How can culture conditions alter the sensitivity and reliability of the Mouse Embryo Assay (MEA), used for detection of toxicity in mineral oil samples?

Summary answer: Culture conditions can greatly alter the sensitivity of the MEA and must be carefully optimized to ensure a reliable detection of embryotoxic mineral oil samples.

What is known already: Mineral oil has been a key component in IVF since Brinster described its use for oocyte/embryo culture in 1963. Overlaying culture medium micro-droplets with mineral oil helps stabilizing temperature, pH and osmolarity during culture, as well as protecting against external contaminants. However, as a petroleum-derived product, mineral oil can harbor undesired components (e.g. peroxides) that may adversely affect fertilization and embryo development. Peroxides can originate during oil manufacturing, and its inadequate storage/handling can result in an increase of peroxides over time. Thus, it is essential to develop quality control assays with enhanced sensitivity to detect toxicity in mineral oil samples.

Study design, size, duration: Mineral oil samples, produced by an ART-medium manufacturer (FertiPro), were exposed to UV-light for different time-periods to induce peroxide formation. Peroxide levels after exposure were determined by potentiometric titration (detection limit <0.1mEq/kg), and samples were MEA tested in our laboratory (Embryotools). Different culture conditions (concentration and type of protein supplement, individual *vs.* group embryo culture, culture dish design) were tested on each oil sample, and results compared to establish optimal parameters for sensitive toxicity detection.

Participants/materials, setting, methods: Experiments were performed with one-cell freshly collected mouse embryos (n = 2066). Three replicates were performed on each oil sample with the different culture conditions established: medium protein supplement type (HSA+Globulins, HSA or BSA) and concentration (5 mg/ml, 0.5 mg/ml), embryo density (individual *vs.* group culture), and culture dish design (Petri vs. time-lapse dish). Embryo development was followed and registered daily until the end-points (Day 5 or Day 6). Blastocysts obtained were fixed and stained for total cell counts.

Main results and the role of chance: A minimum of 80% of expanded blastocysts was obtained in oil with peroxide levels <0.1mEq/kg, regardless of the type and concentration of protein used. By contrast, no blastocysts formed in oil samples with high peroxide concentrations (0.198, 0.503, 0.756mEq/kg).

Interestingly, when an oil sample with peroxide levels close to the detection limit was used (0.128mEq/kg), culture conditions showed a strong influence on

the assay's sensitivity. High embryo density (group culture) and medium supplementation with BSA resulted in higher blastocyst formation rates, indicating a lower capacity to detect embryotoxicity than individual culture and HSA or HSA+Globulins supplements. In HSA and HSA+Globulins groups, lower protein concentrations (0.5 mg/ml) also yielded more blastocysts ($p = 0.0013$) than higher protein concentrations (5 mg/ml), reducing the assay's sensitivity. By contrast, lower concentrations of BSA resulted in diminished blastocyst formation, compared to higher concentrations ($p = 0.0018$). Total cell number was significantly decreased in HSA and HSA+Globulins ($p<0.005$) groups, but not with BSA, once again demonstrating the reduced sensitivity of the latter.

Culturing in a time-lapse dish (Embryoslide®, Vitrolife) improved developmental rates in all protein groups ($p\leq0.005$), possibly because its design entails less contact surface between the culture medium and the oil overlay, hence reducing its sensitivity for mineral oil embryotoxicity detection.

Limitations, reasons for caution: This study confirms that culture conditions must be carefully optimized to successfully detect low concentrations of peroxides in mineral oil. Medium supplementation with BSA, as well as group embryo culture, significantly improved embryo development and can compromise the sensitivity of MEA testing, resulting in undetected embryotoxicity in mineral oil samples.

Wider implications of the findings: Culture conditions significantly modulate embryo development, hence playing a major role in quality control testing. Inappropriate testing of mineral oil derives in diminished sensitivity for embryotoxicity detection. As previously reported, even low traces of peroxides in mineral oil could dramatically affect clinical human IVF outcomes.

Trial registration number: N/A.

P-192 Systematic single embryo transfer after preimplantation genetic screening improves overall results (in terms of "normal" ongoing pregnancies) and should be widely implemented

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Study question: Why do most of specialists transfer 2 (or more) embryos in most of the assisted reproduction procedures, even knowing that multiple pregnancies constitute a real (and sometimes hazardous) complication?

Summary answer: The transfer of a genetically screened embryo after systematic application of blastocyst culture allows the achievement of high pregnancy rates and reduction of multiple pregnancies.

What is known already: Even knowing the complications of multiple pregnancies (MP), 2 (even 3) embryos are transferred in most assisted reproduction procedures in many countries. The main reason for such a policy is to try to achieve high pregnancy rates per transfer even though multiple pregnancies represent a risk both for women and offspring. PGS along with the blastocyst culture allows the selection of the embryo with the highest odds to implant and to become an ongoing, normal and successful.

Study design, size, duration: Retrospective cohort analysis of a total of 530 embryos genetically screened from 117 successive PGS cycles performed during one year period (June,2016-May,2017) at a University associated ART center were analyzed.

Cycles involving PGD for monogenic disease or chromosomal abnormalities were excluded. Also, cycles in donor eggs were used were not included.

Participants/materials, setting, methods: Due to previously reported results (ESHRE Geneva) systematic single embryo transfer (SET) is offered to all patients at our center. After being informed, each patient gave informed consent form for the procedure.

Rates of euploidy depending on the day of embryo biopsy and age of patients were assessed. Efficiency of treatment was defined as the ongoing pregnancy rate (OPR) per transferred embryo and was analyzed depending on age of patients.

Main results and the role of chance: The mean number of microinjected MII microinjected was 12,99. After the fertilization of 766 oocytes, a total of 530 embryos were genetically screened (4,53 per cycle). Euploidy rates were different depending on the day of embryo biopsy: 17,78%, 44,68% and 21,69% of the assessed embryos were genetically normal after embryo biopsies performed on days 3, 5 and 6 respectively ($p = 0,032$). As expected, aneuploidy rates increased with advanced age of patients. Almost half of the embryos (46,94%) from women aged 30-35 were genetically normal, whereas only 26,32% and 8,82% of embryos from women aged 36-40 and 41-45 respectively were euploidic ($p = 0,021$).

Irrespective of the day of the biopsy, the OPR after single embryo transfer of genetically selected embryos was high either among fresh or frozen-thawed embryo transfers. Overall ongoing pregnancy rate per transfer was 67,09%. In the cleavage stage biopsy setting, 12 out of 22 fresh embryo transfers (54,55%) and 8 out 12 frozen-thawed embryo transfers (66,67%) resulted in ongoing pregnancies. Among embryos biopsied at blastocyst stage, the ongoing pregnancy rate was 73,33% (33 out of 45 transferred embryos).

No multiple pregnancy was suffered during the study period.

Limitations, reasons for caution: The number of patients included and the retrospective nature of the study should make us cautious regarding the results.

Randomized trials would be needed to corroborate the results shown in the present study.

Wider implications of the findings: As specialists, we should not accept multiple pregnancies as an "unavoidable" side effect of assisted reproduction in the 21st Century. Furthermore, improvements in embryo culture systems, freezing-thawing procedures and genetic assessment of embryos, among others, should be fully (and really) incorporated into practice.

Trial registration number: Not applicable.

P-193 Safety of fluorescence lifetime imaging microscopy (FLIM) as a non-invasive assessment of embryo metabolism

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Study question: Is the illumination from FLIM-based metabolic imaging safe for embryos?

Summary answer: Using two assays, we found that excessive FLIM illumination dosages caused a transient perturbation to embryos, whereas a limited clinical dosage had no measurable effect.

What is known already: Embryo mitochondrial health is known to be essential for viability. Metabolic imaging uses FLIM to measure autofluorescence of the endogenous molecules, NADH and FAD, which are essential for cellular respiration. We have show that this technique is an effective tool for quantitatively measuring metabolic state; but in order to develop it into a clinical application, we must understand the effects of the technique's illumination on embryos. The primary cause for potential damage using FLIM illumination is the generation of ROS due to the photochemical absorption of light, hence we investigated whether FLIM produced elevated ROS levels.

Study design, size, duration: We conducted a prospective observational study. Mouse embryos were illuminated using carrying degrees of photo dosages (intended clinical dose and varying excessive dosages) and were compared to control groups that received no illumination. We tested four illumination conditions with a total of 155 illuminated embryos and 122 non-illuminated control embryos. Embryo ROS levels were then measured, and metabolic measurements using FLIM were also taken to determine whether illumination had affected metabolic state.

Participants/materials, setting, methods: We used an on-stage incubation system to culture embryos from the 2-cell stage until blastoscyt. During this period, we took metabolic measurements of the embryos at regular intervals. To vary photodosage, we performed this experiment with varying intervals of illumination, from 2 hours to 24 hours. Control embryos were cultured in the same chambers but were not illuminated. After FLIM illuminations, we used an ROS reporter dye (HC-DCFDA) to measure intracellular ROS levels.

Main results and the role of chance: ROS levels were quantified by taking a simple measure of HC-DCFDA intensity, using custom units. According this measure, none of the illumination conditions resulted in significant differences

compared to non-illuminated control groups of embryos (p values < 0.05). The following dosages produced these mean values (± SD). For 44 metabolic measurements: 6.65 ± 1.81 (n = 39) for control and 6.59 ± 1.83 (n = 37) for experimental group; 24 measurements: 5.69 ± 1.22 (n = 32) for control and 5.58 ± 0.7(n = 36) for the experimental; 3 measurements: 4.42 ± 1.07 (n = 33) for control and 4.18 ± 0.80 (n = 33) for experimental group; 1 measurement: 4.42 ± 0.71 (n = 33) for control and 4.18 ± 0.80 (n = 33) for experimental group. We validated our assay by comparing to the positive control of adding H_2O_2 to embryos, which produced a mean value of 11.76 ± 1.97.

Additionally, we performed several experiments in which we took a single metabolic measurement of illuminated and non-illuminated groups. Interestingly, we found our method performed with superior sensitivity, as there were significant, yet transient, shifts for the metabolic parameters for excessive dosages between the illuminated and the control group. These effects where reversed after 24 hours.

Limitations, reasons for caution: Although we did not observe significant increases in ROS levels, our data showed large variations in ROS measurements. We concluded that this popular assay has limited sensitivity to subtle ROS differences. Also, safety data must be obtained using human embryos to further validate this technique for clinical application.

Wider implications of the findings: These data complement previous safety studies showing that FLIM illumination affects neither blastocyst development nor live birth rates in mouse. Together, these results support the safety of using metabolic imaging as a potential embryo selection tool. Future experiments on human embryo samples are planned in the clinic.

Trial registration number: Not aplicable.

P-194 Reproductive outcomes in patients with severe oligospermia undergoing intracytoplasmic sperm injection using testicular versus ejaculated sperms

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Study question: To compare intracytoplasmic sperm injection (ICSI) outcome in patients with severe oligospermia using testicular versus ejaculated sperms.

Summary answer: In patients with severe oligospermia,TESE should be considered as it offers higher pregnancy rates.

What is known already: In infertile men with severe oligospermia (sperm concentration <5 million/mL) undergoing ICSI the choice between using repeatedly ejaculated sperm or recommending use of testicular sperm remains controversial.

Testicular sperm have shown lower levels of DNA damage compared with ejaculated spermatozoa from the same individuals, however testicular sperm exhibit higher rates of chromosomal abnormalities.

Study design, size, duration: Retrospective cohort study.

Patients with severe oligospermia who underwent ICSI cycles with either testicular or ejaculated sperm between January 2014 and December 2016 were included in this study.

205 couples met the study criteria after exclusion of cycles with mixed sperm sources.

Participants/materials, setting, methods: Patients were divided into 2 groups (Group A) included 104 patients in which ejaculated sperms were obtained while (Group B) included 101 patients where fresh testicular sperms were extracted (TESE).

The medical records of the included couples were reviewed and tabulated regarding demographic data, hormonal profiles and different semen parameters.

After micro injection; fertilization rate, cleavage rates, embryo quality, implantation and pregnancy rates were evaluated and compared in both groups.

Main results and the role of chance: There was no significant difference in the fertilization rate between the two subgroups (group A 67.93%, group B 68.01%, p = 0.960), however the cleavage rate of day 2 embryos was significantly higher when testicular sperm cells were used (47.37% vs 42.77%, p = 0.024*).

Implantation rate was slightly lower in group A compared to group B but without statistical significance (25.23% and 29.77% respectively, p = 0.292).

There were no statistically significant differences between both subgroups regarding number and quality of day 3 and day 5 embryos.

Pregnancy rate was significantly higher in group B where testicular sperm cells were used (54.45% vs 40.38, p = 0.044*).

Limitations, reasons for caution: Prospective randomized studies involving larger number of cases are needed to establish whether TESE should be performed as a routine step in infertile males with severe oligospermia or should it be limited to cases with previous ICSI failure.

TESE remains a surgical intervention with possible associated risk.

Wider implications of the findings: Sperm source can lead to better ICSI outcome in this certain group of patients; such finding may extend to include infertile males with abnormal DNA fragmentation testing.

Trial registration number: The study was revised and approved by the official ethical committee of Faculty of Medicine, Alexandria University, Egypt.

P-195 Sequential low oxygen culture improves blastocyst rate and morphokinetics in a randomised controlled study on mouse embryo assays (MEA)

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Study question: Does the sequential low oxygen concentration have better results in embryo development and morphokinetics than the commonly used cell culture condition on mouse model?

Summary answer: Sequential low oxygen concentration significantly improves blastocyst rate and morphokinetics compared to constant low and high oxygen tension on mouse model.

What is known already: Mammalian fertilization and early embryonic development occur in oviduct and uterus. In both organs, oxygen tension rises from day 1 to 3 between the eight-cell-morula stage and blastocyst formation, and then drops when the blastocysts are fully developed and implantation begins. Therefore, this study used time-lapse technique as a tool to investigate the effects of this sequential low oxygen concentration (7% from day 1 to day 2, 2% from day 3 to day 5) in mouse embryo development and morphokinetic analysis of cell division compared to low (5%) and high (20%) oxygen tension, giving insight into potential improvement in ART.

Study design, size, duration: To determine the best oxygen concentrations which have the most beneficial results in ART, we designed a randomized study employing mouse embryos. 314 zygotes were arranged randomly into three parallel groups consisted of high atmospheric oxygen concentration (20%) (n = 93), low oxygen (5%) (n = 129), and mixed (7% from day 1 to 2 and 2% from day 3 to 5) (n = 92). Blastocyst rate and morphokinetic analysis of cell division were recorded using a Primovision-System.

Participants/materials, setting, methods: Hybrid Bl6/CBAca MEA was used to identify morphokinetic cleavage parameters derived from time-lapse imaging. Female mice were sacrificed and zygotes were isolated 20-hours post-insemination. They were cultured in Primovision micro-well culture dishes using GTL Medium (Vitrolife) and observed in the Primovision system that integrates a digital inverted microscope into a standard high volume Memmert IVF incubator. Pictures of embryos were taken at 10 minute intervals for 5 days. Statistical analyses were performed using R program.

Main results and the role of chance: Blastocyst rate at 120 hours in mixed group (91,3%) was significantly increased compared to high (76,34%) and low group (74,42%), p = 0.009 and 0.001, respectively. Moreover, cell division of mixed group was significant faster in almost every cleavage stages comparing to the other groups, including p = 0.014 at 2 pronuclei stage (2PN) (mixed vs high), p = 0.002 at 2-cells stage (2 C) (mixed vs low), p = 0.005 at 5-cell stage (5 C) (mixed vs high), p = 0.009 at 8-cell stage (8 C) (mixed vs high), p = 0.031 and p = 0.034 at morula stage (mixed vs low, and mixed vs high, respectively), p = 0.001 at early blastocyst formation (mixed vs high), p = 0.005 at hatching stage (mixed vs low). Notably, the interim events was also significantly different between the groups, such as p = 0.022 (mixed vs low) and p = 0.002 (mixed vs high) from 3 C to 4 C, p = 0.001 (mixed vs low) and p < 0.001 (mixed vs high) from 3 C to 5 C, p = 0.032 (mixed vs low) and p < 0.001 (mixed vs high) from 4 C to 5 C, and p < 0.001 (mixed vs high) from 2 C to 5 C.

Limitations, reasons for caution: This in-vitro study used surrogate parameters as morphokinetics and blastocyst rates. No data on pregnancy or birth rates can be provided as blastocysts were not transferred due to German law restrictions. The results may differ on embryo development in different laboratory settings such as types of incubator and laboratory procedures.

Wider implications of the findings: If further research shows improved ART outcome in sequential low oxygen culturing, this should enter clinical ART management.

Trial registration number: N/A.

P-196 Female age affects DNA repair gene expression in mouse GV oocytes from stimulated and unstimulated cycles and in MII oocytes matured in-vivo and in-vitro

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Study question: Does female age affect DNA repair gene expression in GV oocytes from stimulated and unstimulated mice and MII oocytes matured in-vivo and in-vitro?

Summary answer: Age has a major impact on DNA repair genes during the GV-MII transition in stimulated and unstimulated cycles.

What is known already: Gene expression patterns in germinal vesicles (GV) and metaphase II (MII) oocytes change with age. Oocyte maturation involves extensive degradation of maternal transcripts suggesting that GVs would have a larger number of mRNA transcripts than MII oocytes. Nevertheless, GVs from aged females show a decrease in numbers of transcripts compared to young GVs, and likewise in old MII oocytes compared to young MII oocytes. DNA repair gene expression patterns may give clearer links to repair competency during early embryo development. Understanding molecular signatures that predict genetically stable oocytes with higher developmental competency may result in better prognosis for reproductive treatments.

Study design, size, duration: GVs were collected from 4 stimulated and 4 unstimulated young (5-8 weeks) and old (42-45 weeks) mice respectively. From the stimulated cycles 20 GVs (stim-GVs) and 20 MIIs (*in vivo*-MII) were collected for each age groups. For unstimulated cycles 20 GVs (unstim-GVs) were used for analysis and a further cohort were matured in order to collect 20 MIIs for analysis (IVM-MIIs). Expression profiles were determined in all 4 groups per female age.

Participants/materials, setting, methods: C57BL6 female mice were used. Unstimulated GVs were *in vitro* matured in culture for 18hrs, and resulting MIIs collected. Oocytes for each treatment were pooled (n = 5), snap frozen and stored at -80C for later cDNA synthesis. cDNA synthesis was performed using the Single Cell-to-Ct Kit (Ambion, Life technologies) and cDNA samples were tested for DNA repair gene expression of 90 DNA repair genes using the Fluidigm Biomark HD system GE 96×96 Standard v2 Biomark Protocol.

Main results and the role of chance: Comparing MII oocytes to GV oocytes in both age groups, significant differences in gene expression were observed between *in vivo*-MII and IVM-MII oocytes (95% Confidence Interval, 95%-IC). The number of significantly down-regulated genes in IVM-MIIs from younger females, were greater than *in vivo*-MII (Young IVM-MII: 38 genes; Young in vivo-MII: 25 genes, 95%-IC). In aged oocytes, the expression pattern was reversed, with more DNA repair genes significantly up-regulated in IVM-MII and *in vivo*-MII oocytes than GVs (Old IVM-MII: 22 genes; Old *in vivo*-MII: 12 genes, 95%-IC). Also, in older females more DNA repair genes were down-regulated in IVM-MII oocytes than in *in-vivo*-MIIs (Old IVM-MII: 13 genes; Old *in-vivo*-MII: 4 genes, 95%-IC), suggesting that IVM has a different effect on MII repair genes than in vivo maturation in stimulated oocytes, with potential repercussions on oocyte quality. The key DNA damage response gene, H2AFX, was always down-regulated in MII oocytes compared to GV oocytes irrespective of maturation type (95%-IC). Confidence intervals were used to establish differential expression in DeltaDeltaCt analysis while Mann-Whitney was used to test relative expression of DeltaCt analysis.

Limitations, reasons for caution: The two age groups in this study were selected to represent women at either end of their reproductive lives, but clinical studies are required to confirm this effect in women. However, challenges in obtaining either unstimulated and/or stimulated MII oocytes for research may limit studies to human GVs.

Wider implications of the findings: Our results show DNA repair gene expression in oocytes is affected in older females, suggesting that this may also be linked with decreased DNA repair capacity in both GV and MII oocytes. These findings will direct future research to develop novel methods to assess the effects of aging on fertility.

Trial registration number: N/A.

P-197 Embryos derived from late maturing oocytes show no increased risk of chromosomal aneuploidy as assessed by Next Generation Sequencing (NGS) and can achieve clinical pregnancies

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Study question: Are oocytes that are immature at cumulus removal but mature by the time of insemination capable of normal fertilization and development into chromosomally euploid embryos?

Summary answer: Late maturing oocytes, inseminated by intraplasmic sperm injection (ICSI), if fertilized, can form chromosomally euploid embryos and are capable of establishing clinical pregnancies.

What is known already: Approximately 20% of retrieved oocytes from controlled ovarian stimulation cycles are found to be immature as either metaphase I (MI) or germinal vesicle (GV) stage at the time of cumulus cell removal. Debate exists as to whether these immature oocytes should be utilised if, after further evaluation, they are observed to extrude a polar body during the period of in vitro culture prior to insemination. The chromosomal constitution and developmental competence of these in vitro matured oocytes has not been adequately explored, with conflicting views on the rates of multinucleation and aneuploidy in the resulting embryos.

Study design, size, duration: A retrospective study was undertaken for 1902 autologous ICSI cycles performed between January-August 2017. Fresh or frozen spermatozoa were used for ICSI insemination.

Participants/materials, setting, methods: Autologous ICSI cycles that had one or more MI oocyte at the time of cumulus cell removal (38 hours post trigger) were included in the study. Embryos were cultured in Vitrolife sequential media and COOK mini-incubators. After laser ablation of the zona on day 3, 5-10 trophectoderm cells were biopsied on day 5 or 6 and embryos immediately vitrified. Genetically euploid embryos were warmed and transferred individually. PGS cycles were performed only at patient request.

Main results and the role of chance: In total 21,138 oocytes were collected from 1902 ICSI cycles. Of these, 75.2% were mature (metaphase II, MII) at the time of cumulus removal and 14.4% were MI. Of the MI oocytes, 42.6% progressed to mature to MII 4-6 hours after oocyte collection and were inseminated via ICSI. Overall, the fertilisation rate after ICSI of the late maturing oocytes was lower than those that were MII at the time of initial cumulus cells removal (41.9% v 64.8%, p<0.05). In total 80 transfers were performed (34 at day 3, 46 at day 5/6) with 51 fresh and 28 frozen embryos resulting in 18 ongoing pregnancies with to date, 2 live births.

A sub-group of embryos resulting from late maturing oocytes were analysed by NGS to assess their chromosomal composition. Thirty one

blastocysts were biopsied and vitrified. Seventeen of the thirty-one embryos (54.8%) were found to be euploid and deemed suitable for embryo transfer in future frozen embryo transfer cycles. To date, six singleton embryo transfers have occurred resulting in four ongoing pregnancies (4/6, 66.7%) in patients aged 44, 43, 39 and 34. These results refute previous reports that suggest late maturing oocytes are more likely to produce embryos with chromosomal aneuploidies.

Limitations, reasons for caution: This study looks at patients of all ages and aetiology. Genetic testing was limited to patients who had selected to undergo a PGS cycle.

Wider implications of the findings: The maturation status of oocytes at the time of cumulus removal does not indicate an increased risk of chromosomal aneuploidy in the resulting embryos. Late maturing oocytes should be utilised for patients undergoing ICSI as they can result in genetically tested euploid embryos and clinical pregnancies.

Trial registration number: Not applicable.

P-198 Optimal timing of trophectoderm biopsy for preimplantation genetic screening (PGS)

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Study question: Is the timing of trophectoderm biopsy associated with successful pregnancy after the embryo transfer of blastocyst subjected to preimplantation genetic screening (PGS)?

Summary answer: Trophectoderm biopsy(PGS) before or after the cryopreservation may be the optimal strategy for the better clinical outcomes compared to the fresh trophectoderm biopsy without cryopreservation.

What is known already: Embryo biopsy and fresh embryo transfer are traditionally performed in the PGS cycle. However, before embryo transfer, the time allowed for genetic analysis of the specimens is restricted, particularly after blastocyst biopsy. Cryopreservation of blastocysts after biopsy instead of fresh transfer permits more sufficient time for performance of molecular diagnosis. The effect of cryopreservation and thawing/warming procedures on clinical outcomes in PGS cycle has not been effectively studied.

Study design, size, duration: This retrospective study included women that underwent IVF with PGS from January 2016 to December 2017. 1469 blastocyst from 306cycles were subjected to trophectoderm biopsy for performing array comparative genomic hybridization (CGH) test. Embryos were cultured to expanded blastocyst stage and underwent trophectoderm biopsy on day 5 to day 6 of embryo development.Cycles with complete PGS diagnosis were 306, 161 of which had embryo transfer. The performance of different groups of PGS patients was evaluated.

Participants/materials, setting, methods: The groups were divided into three; first group (n = 46 transfer/168 cases) contained fresh blastocysts that biopsied for PGS without cryopreservation followed by embryo transfer. In the second group (n = 34 transfer/56 cases), the cryopreserved blastocysts were warmed and biopsied prior to ET. The last group (n = 81 transfer/82 cases), the blastocysts were initially biopsied then proceeded with vitrification and warming before the embryo transfer.

Main results and the role of chance: The total pregnancy rate of fresh blastocyst biopsied group was 47.8% (22/46), the cryopreserved-warmed-biopsied blastocyst group showed 70.6% (24/34) and finally biopsied and cryopreserved-warmed group showed 55.6% (45/81), respectively. First group is significant higher pregnancy rate than second group.(p = 0.043). Also, The second(cryopreserved-warmed –biopsied) group showed slightly higher numerical pregnancy rate than third (biopsied and cryopreserved-warmed) group.

Limitations, reasons for caution: This is a retrospective study and had some limited case numbers for analysis.

Wider implications of the findings: Using currently available data, when faced with the option of fresh embryos, before or after trophectoderm biopsy for PGS, our result supported performing the biopsy before or after embryo cryopreservation and thawing.

Trial registration number: not applicable.

P-199 Synchrony or morphology: embryo-endometrial synchrony is more important than embryo morphology as a predictor of in vitro fertilization outcomes

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Study question: Which one is better as a predictor of in vitro fertilization (IVF) outcomes, synchrony or morphology?

Summary answer: Embryo-endometrial synchronization, represented by proper embryo developmental speed, could be a better predictor of IVF success than good morphology.

What is known already: Embryo morphology including blastomere symmetry and fragmentation is well-known and most commonly used as a predictor of IVF outcomes. Therefore, transferred embryos are usually selected by morphological assessment. Embryo-endometrial synchronization which is represented by developmental speed coincident with embryo stage is also an important predictor of IVF outcomes.

Study design, size, duration: This was retrospective study with fresh embryo transfer on day 3 between January 2015 and December 2016. This study included 428 women who had two transferred embryos of same blastomere number.

Participants/materials, setting, methods: Women transferred two slowly developing day 3 embryos with good morphology embryo were divided into 2 groups by blastomere number.

S1 – two 4 cell embryos with good morphology.
S2 – two 6 cell embryos with good morphology.

Women transferred two properly developed 8 cell day 3 embryos were divided into 3 groups by grade.

G1 – two grade 1 8cell embryos.
G2 – two grade 2 8cell embryos.
G3 – two grade 3 8cell embryos.

Main results and the role of chance: In each group, age and AMH level reflected no differences. Pregnancy rate (PR) of S1 [7.4%(2/27)] was significantly lower than PR of G1[69.3%(138/199), P<0.01] and G2[54.0%(81/150), P<0.01]. It was also significantly lower that of G3[35.7%(10/28), P = 0.02]. PR of S2 [37.5%(9/24)] was significantly lower than PR of G1[69.3%(138/199), P<0.01]. However, it did not show significant difference as compared with that of G2[54.0%(81/150), P = 0.198] or G3[35.7%(10/28), P = 0.876].

Limitations, reasons for caution: This is a retrospective study. The sample size of each group shows difference among groups.

Wider implications of the findings: Implantation potential of slowly developing embryos with good morphology is lower than properly developed low grade embryos. It is probably that slowly developing embryos do not synchronize endometrial implantation window. Therefore, properly developed low grade embryos rather than slowly developing good morphology embryos should be selected for fresh embryo transfer.

Trial registration number: None.

P-200 Testicular sperm of severe oligo-cryptozoospermia patient may expect to improve clinical outcomes compared with ejaculated sperm

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Study question: The purpose was to compare clinical outcomes of in vitro fertilization-intracytoplasmic injection (IVF-ICSI) between sperm retrieved from severe male factor (oligozoospermia, cryptozoospermia, TESE -sperm) patients.

Summary answer: In case of severe male factors, patients with cryptozoospermia showed low fertilization and significantly low clinical pregnancy rates compare to patients with oligozoospermia and TESE-sperm.

What is known already: Sperm from severe male factor contains more DNA damage than sperm from fertile patients as this is due to Reactive Oxygen Species. Several studies reported that sperm are susceptible to damage during passage through the male reproductive tract. When increased DNA damaged sperm is used for assisted reproduction, this sperm may be able to fertilize an oocyte, however, this may result in poor embryo development, implantation and pregnancy rates. The sperm quality may be related to unexplained recurrent pregnancy loss. Some reports states that patients using testicular sperm showed higher implantation and pregnancy rates than patients with severe oligoasthenoteratozoospermia syndrome.

Study design, size, duration: From January 2016 to December 2017, our IVF center performed 9707 IVF-ICSI cycles. Among them, only 299 cycles with severe male factor were analyzed in this retrospective study. The clinical outcomes including fertilization and pregnancy rate were compared and the age of couple was also evaluated in severe male factor groups. Statistical analysis was performed using ANOVA and chi-squared tests.

Participants/materials, setting, methods: Oligozoospermia was defined as spermatozoa under 15×10^6/cc; and cryptozoospermia was defined as spermatozoa observed only in the pellet with sperm concentration under 10^4 after centrifugation (WHO 2010). However, in this study, we considered oligozoospermia as over 0.1×10^6/cc to under 15×10^6/cc. TESE sperm was considered as sperm retrieved from azoospermia patients. In the study, the IVF-ICSI cycles were divided into three groups. Group 1: Oligozoospermia, Group 2: Cryptozoospermia, Group 3: TESE sperm.

Main results and the role of chance: This study showed a significant low fertilization rate in patients with cryptozoospermia (Group 2) compare to other groups (Group 1: 81.67%(820/1004), Group 2: 74.45%(303/407), Group 3: 79.02%(1134/1435); $p = 0.009$). Furthermore, pregnancy rate was also significantly low in cryptozoospermia patients (Group 1: 40.19% (43/107), Group 2: 17.78%(8/45), Group 3: 46.27%(62/134); $p = 0.003$) than others. However, there was no significant difference in fertilization rate and pregnancy rate between Group 1 and Group 3. In addition, there was no significant difference in both the wife's age (Group 1: 36.14 ± 4.09 ($n = 107$), Group 2: 35.04 ± 4.08($n = 45$), Group 3: 35.37 ± 4.35($n = 134$); $p>0.05$), and the husband's age (Group 1: 38.25 ± 5.10($n = 107$), Group 2: 37.60 ± 5.73($n = 45$), Group 3: 37.54 ± 5.66($n = 134$); $p>0.05$) in all three groups.

Limitations, reasons for caution: This is a retrospective study and a limited number of severe male factor in IVF-ICSI cycles were used for analysis.

Wider implications of the findings: The findings of this study indicate that cryptozoospermia patients, using several cryo-sperms with repetitive IVF-ICSI cycle failure and pregnancy failure may need to consider an alternative strategy of using TESE sperm for improved clinical outcomes.

Trial registration number: Not applicable.

P-201 The influence of blastocyst formation time on the pregnancy outcomes of vitrified-warmed blastocyst transfer according to the number of embryo transferred

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Study question: Does the blastocyst formation time before vitrification affect the pregnancy outcomes in single or double vitrified-warmed blastocyst transfer (SET or DET)?

Summary answer: Day 5-DET resulted in similar the pregnancy outcomes compared to day 6-DET while day 5-SET yielded significantly higher the pregnancy outcomes compared to day 6-SET.

What is known already: Zygotes can reach the blastocyst on day 5 or day 6 after insemination. Many studies on fresh IVF cycles have reported higher the pregnancy outcomes with the transfer of day 5 blastocyst compared with day 6. However, it is still unclear whether the blastocyst formation time in vitrified-warmed blastocyst transfer with studies yielding conflicting results affects the pregnancy outcomes.

Study design, size, duration: A retrospective cohort study of 405 SET or DET cycles was performed from January 2013 to September 2017. The cycles with frozen sperm, surgical retrieved sperm, genetic diagnosis, oocyte donation, poor responder, and advanced maternal age (≥38 years) were excluded. We compared the rates of good-quality embryos, clinical pregnancy, ongoing pregnancy, and implantation between day 5 and day 6 vitrified-warmed blastocyst transfer according to the number of embryo transferred.

Participants/materials, setting, methods: The cycles were divided into 4 groups based on the blastocyst formation time and the number of embryo transferred (SET: day 5, n = 107 vs. day 6, n = 104 and DET: day 5, n = 128 vs. day 6, n = 66). The classification of warmed blastocysts quality was evaluated refer to the criteria for classification by Gardner and Schoolcraft.

Main results and the role of chance: Characteristics of the cycles had no statistically significant difference in the female age, male age, infertility diagnosis, number of embryo thawed, survival rate, good quality (≥BB) embryos rate, with the exception of male factor infertility, which was significantly more common in the DET (14.1% vs. 25.8%, p = 0.045). Rates of clinical pregnancy, ongoing pregnancy, and implantation in day 5-SET was higher than those of day 6-SET (Table 1). However, the pregnancy outcomes were not significantly different between day 5-DET and day 6-DET.

Table 1 Comparison of the pregnancy outcomes between day 5 and day 6 vitrified-warmed blastocyst transfer according to the number of embryo transferred.

	Day 5-SET	Day 6-SET	P-value	Day 5-DET	Day 6-DET	P-value
Cycles (n)	107	104		128	66	
Clinical pregnancy rate (%)	43.0	20.2	0.000	50.8	43.9	0.366
Ongoing pregnancy rate (%)	36.4	17.3	0.002	45.3	37.9	0.321
Implantation rate (%)	43.9	22.1	0.001	35.2	29.5	0.266

Limitations, reasons for caution: This is a retrospective study. The number of day 6-DET group was smaller than day 5-DET group. Prospective, further studies are needed in order to support our results.

Wider implications of the findings: The blastocyst formation time could be an independent predictor of the pregnancy outcomes. Although the transfer of blastocyst derived from day 6 remains a viable treatment option, giving precedence to blastocyst derived from day 5 could allow opportunity to successful pregnancy outcomes in SET.

Trial registration number: Not applicable.

P-202 Morphokinetic differences of human embryo development according to the type of morphological defects of sperm

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Study question: Does type of morphological defects of sperm and fertilization method have an impact on morphokinetics of embryo development?

Summary answer: Morphokinetics of embryo development showed various difference according to the type of morphological defects of sperm and fertilization method, such as IVF and ICSI.

What is known already: Sperm morphology is the important parameter for evaluation in predicting male fertility potential. Time-lapse monitoring system offers evaluation criteria of embryo development that can chance to select embryos with higher implantation. In recent years, many studies have been reported that sperm affected in embryo development. But, not well known about the relation between morphokinetics of embryo development and type of morphological defects of sperm according to fertilization method.

Study design, size, duration: We performed a retrospective cohort study on patients undergoing IVF and ICSI cycle from October 2013 to May 2017. A total of 422 zygotes obtained from 60 patients. Exclusion criteria; A women who was over 40 years old or an anti-Mullerian hormone (AMH) less than 1.0 ng/㎖. Sperm parameters were evaluated according to 2010 WHO criteria. Correlation between type of morphological defects of sperm and morphokinetics of embryo development were analyzed.

Participants/materials, setting, methods: Patients were divided into three categories; normozoospermatozoa (≥15 million/㎖, motility ≥40% and normal morphology ≥4%) (Group1), teratozoospermatozoa (normal morphology <4%) with head defects (Group 2), and teratozoospermatozoa with head and tail defects (Group 3). Zygotes were cultured with the time-lapse monitoring system (Primo vision). Morphokinetics were evaluated as below; time of cell division (tS-t8) and interval of cell cycle (tS-tSC, cc1: tSC-t2, cc2; t2-t3, cc3; t3-t5, t2-t5 and s2; t4-t3).

Main results and the role of chance: Time points and durations of cell cycle in the ICSI embryos were similar in Group1 (n = 76) and Group2 (n = 73) except the times of syngamy to first division (tS-tSC) (2.1 ± 0.6, 2.0 ± 0.4, p = 0.0476) and Group1 and Group3 (n = 55) except synchrony in division from 3 to 4 cells (s2) (2.0 ± 3.1, 4.3 ± 5.3, p = 0.0143). There were no significant differences between Group2 and Group3. In IVF embryos, there were significant differences between Group 1 (n = 119) and Group 2 (n = 62) embryos; tS (27.7 ± 3.6, 30.1 ± 4.9, p = 0.0002), tSC (29.5 ± 3.7, 32.2 ± 5.4, p = 0.0001), t4 (41.4 ± 5.0, 43.6 ± 6.2, p = 0.0230) and t8 (57.1 ± 6.8, 61.1 ± 8.9, p = 0.0099). Significant differences were shown between Group 1 and Group 3 (n = 32) embryos; t8 (57.1 ± 6.8, 62.6 ± 9.9, p = 0.0012) and cc3 (11.9 ± 4.1, 14.1 ± 3.3, p = 0.0093). Also, there were significant differences between Group 2 and Group 3 embryos; tS (30.1 ± 4.9, 27.6 ± 3.7, p = 0.0135), tSC (32.3 ± 5.4, 29.5 ± 3.7, p = 0.0122) and t2 (31.6 ± 3.5, 29.9 ± 3.8, p = 0.0466).

Limitations, reasons for caution: This study was conducted with using cleavage stage embryos. Because the remaining embryos after transfer were cultured to blastocyst stage, we excluded it from this study. Based on the this study, more data will be collected in future prospective studies.

Wider implications of the findings: We found that type of morphological defects of sperm affected embryo development in IVF than ICSI. This is probably due to the high probability that ICSI will select the normal sperm. Our results suggest that morphological defects is may be an important factor to consider in the morphokinetics of embryo.

Trial registration number: None.

P-203 Effects of tocotrienol-rich fraction supplementation on the expression of DNA damage response genes in ovary and oocytes quality in mice exposed to corticosterone-induced oxidative damage

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Study question: Does tocotrienol-rich fraction (TRF) supplementation activate the DNA damage response genes in ovary and improves the quality of oocytes in mice exposed to exogenous corticosterone that induced oxidative damage.

Summary answer: Expression of CHEK1 gene that detects DNA damage in ovary is low and oocytes quality improves following TRF supplementation in mice exposed to exogenous corticosterone.

What is known already: Chronic exposure to exogenous corticosterone (CORT) induced-oxidative DNA damage in cells. Female reproductive system is susceptible to the detrimental effects of oxidative DNA damage leading to a decline in biological function of ovaries, deteriorate oocytes quality and eventually contributes to infertility. Tocotrienol-rich fraction (TRF), an antioxidant plays a protective role in oxidative stress-induced damage thus prevent infertility in female. The proposed mechanism of action TRF in protecting female reproductive system from various oxidative damage is by lowering plasma levels of malondialdehyde (an oxidative stress biomarker), reducing DNA damage in embryos and upregulating the expression of anti-aging gene in mouse ovary.

Study design, size, duration: Thirty-two (six-week-old) female mice (Mus musculus) were equally divided into four groups. Group 1: vehicle control was given via intraperitoneal (i.p) injection, Group 2: CORT (10 mg/kg body weight (BW) was administered intraperitoneally, Group 3: vehicle controls were given via i.p injection and oral gavage while Group 4 were administered with CORT (10 mg/kg BW, i.p injection) and concurrently supplemented with TRF (oral gavage) at the dose of 150 mg/kg BW for two weeks.

Participants/materials, setting, methods: At the end of the supplementation period, mice were superovulated and euthanized. The ovaries were collected for total cellular RNA isolation and oocytes were collected for zona pellucida (ZP) thickness measurement to assess its quality. The gene expression analysis of ATM, MPG, CHEK 1, CHEK 2 and MLH3 genes was performed using QuantiGene Plex 2.0 Assay kit.

Main results and the role of chance: Expression of CHEK1, the gene responsible for the detection of DNA damage was significantly higher in the ovary of mice exposed to exogenous CORT as compared to the control group. The expression level of CHEK1 gene in CORT group supplemented with TRF on the other hand was found to be normalized towards control value. However, expression of other DNA damage response genes was not significantly different in all groups as compared to its respective control. The ZP thickness of oocytes in mice exposed to exogenous CORT increased significantly as compared to its control. Conversely, TRF supplementation reduced ZP thickness of oocytes in mice exposed to exogenous CORT towards control. Upregulation of DNA damage response genes play a significant role in the maintenance of cell by activating various signalling network that detect and repair the oxidative stress induced-DNA damage in mice ovary exposed to exogenous CORT thus protect the ovary and maintain the production of good quality oocytes.

Limitations, reasons for caution: Gene expression analysis can only be done using ovarian tissue and not using oocytes as large quantity of oocytes is required for gene expression analysis.

Wider implications of the findings: Tocotrienol-rich fraction supplementation blocks the adverse effect of exogenous CORT in inducing oxidative DNA damage, as indicated by the low expression of CHEK1 gene that detects DNA damage in mice ovary. Consequently, by protecting ovary from oxidative damage, TRF maintain the quality of oocytes and hopefully will potentially improve fertility.

Trial registration number: N/A.

P-204 Is vitrification-warming of embryos developed from vitrified oocytes (double vitrification) an effective procedure for heterologous IVF program with egg donation?

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Study question: To evaluate the efficacy of transfer of *vitrified-warmed (V-W) embryo* derived from V-W oocyte (double vitrification) in heterologous IVF programs with egg donation.

Summary answer: Transfer of V-W embryo derived from V-W oocyte allows to obtain results statistically comparable to those obtained with fresh embryo derived by V-W oocytes.

What is known already: Advances in reproductive techniques, i.e. the introduction of oocytes vitrification, have provided opportunity to conceive from oocytes banks. The vitrification of oocytes and embryos is the gold standard method used worldwide for cryopreservation in infertility management and fertility preservation. The IVF outcome, in terms of pregnancy and implantation rate, are comparable with those obtained with fresh oocytes/embryos.

Study design, size, duration: This is a prospective study aimed to evaluate the efficacy of double vitrification in heterologous IVF cycles with oocytes donation at the IVF clinic of Careggi University Hospital of Florence, from 2016 to 2017. We compared pregnancy and implantation rates of transfer of V-W embryos derived from V-W oocytes (double vitrification) with transfer of fresh embryos derived from V-W oocyte (single vitrification), that represent our control population.

Participants/materials, setting, methods: From January 2016 to December 2017 we performed 49 transfer of V-W embryos derived from V-W oocytes; as controls we included 329 transfer of fresh embryos obtained from V-W oocytes within our egg donation program. We used the Kitazato vitrification-warming protocol; embryo transfers were performed in day 3 or day 5.

Main results and the role of chance: The pregnancy rate per ET cycle was 40,0% in the double vitrification group and 37,0% in the control group ($p = 0,751$). The implantation rate was similar in the double vitrification group and in the control population (29,7% and 24,7% respectively; $p = 0,562$).

Limitations, reasons for caution: The age of recipients in our oocytes donation program is usually advanced (even if less than 50 years old), so this could influence uterine receptivity due to several age-related pathologies (such as fibroids, menopause, endometritis).

Wider implications of the findings: Our findings confirmed the efficacy of the double vitrification-warming procedure (at oocytes and embryos stage) that could be used for supernumerary embryos or to postpone embryo transfer when the endometrial pattern is suboptimal.

Trial registration number: Not Applicable.

P-205 Cell-lineage specific effects of granulocyte macrophage colony-stimulating factor (GM-CSF) in mouse blastocysts

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Study question: Does GM-CSF induce cell lineage specific differences during pre-implantation development?

Summary answer: Embryos cultured in medium supplemented with GM-CSF show significantly increased numbers of cells in the pre-implantation state. The effect is strongest in the trophectoderm.

What is known already: GM-CSF is a cytokine influencing the maternal-fetal interface and supporting placental development in mouse and human. It is expressed in epithelial cells of the endometrium under regulation of estrogens. A large clinical trial showed that GM-CSF supplemented medium leads to an increased survival of transferred embryos up to week 12. Especially in women with previous miscarriages GM-CSF supplementation of culture medium may improve ongoing implantation rates. However, a research study in mice found that high concentrations of GM-CSF may have a negative impact on blastocyst development and total cell numbers of the preimplantation embryo.

Study design, size, duration: Thirty female mice (5-10 weeks pp) were stimulated for zygote isolation. After 4.5 days of culture in defined concentrations (1, 2 or 5 ng/ml) of GM-CSF a total of 277 embryos were collected and subjected to immunohistochemical staining. Markers against the three cell lines trophectoderm (CDX2), epiblast (NANOG) and primitive endoderm (SOX17) enabled selective counting of cell numbers. 244 embryos were eligible for further analysis.

Participants/materials, setting, methods: Female mice (B6C3F1) were superovulated by injection of 5 IU PMSG and 10 IU hCG and mated to males (C57BL/6J). Zygotes were isolated on day 0.5 post coitum, pooled and randomly distributed to KSOM(aa) without GM-CSF and medium supplemented with increasing concentrations of GM-CSF. Zygotes were cultivated until blastocyst stage with a medium refreshment step at day 2.5.

Main results and the role of chance: Total cell numbers of blastocysts showed significant differences between KSOM(aa) without GM-CSF and KSOM (aa) containing 2 or 5 ng/mL GM-CSF (mean: KSOM(aa): 81.7 cells; KSOM(aa) +1 ng/mL GM-CSF: 84.5 cells; KSOM(aa)+2 ng/mL GM-CSF: 90.9 cells; KSOM(aa)+5 ng/mL GM-CSF: 91.4 cells). These differences were mainly due to changes in trophectoderm cell numbers; we found significant differences in the number of CDX2 positive cells between embryos cultured without GM-CSF and the two groups containing 2 and 5 ng/mL GM-CSF (mean: KSOM(aa): 65.6 cells; KSOM(aa)+1 ng/mL GM-CSF: 69.5 cells; KSOM(aa)+2 ng/mL GM-SCF: 74.1 cells; KSOM(aa)+5 ng/mL GM-SCF: 73.8 cells; $p \leq 0.01$). Additionally, more positive primitive endoderm (SOX17) cells were found at higher concentrations of GM-CSF (mean: KSOM(aa): 9.21 cells; KSOM(aa) +5 ng/mL GM-CSF: 11 cells $p>0.5$).The amount of epiblast (NANOG positive) cells showed almost no variance at different concentrations of GM-SCF. However, the proportion of trophectoderm, epiblast and primitive endoderm cell numbers did not change among the embryos of the different groups (80-82% trophectoderm; 11-12% primitive endoderm; 7-8% epiblast).

Limitations, reasons for caution: This study was performed in a mouse model and would need further investigation in other animal models or experiments with human embryos to elucidate and validate the influence of GM-CSF on trophectoderm cell numbers during preimplantation development.

Wider implications of the findings: GM-CSF increases the number of trophectoderm cells which may lead to an improvement in the implantation process and embryo or placental development and also may increase survival rates of transferred embryos in a clinical application.

Trial registration number: DFG NO 413/3-3 and BO2540/4-3

P-206 A prospective sibling oocyte study; do sequential and single step media perform comparably

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Study question: Do sequential and single step media perform comparably in terms of blastocyst formation, quality and pregnancy rates when using sibling oocytes?

Summary answer: There is an increase in blastocyst formation with single step media, however this does not appear to translate to an increase in clinical pregnancy rate.

What is known already: Recent years have seen the increased utilisation of culture in time lapse systems. Associated with this is the rise in the use of Single Step media for IVF culture. Using sibling oocytes we are validating the use of Sage Single Step (SS) media alongside Sage Sequential (SQ) media, with the eventual aim of introducing SS with the use of time lapse culture.

Study design, size, duration: From August 2017 until Decmeber 2017 103 patients who were being treated with IVF or ICSI who had 4> normally fertilised oocytes were included in the study. 85 patients reached transfer (the remainder had freeze all). Embryo selection for transfer is based on development and quality only not on media type. Decision to transfer on day 3 or culture on is based on embryo development and quality on the morning of day 3.

Participants/materials, setting, methods: The fertilised oocytes are divided between Sage Single Step (SS) media and Sage Sequential (SQ) media; Cleavage media, that is changed to Blastocyst media at approximately 68 hours post insemination. 50% that are cultured in Sage SS media that is refreshed at approximatley 68 hours post insemination. Our programme aimed to transfer a

single embryo unless patient history or embryo quality indicate a benefit from double embryo transfer.

Main results and the role of chance: Our inital analysis of the data suggests an increase of blastocyst formtion rate in SS embryos compared to SQ (68% vs 54% $P<0.05$). This is reflected in the increase seen in SS cultured embryos being selected for transfer over SQ derived embryos (50.5% vs 44.7%) the remaining 4.8% of transfers reflects a mix of SQ and SS embryos. Top quality blastocyst rates (SS 44% vs SQ 47%) and number of embryos frozen (SS 40% vs SQ 42%) are comparable. An increase is seen in positive pregnancy test for SQ transfers (53% vs 60% per embryo transfer), on going clinical pregnancy rates are comparable (SS 44% vs SQ 45%) and per embryo transferred (SS 34% vs SQ 32%).

Limitations, reasons for caution: The limitations of this study are the numbers of patients who have so far been recruited. As this is an ongoing study we hope to provide more data to increase its power.

Wider implications of the findings: This ongoing study indicates an increase in blastocyst formation for embryos cultured in SS media over SQ media. This increase does not currently translate into an increase in quality or clinical pregnancy outcome or increase in the number of embryos frozen.

Trial registration number: not applicable.

P-207 Embryo secretome profile from infertile patients undergoing assisted reproductive techniques can be affected by infertility factors

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Study question: Does the embryo secretome vary in according with infertility factor of women undergoing assisted reproductive techniques (ART), as endometriosis, polycystic ovarian syndrome or tubal factor?

Summary answer: Embryonic secretome from patients presenting deep infiltrating endometriosis (DIE), polycystic ovarian syndrome (PCOS), and tubal factor (TF) presented divergent protein profiles evaluated through proteomics approach.

What is known already: The field of human ART would benefit from more quantitative methods of determining embryo viability and implantation potential. It is known that soluble ligands and it receptors mediate human preimplantation embryo development and implantation. A number of researchers has been used proteomics approach related to human reproduction in several subareas. Various proteins has been investigated as embryo biomarker in the spent culture media, given the embryo culture medium secretome reflect the embryo development. We hypothesize that protein profiles are affected according with infertility factors, which can be responsible for detrimental oocyte and embryonic developmental, ensuing on lower IVF success rates.

Study design, size, duration: We studied embryo culture media from patients presenting DIE (n = 14) and PCOS (n = 7), which are the most frequent female factors of infertility. The control group was constituted by TF (n = 6) patients. It were included in the study embryo culture media samples obtained from patients submitted to IVF cycle. Patients underwent IVF treatment as routine and oocytes were fertilized by ICSI. The embryos were group cultured until D3 and after transfer, the culture media were collected.

Participants/materials, setting, methods: For the proteomics analysis, two pools of samples were prepared for groups CONTROL and PCOS, and 4 pools for group DIE. Samples were prepared to deplete high abundant proteins and followed evaluated by high throughput proteomics approach. Raw data acquired were searched against the European Bioinformatics Institute's (EBI) universal protein resource database (UniProt) using Mascot (version 2.2). Peptide and protein validations were performed using the Scaffold platform (version 3.00.08).

Main results and the role of chance: From 2904 proteins identified, decoy proteins that are considered false identification were excluded. Two sets of analysis were carried out with remaining proteins using the Ingenuity Pathway Analysis Software. In the DIE group, 17 proteins were exclusively expressed, and two were over expressed compared to CONTROL. The canonical pathways identified were associated with calcium metabolism (calcium signaling and transport and calcium induced T –lymphocyte apoptosis) and EGF signaling. The PCOS group presented 284 proteins exclusively expressed and one overexpressed compared to CONTROL, which were associated with the following canonical pathways: Protein Kinase A signaling and calcium signaling were downregulated, and GADD45 signaling, hydrocarbon receptor signaling and GDP-L fucose biosynthesis II were upregulated. Based on proteins identified, the CONTROL group had the following cellular and molecular function highlighted: cellular development, cellular movement, amino acid metabolism, small molecule biochemistry, cellular assembly and organization, which were function associated to general cellular development. Also, the embryonic, organ and tissue development were physiological functions activated based on proteins identified in the three study groups.

Limitations, reasons for caution: We did not correlate proteins with the embryo characteristics, as samples come from group cultured embryos. Also, due to high concentration of contaminants in the culture media, samples were submitted to a number of process which might depleted other less abundant proteins. The validation of proteins found is necessary.

Wider implications of the findings: The DIE group present a high calcium activity while PCOS showed a decreased calcium action, which may be related to embryo developmental competence or plasticityEmbryonic secretome will advance our knowledge and could lead to improved selection of embryos for transfer warrants further investigation.

Trial registration number: not applicable.

P-208 Oviduct-embryo interaction in cattle: Effect of asynchrony between the embryo and the oviduct on subsequent embryo development

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Study question: Does maternal-embryonic asynchrony in the oviduct have an impact on subsequent development?

Summary answer: Asynchrony between the developing embryo and the oviduct has a negative impact on embryo survival and development.

What is known already: In cattle, which share a similar chronology of early embryo development with humans, while fertilisation of the oocyte occurs successfully in the majority of cases, a large proportion of embryos die subsequently, mostly in the first 2-3 weeks. Using state-of-the-art assisted reproduction techniques, this study aims to determine the influence of maternal-embryonic asynchrony in the oviduct on embryo development using the bovine model. Specifically, we investigated how the oviductal environment affects early development up to 8- to 16-cell stage embryos (Day 4, embryonic genome activation), blastocyst stage (Day 7, cell differentiation) and Day 15 conceptus stage (maternal recognition of pregnancy).

Study design, size, duration: Day 1 in vitro-produced bovine zygotes (IVF=Day 0) were endoscopically transferred to the oviducts of heifers (40 cross-bred beef heifers) which were either (i) synchronous with the embryos (i.e., at Day 1 after ovulation) or asynchronous and ahead of the embryo (i.e., at Day 3 after ovulation). A proportion of the heifers were slaughtered in a commercial abattoir 3, 6 and 14 days after transfer to assess embryo development at key embryo developmental stages.

Participants/materials, setting, methods: Fifty presumptive zygotes were transferred endoscopically into the oviduct ipsilateral to the corpus-luteum of heifers (n = 40, total of 2000 zygotes) which were synchronous or asynchronous with the embryo. Embryos on Day 4, 7 and 15 were recovered and

assessed; location within the reproductive tract, survival (based on morphology) and developmental stage (cell count by fluorescence microscopy or conceptus size in Day 15 embryos) were recorded. Data were analysed by Chi square and Student's t-test analysis.

Main results and the role of chance: Overall recovery rates were: Day 4 (83.5% vs 57.0%; p<0.05), Day 7 (70.0% vs 71.3%) and Day 15 (4.0% vs 12.0%) for asynchronous vs synchronous transfer, respectively. Transfer of an embryo to an advanced oviduct (asynchrony) resulted, on Day 4, in fewer embryos at the expected location (oviduct) (6.0% vs 42.2%), more degenerated (95.2% vs 74.4%) and less developed (8- to 16-cells: 9.7% vs 22.7%) embryos with a lower total cell number (10.8 ± 0.8 vs 13.7 ± 0.8) compared to synchrony group (P<0.05).

On Day 7, the recovery rate at the expected location (uterus) was lower following asynchronous transfer (86.7% vs 98.1%) with more degenerated (96.7% vs 84.1%) and less developed embryos (morula/ blastocyst stage: 14.8% vs 33.2%) compared to the synchrony group (p<0.05). However, in terms of quality the total embryo cell number was similar among asynchrony (78.3 ± 5.9) and synchrony (77.3 ± 3.6) groups.

On Day 15, only 50% of the asynchronous heifers yielded conceptuses vs 100% in the synchrony group. Day 15 conceptuses were longer following asynchronous tranfers (33.7 ± 4.4 vs 17.2 ± 1.9 mm, P <0.01), likely due to a progesterone-mediated effect in the uterus rather than an oviduct effect.

Limitations, reasons for caution: While the transfer of multiple embryos has been successfully used to study early embryo-maternal communication in the past, it should be noted that the presence of 50 embryos in the bovine oviduct is not the physiological norm.

Wider implications of the findings: Understanding how the early embryo interacts with the the oviduct will improve our knowledge of the factors regulating embryo development and survival and may lead to improved *in vitro* culture systems. Also, results may provide valuable information regarding the correct timing for embryo transfer.

Trial registration number: Project authorised by the Health Products Regulatory Authority (www.hpra.ie). Project Authorisation Number: AE18982/P120.

P-209 Laboratory and clinical outcome with embryos cultured in continuous single step media containing reduced lactate: A prospective sibling oocyte study

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Study question: Does embryo culture media with reduced lactate create any disturbances/benefits in terms of human embryo development and clinical outcome in comparison to conventional single step media?

Summary answer: Simultaneous comparison of two single step embryo culture media containing control and low lactate on sibling oocytes shows comparable and acceptable laboratory and clinical outcomes.

What is known already: Pyruvate, lactate and glucose are the primary energy substrates for human preimplantation embryos. Although its concentration greatly varies in vivo during the course of preimplantation embryo development, lactate concentration in commercially available human single step embryo culture media is usually kept between 4-10 mM. Recent studies indicate that blastocyst development and viability can be improved in environments having low lactate concentrations (<5 mM). Also, high lactate concentration in the culture media has previously been shown to change pyruvate uptake and may cause metabolic disturbances during zygote and early cleavage stages.

Study design, size, duration: This study is a prospective sibling oocyte study, performed between February – November 2017, including 1101 mII oocytes of 55 freeze-only ICSI cycles. After hyaluronidase treatment, mII oocytes of each case were equally (where available) allocated in two commercially available complete culture media, namely Continuous Single Culture Media (CSCM) and CSCM-NX (Low lactate) from the same producer (Irvine Scientific). They were individually cultured before and after vitrification/warming under 6% CO_2 and low O_2 (5%) conditions.

Participants/materials, setting, methods: Mean female age and oocytes collected per case were 31,6 ± 5,6 and 22,8 ± 6,1 respectively. A total of 546 and 555 mII sibling oocytes were inseminated by ICSI and cultured in either CSCM or CSCM-NX. In all cycles, developing embryos were cryopreserved by vitrification on either day 3 or day 5 of embryo development and subsequently warmed/cultured until ET. Fertilization, early embryo cleavage, blastocyst development as well as clinical pregnancy/delivery outcomes were compared between the groups.

Main results and the role of chance: Data regarding main laboratory KPIs such as fertilization rate, percentage of slow (<6 cells) and fast (>8 cells) developing embryos on day 3 as well as blastocyst development on day 5 were 76.9%, 19.5%, 19.0% and 44.0% for CSCM Group and 78.4%, 16.1%, 24.3% and 46.9% for embryos in CSCM-NX Group respectively. Although a slight increase in cleavage rates towards blastocyst stage was observed in CSCM-NX Group, no statistically significant differences were observed for all laboratory variables documented and compared in the current study (p>0.5). Of 55 freeze-only cycles, a subsequent FET was performed in 47 cycles by utilizing embryos from either CSCM Group (n = 21) or CSCM-NX Group (n = 26). Clinical pregnancy, ongoing pregnancy and implantation rates in each group were 66,6%, 57,1% and 64% for CSCM Group and 69,2%, 65,4% and 69,7% for CSCM-NX Group respectively.

Limitations, reasons for caution: Since this study mostly included good responder patients, our preliminary results should not be generalized for other patient groups with varying degree and spectrum of infertility indications. More studies with larger sample sizes are needed to compare these results in general patient population.

Wider implications of the findings: Lowering the lactate concentration in routine single step culture media displays similar embryo cleavage and developmental kinetics during early as well as post-compaction embryo development on embryos generated from sibling oocytes of good responder patients.

Trial registration number: None.

P-210 Spindle transfer can enhance the potential of developmentally compromised human oocytes to reach the blastocyst stage: proof of concept with donor oocytes

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Study question: Can spindle transfer represent a valuable strategy to enhance the potential of developmentally compromised human oocytes?

Summary answer: Spindle transfer can overcome embryo development arrest and enhance the potential of human oocytes with compromised quality to reach the blastocyst stage.

What is known already: Spindle transfer (ST) is a mitochondrial replacement therapy that has been proposed to prevent the transmission of maternally inherited mitochondrial diseases. In a recent study, we showed that ST could also be successfully used to overcome embryo development arrest and improve implantation rates in a mouse model with an intrinsic poor reproductive performance. The feasibility of using this technique for the purpose of treating infertility problems should be further explored, as it could offer patients with poor oocyte quality a last chance of having a child genetically related to them.

Study design, size, duration: Experiments were authorized by the Greek National Authority of Assisted Reproduction and approved by the IRB of the IASO Maternity Hospital. Informed consent was obtained from the 18 donors participating in the study, which was carried out at the Institute of Life (Athens) between June and December 2017. Experimental design aimed to compare the

efficiency of two membrane fusion methods and explore the feasibility of the technique using donor oocytes with different developmental competence.

Participants/materials, setting, methods: Micromanipulation procedures were performed on a heated stage under an inverted microscope (Olympus-IX73) equipped with an image birefringence system to confirm spindle removal. Karyoplast-cytoplast fusion was induced by exposure of the reconstructed oocytes to either a chemical solution or an inactivated protein extract (HVJ-E). Manipulated and non-manipulated control oocytes were inseminated by ICSI with a donor's sperm sample and cultured (Embryoscope$^+$, Vitrolife) in single medium (LifeGlobal) until Day6. Blastocysts were used for molecular analysis.

Main results and the role of chance: After careful optimization, enucleation of MII-oocytes was 100% in all oocytes presenting a birefringent spindle. The volume of cytoplasm removed was estimated in <1%(judged by microscopic measurements). In the first set of experiments, aimed at comparing two fusion methods, a total of 54 oocytes were used. HVJ-E-mediated fusion rates were significantly higher (98.2%) than those in the chemical method (71.7%, p<0.01). Fertilization rates were similar between the control (70%), HVJ-E (75%) and chemical-fusion (66.7%) groups. No differences (p>0.05) were found in blastocyst rates between controls (85.7%), HVJ-E (73.3%) and chemical-fusion (60.2%) groups. In the second set of experiments, ST was performed in 104 donor oocytes with differing developmental competence collected from normal and high responder donors, which included: fresh, vitrified, 'PCO-like"-morphology and in vitro matured oocytes. Overall, fertilization and blastocyst formation rates varied greatly depending on the quality of the recipient cytoplasm. When spindles were transferred from in vitro matured or PCO-like morphology oocytes into good quality cytoplasts, fertilization (66.7%-71.4%) and blastocyst (75.0%-60.0%) rates were significantly (p<0.05) improved compared to those of the corresponding non-manipulated controls (50.0%-33.3% and 0.0%-0.0%, respectively) or the reciprocally reconstructed (25.0%-0.0% and 37.5%-33.3%, respectively) oocytes. Preliminary molecular analyses from 8 ST-reconstructed blastocysts resulted in euploidy rates >60%.

Limitations, reasons for caution: Spindle transfer is a technically demanding procedure with multiple steps that need to be carefully optimized and validated to ensure its effectiveness. Genetic analyses are currently being performed to determine the aneuploidy rates and mtDNA carryover levels in a larger number of blastocysts.

Wider implications of the findings: This study shows the feasibility of ST to enhance the potential of compromised oocytes to develop up to the blastocyst stage. These promising preliminary results prompt further investigation under regulated controlled trials to confirm the effectiveness of the technique and identify groups of patients that could benefit from its application.

Trial registration number: Not applicable.

P-211 Embryonic exposure to Mono-2-ethylhexyl phthalate (MEHP) inhibits invasion and alters metabolism in mouse blastocysts

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Study question: MEHP is lipophilic, tends to accumulate in adipose tissue and disturbs energy metabolism. Here we aim to investigate the influence of MEHP on embryonic metabolism and mitochondrial function.

Summary answer: Exposing to MEHP could negatively affect embryo implantation potential and viability by inhibits invasion and alters metabolism, suggesting possible risks for female reproductive health.

What is known already: In Human studies and animal exposure experiments, there is increasing evidence showing that exposure to PAEs elicits adverse effects on female reproduction.

Study design, size, duration: A prospective RCT of experimental animal study in a university hospital.

Participants/materials, setting, methods: The 2-cell mouse embryos were cultured in HTF supplemented with MEHP at range levels (0-50 μM) for 48 h (blastocyst stage) then blastocysts were collected from each groups. The expression levels of proteins involved in antioxidant enzyme (MnSOD), implantation (MMP-2 and MMP-9) and metabolism (lipin 1 and Glut3) were validated by Immunofluorescence analysis. The Lipid droplet abundance, ATP content and mitochondrial activity also assessed by sting BODIPY and MitoTracker, respectively.

Main results and the role of chance: Lipid droplet and antioxidant enzyme (MnSOD) was significantly increased ($P< 0.05$) in blastocyst by exposure MEHP (50 μM) than control. Moreover, MEHP were up- regulation of lipin 1 expression in dose-dependent manner (in 25 and 50 μM, $P< 0.05$). Meanwhile, ATP content and MMP-9 were significantly decreased in MEHP-treated blastocysts ($P< 0.05$).

Limitations, reasons for caution: Our study was based in mouse model and further studies on implantation or birth rate are required to confirm our findings.

Wider implications of the findings: This study provided new evidence of PEAs negative impact on embryo implantation and metabolism. It may contribute to early pregnancy loss in women.

Trial registration number: NA.

P-212 Aberrant expression of ribosomal proteins results in the developmental failure of human preimplantation embryo

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Study question: To investigate the underlying molecular mechanism of 8-cell arrested embryos without chromosomal abnormalities and morphokinetic abnormalities.

Summary answer: The decreased expression of ribosomal proteins (RPs) genes activate p53 pathway by nucleolar stress, which induced human embryo arrest at 8-cell stage.

What is known already: During in vitro fertilization (IVF) treatment, about 50-70% of normally fertilized human embryos cannot form blastocyst. Chromosomal abnormalities including uniformly aneuploid, polyploid, haploid, mosaicism chaotic chromosomal complement, and morphological abnormalities, such as multinucleation, cytoplasmic fragmentation, and unequal cleavage are the main causes of early embryonic development block. However, a small number of embryos with normal chromosome and morphological features still cannot reach the blastocyst stage.

Study design, size, duration: A total of 26 in-vitro matured metaphase (MII) oocytes were collected to perform ICSI. Then 8-cell embryos with normal cleavage in the first three times cleavages were collected, and single blastomere from 8-cell embryos was biopsied for transcriptome sequencing. According to whether the 8-cell embryos form blastocyst, they were divided into two groups, the blastocyst and the arrested embryo. Test the chromosomal constitution and compare the gene expression differences between the two groups.

Participants/materials, setting, methods: Monitor the morphological features and kinetic parameters of the two groups embryos via time-lapse technology, and analyze gene expression of single blastomere from 8-cell embryos by single-cell RNA-SEQ. Trophectoderm (TE) of the blastocysts and the arrested embryos were biopsied and detected the chromosomal makeup by next-

generation sequencing. And then the remaining of blastocyst were stained by immunoflurescence for SOX17 and NANOG.

Main results and the role of chance: A total of 3 embryos were collected in the blastocyst and the arrested group, respectively. 6 embryos are high-quality embryo at day 3 which graded 8cell-I or 8cell-II, and they had normal chromosome compositions at day 5. In the blastocyst group, all embryos had a significant expansion blastocoelic cavity and inner cell mass (ICM), and the average cell number of TE and ICM are 226 and 33, respectively. In the arrested group, 3 embryos arrested at 8-cell stage, with compaction of a few of cells and embryo fragmentation.

The average of total gene number for the blastocyst and arrested group were 15785 and 13907, respectively. 420 genes of 675 differentially expressed genes (DEGs) were identified as downregulated genes, and 255 DEGs were upregulated. The majority of 420 downregulated genes had a low level of expression, except RPs. And KEGG pathway analysis showed the most apparent enriched pathway was ribosome, including 80 genes, 23 of which were RPs. Likewise, the PRAME family genes were the higher expression among the 255 upregulated genes. Meanwhile, MDM2 related to nucleolar stress was upregulated. This indicates the decreased expression of RPs induces nucleolar stress indispensable for p53 activation, resulting in human embryo arrest.

Limitations, reasons for caution: A limitation is the sample size of this study. In addition, the preliminary results of the analysis will need to be further investigated by the more arrested embryos experiment.

Wider implications of the findings: During IVF/ICSI, 8-cell high-quality embryo without morphokinetic abnormalities may not have a capable of implantation at day 3 and it is necessary to perform more studies for accurate evaluation. And that besides the primary ribosome biogenesis, ribosomal proteins could regulate early human embryo development.

Trial registration number: NA.

P-213 Editing of the coatomer protein complex subunit alpha gene in bovine blastocysts using CRISPR/Cas9

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Study question: Is CRISPR/Cas9-mediated editing of the coatomer protein complex subunit alpha (COPA) gene possible in bovine zygotes?

Summary answer: Microinjection of CRISPR/Cas9 in presumptive zygotes led to gene edited bovine blastocyst stage embryos.

What is known already: Successful genome editing to term has been accomplished in cattle by somatic cell nuclear transfer (SCNT). CRISPR/Cas9 microinjection of zygotes has been done in a limited number of attempts in ruminant species, mostly using RNA. Here we report successful editing of the COPA gene through plasmid delivery of CRISPR/Cas9 in bovine zygotes.

Study design, size, duration: Presumptive zygotes (n = 350) were microinjected with a CRISPR/Cas9 plasmid. Injected embryos were put in *in vitro* culture until blastocyst stage. At day 8, embryos were selected based on GFP fluorescence and frozen individually for sequence analysis. A separate IVF run with identical parameters was used as negative control without microinjection to compare development rates.

Participants/materials, setting, methods: Presumptive zygotes derived from slaughterhouse oocytes by *in vitro* maturation and fertilization were microinjected with the pX458 plasmid (Addgene #48138) containing a guide RNA recognition sequence for the 6th exon of the COPA gene. GFP positive structures at day 8 were selected as candidates for successful editing. Editing was assessed through T7 endonuclease assay, subsequently followed by Sanger sequencing.

Main results and the role of chance: Compared to the control (32.3%), the injected group showed a day 8 blastocyst rate of 27.7%. Fluorescence rate at day 8 was 7.6%. GFP positive blastocyst rate was 2.5%, totaling 7 blastocysts. T7 endonuclease assay revealed editing in blastocysts and morulae as well. Structures that were selected for editing through T7 assay showed a high variability of editing events neighboring the guide RNA target site. Deletions as long as 94 base pairs were found in blastocysts, as well as the absence of editing. Multiple editions found in structures as well as wild-type sequences suggest mosaic editing of blastocysts. This implies that editing of the COPA gene is possible in bovine zygotes and did not affect embryo development *in vitro*.

Limitations, reasons for caution: Editing of the COPA gene in bovine embryos is possible with plasmids but limited because of mosaic editing. Observation of genome editing without wild-type sequences persisting through development is crucial to imply that complete editing of COPA is not lethal.

Wider implications of the findings: This research sheds light on how CRISPR-Cas9 plasmid delivery in zygotes behaves and how it could circumvent cloning of edited cells. Results also indicate that the COPA gene may not be required for early embryo development.

Trial registration number: This study was funded by project number 309-70102 of the University of Bonn. The authors declare.

P-214 Effects of sperm direction (head first injection, tail first injection) in Piezo-ICSI on oocyte survival, fertilization and embryo morphokinetics in humans

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Study question: Does the sperm direction (head first injection, tail firstinjection) in Piezo-ICSI have an effect on oocytes survival, fertilization and embryo morphokinetics in humans?

Summary answer: Sperm's tail first injected oocytes showed significantly higher fertilization as compared to the sperm's head first injected oocytes, but the fertilized embryo morphokinetics were similar.

What is known already: For the application of ICSI to human oocytes, it is believed that the sperm should be injected head first into the cytoplasm for fertilization because the internalization of sperm in the cytoplasm is initiated from the sperm head during natural fertilization. However, in the ICSI procedure, sperm are directly injected, bypassing the internalization of the sperm head in the cytoplasm; accordingly, oocytes could be fertilized if the sperm is injected tail first into the cytoplasm. However, little is known about the effect of the sperm direction on ICSI results, including embryo morphokinetics.

Study design, size, duration: We retrospectively investigated 75 mature oocytes retrieved from 26 patients were microinjected from sperm's head first (H-first) and 145 mature oocytes retrieved from 40 patients were microinjected from sperm's tail first (T-first) between May 2016 and April 2017. Of these. Only metaphase II oocytes with a visible meiotic spindle at the time of ICSI were microinjected to minimize the potential influence for fertilization (Heindryckx B et al. Hum Reprod. 2011: 26: 535-44).

Participants/materials, setting, methods: The survival, fertilization and good-quality day-3 embryo rates after Piezo-ICSI by H-first injection group and tail T-first injection group were compared. In addition, the time of second polar body emission, vanishing the two pronucleus and the first cleavage of fertilized oocytes were monitored by a time-lapse microscopy system (CCM-iBIS). Times were expressed as mean ± SD hours post-insemination and analyzed, where appropriate, by Unpaired T Student or Fisher's exact tests.

Main results and the role of chance: The survival rates of H-first and T-first groups were 97% (73/75) and 99% (143/145). No significant difference was observed. The fertilization rates of H-first and T-first groups were 85% (64/75) and 95% (137/145). A significantly higher fertilization rate was achieving in T-first group (P = 0.0397). The 0PN (no pronucleus) rates of H-first and T-first groups were 11% (8/75) and 2% (3/145). A significantly lower 0PN rate was achieved in T-first group (P = 0.0087). The 1PN (mono pronucleus) rates of H-first and T-first groups were 0% (0/75) and 1% (1/145). No significant difference was observed. The 3PN (three pronucleus) rates of H-first and T-first group were 1% (1/75) and 1% (2/145). No significant difference was observed. The good quality day-3 embryo rates per microinjected oocytes of H-first and T-first groups were 52% (39/75) and 68% (98/145). A significantly higher good quality day-3 embryo rate was achieving in T-first group (P = 0.0280). Among the H-first and T-first groups, there were no significant differences when comparing the time of second polar body emission (2.6 ± 0.8 vs. 2.8 ± 0.7 h), two

pronucleus vanishing (23.0 ± 2.9 vs. 22.5 ± 2.7 h) and first cleavage (25.4 ± 2.9 vs. 25.1 ± 2.8 h) of fertilized oocytes.

Limitations, reasons for caution: The morphokinetic parameter of first cleavage assessed in this study may require better definition to reduce inter-operator annotation variability.

Wider implications of the findings: Sperm tail first injection associated with higher fertilization and good quality day-3 embryo rates as compared to sperm head first injection without detrimental effect on embryo morphokinetics. Interestingly, sperm tail first injection might be used as a new technique for human ICSI to improve the effective utilization rate of oocytes.

Trial registration number: Not applicable.

P-215 A prospective study of oocyte Zona Pellucida mechanics as a predictor of embryo implantation: a novel tool for embryo selection

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Study question: Does the zona pellucida's ability to resist shape change (shear modulus) predict embryo implantation potential?

Summary answer: Zona pellucida (ZP) shear modulus can predict implantation rate, regardless of ZP thickness, embryo diameter or patient's age.

What is known already: The ZP undergoes extensive mechanical deformation at the time of blastocyst expansion and hatching. To the best of our knowledge, no study examined the effect of mechanics properties of the ZP on embryo hatching and implantation ability. Furthermore, most studies assume that the ZP can be described as a linear elastic material and employ analytical models that mimic the oocyte deformation. This assumption may be applicable to small deformations but not to extensive ones when ICSI is performed. Although ZP hatching is at least partly a mechanical phenomenon, oocyte mechanics is not taken into consideration during embryo selection.

Study design, size, duration: A non-randomize prospective study performed during 2015 – 2017 designed to examine the deformation of the ZP during the ICSI procedure. One hundred and sixty-four oocytes were aspirated from 28 IVF patients. The patients were tested for ZP mechanical properties. Thirty nine embryos were transferred and the implantation rate was measured.

Participants/materials, setting, methods: The study was conducted at an IVF unit and the mechanical engineering department. Images from ICSI procedures were used to construct computational models using a non-linear model applicable to large deformations. The computed ZP deformation was compared to deformation observed in ICSI during oocyte suction. This comparison enabled the determination of ZP-shear modulus which represents the ability to resist shape change. Implanted embryos were compared to non-implanted embryos regarding the shear modulus using chi-square test.

Main results and the role of chance: The data show that a great variance exists in the shear modulus of oocytes aspirated from the same patient (0.0001-0.00065 Mega Pascale-MPa). A significant correlation was found between the value of the ZP shear modulus and implantation rate. The total implantation rate of the transferred embryos in the study was 30.7% (12/39). A significant difference was found when comparing implantation rate inside and outside of a specific shear modulus range (0.0002-0.0004 MPa). Ten out of the 16 (62.5%) implanted embryos, displayed shear modulus values in the specific range, with only 2 implanted embryos out of 23 were outside the range (8.7%) P = 0.037. No correlation was found between ZP shear modulus and embryo quality regarding blastomere's number and morphological index. The age of the patients inside and outside the range of the ZP shear modulus was comparable. Other mechanical properties such as the thickness of the ZP and oocyte diameter were not associated with implantation rate.

Limitations, reasons for caution: The limitations of the study: a small sample size.

Wider implications of the findings: The ZP shear modulus which is determined early at the time of sperm injection can be used as a novel method for embryo selection, consequently leading to a higher embryo implantation rate. Our methodology can be implemented with no major changes in the current ICSI procedure.

Trial registration number: 0041-14-SOR.

P-216 Human blastocyst development and implantation rate with human recombinant growth factors supplementation of culture media

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Study question: Could growth factors promote early embryonic development and implantation in IVF treatment?

Summary answer: Growth factors promote blastocyst quality; this may help choosing embryo for transfer and improve implantation chance.

What is known already: Early studies using animal models demonstrated enhanced early embryonic development and survival when culture media were supplemented with individual growth factors (GF), including insulin-like growth factor-I (IGF-I), granulocyte-macrophage colony-stimulating factor (GM-CSF), epidermal growth factor (EGF) and others. Supplementation of culture media with key autocrine/paracrine growth factors promoted the development of tripronuclear human zygotes to blastocyst, promoted blastocyst formation of day 3 cryopreserved embryos and increased the proportion of high quality blastocyst. Also, growth factors could improve normally fertilized hatching blastocysts outgrowth *in vitro*.

Study design, size, duration: Patients undergoing IVF treatment were randomly divided into two groups (Group 1, n = 44, 34.2 ± 4.4 years; Group 2, n = 58, 34.1 ± 4.0 years). All obtained two-pronuclear zygotes were cultivated without GF (group 1, 196 embryos) or with recombinant human growth factors mix (group 2, 247 embryos) to blastocyst stage. Received good and high quality embryos were transferred or cryopreserved on fifth day. Both groups treatment, cultivation and embryo transfer (ET) were used with informed consent of the patients.

Participants/materials, setting, methods: After fertilization embryos were cultivated in one-step media with human serum albumin (Global total, LifeGlobal, US) individually in 30 ml microdrops without or with IGF-I and GM-CSF mix (each at 5 ng/ml) at equal conditions (37°C, 6.5% CO_2, pH 7.2-7.25). Blastocyst were assigned scoring based on the Gardner blastocyst grading scale (AA blastocyst were scored 5; AB, BA and BB - 4; CB, BC, CC - 3; and arrested blastocyst were scored 2) for statistical analysis.

Main results and the role of chance: Group 1 and 2 has no difference in blastocyst formation (50 ± 45% and 51 ± 44% to zygotes quantity, respectively, p = 0.82) but in the group with GF high quality blastocyst proportion was significant increased (morphological score was 3.6 ± 0.9 in group 1, and 4.0 ± 0.9 in group 2, p = 0.0035). In group 1 was 42 transfers (1.38 embryo on ET) and 56 ET were in group 2 (1.46 embryo on ET) inclusion of growth factors mix showed rising trends in implantation rate without significantly differences in groups (14 ± 5% and 22 ± 5% to the number of transferred embryos, respectively, p = 0.20). The ET success was evaluated at 8-9 gestation week by ultrasound screening with a heart rate examination. Two miscarriages cases were in group 1 (one frozen pregnancy and one trisomy 22) and an anembryonic pregnancy in group 2. All miscarriages were not included in success ET cases. All samples in groups were compared by T-test for Independent Samples, and values were showed in Mean±SD format.

Limitations, reasons for caution: Although this experimental design is based on the supplementation of recombinant growth factors diluted during embryo cultivation, future studies on the potential side effects of these factors on chromosomal numbers, genomic integrity, proteomic changes, and epigenetic modifications are essential for wide clinical use.

Wider implications of the findings: Though, growth factors not influence on the blastocyst formation rate, but increase proportion of high quality embryos. Probably GF using not evaluate on blastocyst quantity, but assist to grow and choose better morphological grade blastocyst for ET, this finding could potentially increase implantation chance for every one transferred embryo.

Trial registration number: Not applicable.

P-217 The effect of changing culture media on aneuploidy rates seen in preimplantation embryos

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Study question: To examine the effect of two different embryo culture media, in the same clinic using the same reference lab, on blastocyst ploidy.

Summary answer: Aneuploidy rates vary with different culture media for specific patient populations. However, this may be a manifestation of different blastulation rates.

What is known already: Aneuploidy screening of embryos has been proposed to increase implantation rates and decrease miscarriage rates in women undergoing IVF. While maternal age is the strongest predictor of embryonic aneuploidy, these and mosaicism rates have been observed to vary between clinic and reference labs. These variations have been seen in donors and cannot be explained by maternal age alone. The embryo culture system was changed from sequential media to a single step medium in our lab in 2017.

Study design, size, duration: This was a retrospective cohort study comparing aneuploidy rates between two five month periods in a single clinical lab in which the culture system was different (Period I: Sage sequential plus 10% SPS, Period II: Sage 1-step media). All patients undergoing PGS during these periods were included, consisting of 212 patients and 465 blastocysts cultured in sequential media, and 176 patients and 476 blastocysts cultured in 1-step media.

Participants/materials, setting, methods: Patient age, blastocyst development rates and pre-implantation genetic screening results were compared between two periods during which different culture media were used. CO2 levels were adjusted to maintain the same media pH of both media and no other changes in culture SOP were made between these two periods. All biopsies were performed at the blastocyst stage on day 5-6 and sent to a single reference lab for aneuploidy testing using NGS.

Main results and the role of chance: A total of 212 patients and 465 blastocysts biopsied over a 5 month period after culture in sequential media. A total of 176 patients and the corresponding 476 blastocysts were biopsied after culture in single step media. Mosaicism rate was < 2% in all groups throughout the study period and therefore no statistical analysis was possible. There were significantly more usable blastocysts in the single step medium (Sequential Media 48 % usable Blast/2PN vs. Single Step: 56 % usable blastocysts/2PN, $p<0.05$). Euploid/Aneuploidy rates were similar between media groups for patients in age groups <30, 30-35, 36-37, 38-39. There were significantly higher euploidy rates for blastocysts cultured in single step media for patients age 40-41 (54% vs. 32%, $p<0.05$) than blastocysts cultured in sequential media, and a similar trend approaching significance for patients ≥42 (6% vs. 0%, $p<0.05$) favoring blastocysts cultured in single step media.

Limitations, reasons for caution: This studies retrospective design and the difference in blastulation rates between culture systems prevents concluding whether the media affects embryo ploidy rather than changing the number of embryos available for biopsy.

Wider implications of the findings: There was an increase in euploidy rate after culture in single step media compared to sequential media for women >40. This finding suggests that culture conditions may influence aneuploidy rates, directly or indirectly by increasing the number of blastocysts available for biopsy or other less understood mechanisms.

Trial registration number: Not applicable.

P-218 Investigating the efficacy of non-invasive pre-implantation genetic testing for aneuploidy (NI-PGT-A) using spent culture media as a source of embryonic DNA

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Study question: Are spent media samples, derived from different culture systems/ conditions, a reliable non-invasive source of embryonic DNA for the purpose of NI-PGT-A?

Summary answer: Spent media samples contain embryonic DNA but the nature and content of that DNA varies according to their corresponding culture conditions.

What is known already: Currently, analysis of DNA from trophectoderm biopsy is the gold standard for comprehensive chromosomal screening (CCS) as well as pre-implantation genetic diagnosis for monogenic disorders. However, this approach is invasive and necessitates highly skilled personnel along with expensive micromanipulators to perform the biopsies. In recent years, non-invasive sources of embryonic DNA such as blastocoel fluid and spent culture media have been explored with the ultimate aim of developing non-invasive chromosomal screening and genetic diagnosis methods.

Study design, size, duration: Standard DNA amplification methods, including Sureplex and multiple displacement amplification (MDA), along with a modified MDA method were used to explore genetic content of 84 spent culture media samples derived from either fresh embryo cultures (n = 51) or vitrified-warmed embryo cultures (n = 33).The samples were subjected to next generation sequencing (NGS) and subsequently analysed for chromosomal copy number. Results were compared to those obtained using well-established invasive methods involving trophectoderm (TE) biopsy followed by CCS.

Participants/materials, setting, methods: For spent media samples from fresh embryo cultures (n = 51), a changeover of media was performed on Day-3 post fertilisation to a new 3 µl culture droplet (Sage Quinn) and the samples were subsequently collected on Day5/6 post fertilisation. Previously vitrified blastocysts (n = 33) were warmed in ~3-5 µl of culture media and collected after overnight incubation. The samples were subjected to whole genome amplification (either Sureplex, MDA or modified MDA) and subsequently sequenced using Illumina's Miseq platform.

Main results and the role of chance: Overall, the DNA amplification rates were superior in the samples amplified with the modified MDA method compared to those amplified by Sureplex and MDA methods: 93.48% (43/46) versus 47.37% (9/19) and 15.78% (3/19) respectively ($P<0.05$). Of note, the media samples which failed to amplify mainly belonged to a cohort frozen (at -20°C) for over a month before amplification. The aneuploidy/euploidy concordance rate between spent media samples and the corresponding embryo biopsy was found to be 95.35% (42/43) for modified MDA samples, 88.8% (8/9) for Sureplex amplified samples and 33.34% (1/3) for MDA samples. There was 99.3% concordance at the individual chromosome level for modified MDA samples (over 1000 chromosomes assessed), 99.1% for Sureplex samples and 94.4% for MDA samples.

For spent media samples amplified using modified MDA, all the female embryos were correctly identified with respect to gender of the corresponding TE biopsy (female embryos concordance: 27/27; 100%). However, one male embryo was misidentified as a female embryo (male embryos concordance: 15/16; 94%).

Limitations, reasons for caution: The sample size for present study is relatively small and the results need to be verified on a larger sample population. The ploidy status of the spent media samples was compared with a TE biopsy from the corresponding embryo and not the entire embryo.

Wider implications of the findings: The results confirm that the analysis of spent media represents a promising strategy for non-invasive PGT-A, frequently yielding genetic material which is representative of the embryo when appropriately amplified. While further experiments need to be performed to verify this data on larger sample sizes, the preliminary results are encouraging.

Trial registration number: Not applicable.

P-219 Determination of c-Abl tyrosine kinase and mTERT catalytic subunit of telomerase expression level during mouse prenatal and postnatal gonadal development

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Study question: Do c-Abl tyrosine kinase and mTERT catalytic subunit play an important role in a successfull gonadal development?

Summary answer: c-Abl tyrosine kinase and mTERT telomerase catalytic subunit may have curriciale role during mouse prenatal and postnatal gonadal development.

What is known already: c-Abl is a non-receptor tyrosine kinase that plays an important role including cell adhesion, cell proliferation, growth and development. Telomerase is a ribonucleoprotein complex and a part of this complex as telomerase catalytic subunit telomerase reverse transcriptase (TERT). The maintenance of telomerase activity is necessary for the survival of proliferating cells. c-Abl and mTERT have important role in regulation of telomerase function. We aimed to compare and demonstrate the localization of c-Abl tyrosine kinase and mTERT telomerase catalytic subunit during periods of known prenatal and postnatal ovary-testis development.

Study design, size, duration: Gonads were dissected from prenatal 10.5, 11.5, 12.5 and 13.5 embryonic days and dissection was performed on days determined from different mothers. Ovary and testis tissues from postnatal days 1, 3, 5 and 9 were dissected by accepting the day when the pups were born. Additionally, 12-week old adult ovary and testis were removed from female and male mice, respectively.

Participants/materials, setting, methods: Hematoxylin-eosin staining was performed to compare ovarian and testicular morphology in prenatal and postnatal developmental periods in mice. Subsequently, localization of c-Abl and mTERT were detected by immunofluorescence staining with confocal microscope. *c-Abl* and *mTERT* expressions were detected at the mRNA level by qRT-PCR.

Main results and the role of chance: The morphological evaluation was completed with hematoxylin and eosin staining and gonadal areas were detected under a light microscope. c-Abl and mTERT protein localization were observed in germ cells and somatic cells in gonads. In the embryonic 10.5, 11.5, 12.5 and 13.5 days, c-Abl and mTERT expression were observed in germ cells and follicular cells surrounding germ cells. c-Abl expression in postnatal day 1, 3, 5, and 9-day ovaries was observed in only oocyte cytoplasm while it was observed in both oocyte and granulosa cells in adult ovaries. mTERT expression was weakly observed on days 1, 3, and 5, whereas a significant increase expression was observed in granulosa cells on day 9 in testicular tissue. c-Abl immunoreactivity detected in Sertoli cells. PN 1, 3, 5 ve 9 days. c-Abl immunoreactivity seen in Leydig cells on day 5. mTERT expression was not observed on days 1 and 3 in the testes, whereas expression was observed in Sertoli cells and Leydig cells on days 5 and 9. In the adult testis, c-Abl and mTERT expression were seen in spermatid cells found in the seminiferous tubule lumen.

Limitations, reasons for caution: Additional studies related inhibition of *c-Abl* gene expression might confirm the association between c-Abl and mTERT during gonadal development.

Wider implications of the findings: Interaction between c-Abl and mTERT genes can contribute to the explanation of gonadal developmental abnormalities.

Trial registration number: not applicable.

P-220 Elective frozen over fresh blastocyst transfer highly increases pregnancy rate in patients over 35 years-old

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Study question: Is elective frozen blastocyst transfer an advantageous strategy for patients of all ages?

Summary answer: Chance of pregnancy in female patients, over 35 years-old, is increased by performing frozen embryo transfer.

What is known already: Researchers have recently focused on understanding endometrial receptivity, providing tools that are meant to evidence the best moment to perform embryo transfer, however oocyte and embryo quality are key factors that are not to be disregarded.

Due to the success on embryo vitrification, discrepancy has been raised over the point of whether the strategy of freeze-all embryos and subsequent frozen embryo transfers are meant for all cases.

In this study, we analyze our data of the past 6 years, trying to provide an answer on which patients may benefit of a freeze-all policy and a subsequent frozen embryo transfer.

Study design, size, duration: This retrospective cohort study includes infertile patients aged 30 to 41 years old, without previous history of recurrent failure of ART (including recurrent miscarriages). Enrolments took place between January 2012 and December 2017 and cycles with oocyte donation were excluded. Patients were transferred with either 1) embryos from a fresh cycle (ET) or 2) embryos that were frozen in a freeze-all policy (FET).

Participants/materials, setting, methods: Only patients with transfer of 1 or 2 blastocysts were included. PGD cycles were excluded. Moreover, following a freeze-all policy, pregnancy rate of the first FETs was compared to the outcome of the ETs. Transfers of Top and/or good quality embryos were included in the study.

The number of patients complying with the inclusion criteria were: 391 patients in 403 ICSI cycles (ET); and 216 patients in 220 transfers of frozen embryos (FET).

Main results and the role of chance: Mean age of patients with ET or FET was similar (35.0 ± 3.9 years-old vs 35.7 ± 3.9; mean ± SD; respectively).

Pregnancy rate was 47.6% and 58.6% (ET and FET, respectively). Based on our own observations and what has been reported in literature, pregnancy rate, following ET, undergoes a progressive decline at 35 years-old, therefore our patient population was stratified in four age ranges (30-32, 33-35, 36-38, 39-41 years-old).

No differences between ET vs FET were observed among 30-32 and 33-35 groups (45.7% vs 60% and 58.0% vs% 58.5%, respectively). However, FET significantly increased pregnancy rate in the 36-38 group (68.5% vs 48.6%), non-significant in the 39-41 group (54.1% vs 41.7%). These contrasting results were attributed to the population size in the oldest group (n = 37 for FET).

This latter result led us to further re-stratify the population in two groups (30-35 & 36-41 years-old).

In the group 30-35, no difference was observed between ET and FET. However, pregnancy rate was significantly lower following ET vs FET for the 36-41 group (46.0% vs 62.6%, respectively) (odds ratio 1.97; 95% CI 1.19-3.27). This result translates into a 36% higher chance of pregnancy when choosing FET over ET for the 36-41 group.

Limitations, reasons for caution: Although the authors consider that the patient population is of optimal size, a detailed analysis of the stimulation protocol and hormonal values (estradiol and progesterone) during treatment, and its potential relation to the outcomes, should follow.

Wider implications of the findings: Considering the discrepancy on whether applying a freeze-all policy to all patients or not, our data suggests that elective frozen embryo over fresh transfer is advantageous for the older patient population (advanced maternal age); enhancing the chances to pregnancy in 36%.

Trial registration number: not applicable.

P-221 Spectroscopic characterization of Granulosa Cells correlates with oocyte fate

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Study question: Is it possible to outline the fate of an oocyte by analysing the corresponding granulosa cells?

Summary answer: By evaluating spectral Granulosa Cells biomarkers, here identified and validated, it is possible to outline the fate of the corresponding oocyte.

What is known already: In ART, oocyte selection is mainly based on the subjective observation of its morphological features; however, the success rate of this practice is still unsatisfactory.

Granulosa Cells (GCs) play a key role in regulating oocyte development and in maintaining the appropriate microenvironment for the acquisition of its competence. GCs produce estradiol and progesterone, together with essential nutrients for oocyte development.

Fourier Transform Infrared (FTIR) spectroscopy is a well-established vibrational technique, applied in life sciences for the study of the biomolecular building and composition of cells. Recently, FTIR was applied to evaluation of oocyte quality in several experimental models including human.

Study design, size, duration: The study has been conducted between March 2016 and September 2017 on 55 women undergoing a COH for IVF treatment at 9.baby, Bologna Italy. Inclusion criteria: age <40 years; no ovarian infertility diagnosis (only tubal, idiopathic and male infertility); regular ovulatory menstrual cycles (25-30 days) with FSH < 10 IU/l on day 3 of the menstrual cycle; sperm sample with a total motility count after treatment ≥ 300.000; number of oocytes retrieved ≥ 8; non-smokers.

Participants/materials, setting, methods: GCs from single-follicle aspirates were classified based on the clinical outcome of the corresponding oocyte: clinical pregnancy(A), fertilization failure(B), embryo development failure(C), and implantation failure(D). FTIR analysis provided a set of macromolecular data characterising GCs; then, feature selection procedures were applied to derive the spectral biomarker signature for different oocyte fates. ANOVA, PERMANOVA, Factorial Discriminant Analysis, FDA, and Canonical Analysis of Principal Coordinates, CAP statistical tools were applied to validate the identified spectral biomarker signature.

Main results and the role of chance: An innovative FTIRM-based method was developed to characterize GCs macromolecular fingerprint and to retrospectively relate this FTIR information to the corresponding oocyte clinical outcome. Several feature selection procedures ('Leave-one-out' method on FDA, Variable Characterization method, and Logistic Regression method; significance set P<0.0001) let identify 17 FTIR-biomarkers, singularly analyzed by univariate statistics and validated by multivariate tools.

ANOVA (P<0.05) analysis pinpointed that each experimental group showed specific macromolecular traits, ascribable to different biological and metabolic characteristics of GCs, and that not only the correct amount of macromolecule supplied by the GCs but also the quality could make the difference for the fate of the surrounding oocyte.

By applying multivariate tools, we validated the ability of the 17 FTIR-biomarkers to segregate GCs samples in the four experimental groups. FDA showed a clear separation along F1-axis (62.75% of discrimination) between GCs from oocytes able (A,Dgroups) or not (B,Cgroups) to develop into embryos; F2-axis (24.14% of discrimination) segregated the embryos that gave pregnancy (A) from those that failed implantation (D). The confusion matrix (total percentage of correctness = 80.25%) obtained from this analysis pinpointed that GCs from oocytes unable to develop into embryos (B,C) were better characterized than those from oocytes able to give viable embryos (A,D).

Limitations, reasons for caution: This study was conducted on a cohort of patients selected based on tight inclusion/exclusion criteria. Further analyses should also include elder women and/or presenting with ovarian infertility diagnosis.

Wider implications of the findings: The 17 identified and validated biomarkers could be the basis for the development of a data standardization system based on machine learning techniques, with the final scope to establish a reliable, non-invasive and objective tool to predict oocyte fate by analysing the biochemical composition of the surrounding GCs.

Trial registration number: Not requested as Basic Study.

P-222 Sperm derived from testicular sperm extraction is associated with differences in time-lapse morphokinetics of resulting human embryos

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Study question: Is there a difference in developmental kinetics between embryos resulting from intracytoplasmic sperm injection (ICSI) with testicular derived sperm compared to ejaculated sperm?

Summary answer: The first cell cycle, from pronuclear appearance to disappearance, is significantly longer in embryos fertilized by testicular sperm, whereas the second cell cycle is faster.

What is known already: Embryo selection based on time-lapse morphokinetics is a promising method to improve implantation rates and time-to-pregnancy. However, morphokinetics can be influenced by culture conditions and patient characteristics. So far, little is known about the impact of the origin of the sperm cell used for fertilization on embryo morphokinetics. One earlier study showed that embryos from testicular sperm reached the 3- and 8 cell stage significantly later than embryos from epididymal sperm. Another study showed a delayed morula formation and blastocyst hatching in embryos derived from surgically retrieved sperm compared to ejaculated sperm. The clinical relevance of these observations is unclear.

Study design, size, duration: This is a retrospective study of couples (n = 299) undergoing their first intracytoplasmic sperm injection (ICSI) cycle with frozen-thawed testicular sperm (TESE-ICSI; n = 112) or fresh ejaculated sperm (normal ICSI; n = 187) between 31 January 2012 till 4 August 2017 at the Erasmus MC. A total of 798 oocytes were injected with testicular sperm and 978 oocytes with ejaculated sperm. Only transferred and cryopreserved embryos were annotated in the TESE-ICSI group (n = 310) and ICSI group (n = 496).

Participants/materials, setting, methods: All patients underwent ovarian stimulation, ICSI, TESE-ICSI and embryo transfer according to standard protocols. After injection, fertilized oocytes were cultured in the EmbryoScopeTM time lapse incubator in either Vitrolife G-series culture media (from January 2012 to 15 November 2014) or Sage 1-step (after 17 November 2014). Transfer was performed on Day 3 and embryos were frozen on Day 4. Embryos were annotated for pronuclear appearance and –fading, and for timepoints in reaching the 2-8-cell stage.

Main results and the role of chance: Significant differences were observed between the group that underwent normal ICSI vs. TESE-ICSI in female age (median age of 33.0 vs 31.6 years; p-value 0.045) and culture medium used (55.1% vs 97.3% Sage1, 44.9% vs 2.7% Vitrolife G-series; p-value <0.001). TESE-ICSI resulted in significantly less fertilized oocytes than ejaculated sperm (respectively 57% vs 75%; p-value <0.001). Implantation and live birth rates were comparable between normal ICSI and TESE-ICSI (41.2% vs 41.1% and 24.6% vs 24.1%, respectively). A linear mixed model was used to correct for clustering of embryos from one patient and for differences in age. TESE-ICSI embryos needed more time from pronuclear appearance to disappearance compared to embryos from normal ICSI (beta 0.74 h, 95% CI [-0.07 to 1.55]; p-value 0.073). These results suggest that embryos from TESE-ICSI need more time before nuclear envelope breakdown, possibly due to repair of DNA damage or changes in epigenetic profiles. Remarkably, despite the initial delay, TESE-ICSI embryos reached the six cell stage significantly earlier (beta -2.68 h, 95% CI [-4.10 to -1.25]; p-value <0.001). Also, the interval between pronuclear fading and the 3-cell stage (t3-tPNf), was shorter (-1.25 h 95% CI [-1.97 to -0.53]; p-value 0.001), an important parameter in morphokinetic selection models.

Limitations, reasons for caution: When adding culture medium to our mixed model analysis results become less significant. More research is needed on the specific effect of culture medium on morphokinetics. The testicular sperm cells were frozen, whereas the ejaculated sperm cells were fresh. However, freezing was previously not observed to have an effect.

Wider implications of the findings: Prediction models based on time-lapse annotations become a more common tool for embryo selection. In our study TESE-ICSI embryos developed significantly faster without impacting on implantation potential. However, using the previously published KIDscore Day 3 selection model would lead to significantly more unjustly discarded embryos in the TESE-ICSI group.

Trial registration number: not applicable

P-223 Importance of multinucleation at the two-cell stage on embryo development

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Study question: To study the impact of multinucleation at the 2-cell stage in human embryos regarding development, implantation and abortion rate.

Summary answer: In our study no correlation was found between multinucleation at two-cell stage and embryo development, implantation status or abortion.

What is known already: According to literature, multinucleation is related to aneuploidies, detrimental effect on implantation and higher abortion risk. However, time-lapse technology has shown that an important number of the cultured embryos are multinucleated at the 2-cell stage. This has led to reconsider the impact of this event in normal embryo development.

Study design, size, duration: A retrospective morphokinetical analysis of 323 Known Implantation Data (KID) embryos was performed. Analysis consisted in comparing KID and abortion relative to 2-cell stage multinucleated versus non-multinucleated embryos. Here, both clinical and biochemical abortions were considered.

Participants/materials, setting, methods: We retrospectively analyzed the implantation data of 323 embryos which were cultured at MIRI-TL® incubator with Continuous Single Culture Media (Irvine®). This analysis consisted in comparing embryo implantation and abortion rates according to whether they had multinucleation in 2-cell state. Data were analyzed by Student´s t-test using SPSS 22.0 v. statistics software.

Main results and the role of chance: From the 323 KID embryos, 76 were multinucleated at the 2-cell stage (23,5%). Among them, 26 were KID+ and 50 KID- (34,2% vs. 65,8% respectively). The other 247 embryos (76,5%), showed no multinucleation, 61 were KID+ (24,7%) and 186 were KID- (75,3%). No significant differences were found between implantation status (KID+/KID-) of multinucleated and non-multinucleated embryos. Regarding abortion, no significant statistical differences were found between multinucleated (26,9%) and no multinucleated (27,9%) 2-cell embryos.

Limitations, reasons for caution: It would be necessary to increase the number of analyzed embryos to confirm this hypothesis.

Wider implications of the findings: According to these results it is reasonable to consider multinucleation at the two-cell stage as not informative or predictive for implantation potential or abortion risk.

Trial registration number: No

P-224 Lipid and antioxidant supplements improve cryotolerance and embryonic quality of Metaphase II mouse oocytes undergoing vitrification

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Study question: May lipid and antioxidant added to vitrification media improve cryotolerance and embryonic quality of blastocyst originating from metaphse II (MII) mouse oocytes that underwent vitrification?

Summary answer: Short-term exposure of MII mouse oocytes to lipid and antioxidant biomolecules during vitrification safeguards blastocyst inner cell mass (ICM), which improve safety of oocyte cryopreservation.

What is known already: Clinical application of oocyte vitrification was approved only in 2013. Preservation of oocytes by vitrification had significantly higher survival rate, clinical pregnancy, and implantation rates than slow freezing. Although recently there has been a consistent improvement in efficiency of vitrification with live birth rates similar to fresh oocytes, safety remains obscure. The scientific literature anticipates that lipids, serum derivatives and antioxidants added to sperm freeze medium have improved sperm viability and pregnancy rates. However, the state-of-the-art does not yet contemplate the development of new cryoprotective media for human oocytes containing lipids and antioxidants with greater biosafety and efficacy.

Study design, size, duration: The total number of vitrified MII oocytes was 484. Three cryoprotectant formulations were investigated: Tvitri-4 (control/standard vitrification media n = 127), Tvitri-4-AX (T4-Antioxidants n = 133), Tvitri-4-LIPID-AX (T4-LIPIDS-Antioxidants n = 111) and Tvitri-4-LIPID-AX-PL (T4-LIPIDS-Antioxidants-Phospholipids n = 113). IVF was performed with oocytes surviving the thawing process: CT (191), Tvitri-4 (127), Tvitri-4-AX (129), Tvitri-4-LIPID-AX (107) and Tvitri-4-LIPID-AX-PL (109) and blastocysts rates were analyzed. ICM cells, TE (trophectoderm) and total cells were compared from a total of 20 blastocysts per group.

Participants/materials, setting, methods: Females from 8-12-week-old C57Bl/6 were submitted to superovulation with pregnant mare's serum gonadotropin and human chorionic gonadotropin (hCG) for obtaining of *in vivo* matured oocytes. The oocytes were distributed in following groups: Fresh control; Tvitri-4; Tvitri-4-AX; Tvitri-4-LIPID-AX and Tvitri-4-LIPID-AX-PL. After devitrification, oocyte survival rates were observed and in vitro fertilization was performed. Blastocyst rates and embryonic quality evaluated by ICM, TE and total cell number were compared among groups using ANOVA and Tukey's multiple comparisons.

Main results and the role of chance: The blastocyst formation rate from CT oocytes was 58%. The survival rates ($p = 0.2275$) and blastocyst rates (0.4274) of the vitrified eggs in Tvitri-4, Tvitri-4-AX, Tvitri-4-LIPID-AX, Tvitri-4-AX-LIPID-PL were 100% and 48%, 96.7 and 47.4%, 96 and 57% and 96.9 and 51.3%, respectively. The number of ICM cells, TE and total cells of the blastocysts were: CT (19.5 ± 3.48, 51.45 ± 6.56, 70.95 ± 9.63), Tvitri-4 (14.85 ± 2.28, 33.0 ± 5.78, 47.85 ± 6.76), Tvitri-4-AX (16.05 ± 4.11, 34.7 ± 8.01, 50.75 ± 11.4), Tvitri-4-LIPID-AX (17.85 ± 3.95, 34.95 ± 7.48, 52.80 ± 9.78), Tvitri-4-LIPID-AX-PL (17.80 ± 3.69, 35.6 ± 7.11, 53.4 ± 9.75). The mean number of ICM cells was similar among CT and lipid plus antioxidant-supplemented media (Tvitri-4-LIPID-AX, and Tvitri-4-LIPID-AX-PL groups) ($p>0.05$). Inversely, ICM cells of CT were higher than Tvitri-4 and Tvitri-4-AX ($p = 0.0009$). Regarding the number of TE and total cells, blastocyst from CT have better parameters than others experimental groups ($p<0.0001$).

Limitations, reasons for caution: The clinical applicability of these findings is challenged by the small sample size but positively affected by highly consistent and reproducible in vitro outcomes, with excellent survival of vitrified-thawed oocytes and blastocyst rates in a mouse model widely used in pre-clinical studies and quality control in ART laboratory.

Wider implications of the findings: Adequate lipid supplementation during vitrification seems to be crucial to embryo development of cryopreserved oocytes. The primary endpoints were concluded and reinforced the potential for efficacy and safety of the new cryoprotectant formulation containing lipid and antioxidant biomolecules, which would have clinical applicability by improving the safety of this technique.

Trial registration number: Ethics committee on the use of animals - Medical School of Ribeirão Preto - University of São Paulo - 233/2014

P-225 The proportion of immature/compromised oocytes impairs ooplasmic maturity of the mature cohort retrieved

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Study question: Can the performance of the retrieved mature oocytes be influenced by the fraction of immature and/or compromised cohort retrieved?

Summary answer: An increasing share of immature/compromised oocytes among the retrieved cohort may foretell embryo developmental competence and subsequent live birth rate.

What is known already: The achievement of complete oocyte maturation is a prerequisite to accomplish fertilization ability and yield conceptuses capable of implanting. The meiotic progression and ooplasmic maturity are integral events allowing the attainment of oocyte competence. While oocyte nuclear maturity is assessed by the extrusion of the first polar body, indeed ooplasmic readiness is a subtle phenomenon that has been known for some time but has still been poorly detected.

Study design, size, duration: Couples treated by ICSI between 1993 and 2017 involving young female partners (≤ 35 years old) and men with adequate semen parameters were included. Cycles were divided into four groups according to the proportion of MII oocytes at time of retrieval: *Optimal* (76-100%), *adequate* (51-75%), *partial* (26-50%), and *minimal* (0-25%). Ovarian stimulation and ICSI were performed in a standard fashion. Fertilization and pregnancy characteristics was compared between the four groups.

Participants/materials, setting, methods: The average maternal age was 31.9 ± 3 years while paternal age was 35.1 ± 5 years. A total of 8,861 ICSI cycles in which 87,029 MII oocytes were injected using ejaculated spermatozoa were included. T-test was used to compare the mean values of the number of injected oocytes. Chi-squared test was used to compare the percentages of categorical variables.

Main results and the role of chance: Cycles were allocated into the following four oocyte maturity ratio groups: *optimal (*n = 5516), *adequate* (n = 2599), *partial (*n = 635), and *minimal* maturation (n = 111). Maternal age was comparable among the four groups (31.9 ± 2, 31.9 ± 2, 32.0 ± 2, and 31.8 ± 2 years, respectively). The mean numbers of injected oocytes were 11, 8.9, 4.8, and 1.4, respectively ($P<0.0001$). The decreasing proportion of MII oocytes was associated with a significant increase of germinal vesicle (GV) from 5.5% to 48% ($P<0.0001$). The number of degenerated oocytes at time of retrieval increased from 1% to 10.3% ($P<0.0001$). Consequently, the minimal group presented the lowest rates of normal fertilization (2-pronuclei) (78.6% to 63.2%, $P<0.0001$) and corresponding highest rates of abnormal fertilization (digynic 3-pronuclei) (2.7% in the *optimal* group versus 4.3% in the *minimal* group, $P = 0.03$). The clinical pregnancy rates (52.2 to 32.0%, $P<0.0001$), and live birth rates (49.1% to 30.0%, $P<0.0001$) were also affected by the decreasing proportion of MII oocytes among the total cohort retrieved.

Limitations, reasons for caution: To control for the confounding effect of oocyte aneuploidy, this study only included young female partners. In this study, while we prove the obvious synergy between nuclear and ooplasmic maturity required for optimal outcomes, we did not provide a direct marker of the latter but only an indirect evidence.

Wider implications of the findings: This study indicates that cytoplasmic component needs to complement nuclear maturity to achieve successful ART outcomes. It provides guidance toward superovulation protocols aimed at achieving lower proportion of MI, GV, and degenerated oocytes in order to yield a higher fraction of more competent MII sibling oocytes.

Trial registration number: N/A.

P-226 Era of frozen blastocyst embryo transfer might replace that of fresh blastocyst embryo transfer—A large retrospective cohort study

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Study question: Does frozen blastocyst transfer have a better clinical outcome compared to fresh blastocyst transfer in all patients undergoing IVF/ICSI treatment across all age groups?

Summary answer: This study demonstrates that frozen blastocyst on (day 5/6) compared to a fresh blastocysts transfer resulted in significantly better pregnancy outcome irrespective of age.

What is known already: Elective frozen-embryo transfer has been shown to result in a higher live-birth rate than fresh-embryo transfer among anovulatory women with the polycystic ovary syndrome. The hormonal effect of ovarian stimulation on the endometrial lining has been attributed as one of the possible causes for the lower pregnancy rates during fresh transfer in this subcategory of patients. Pregnancy and perinatal outcomes of frozen embryos have shown to have similar outcome to that of fresh in women. In addition, Day 5 blastocyst transfer result in a better pregnancy rates compared to day 6 blastocyst transfers.

Study design, size, duration: Retrospective single centre cohort study evaluating the pregnancy outcome of total of 4049 blastocyst embryo transfers (fresh and frozen D5/6) between January 2014 to December 2017, was performed. During that period 2272 frozen embryo transfers and 1777 fresh embryo transfers were done.

Participants/materials, setting, methods: Patients undergoing fresh and frozen blastocyst transfers were included. Study was conducted at a single UK centre, CRGH London. Clinical pregnancy rates were calculated for fresh day 5, fresh day 6, frozen day 5 and frozen day 6 blastocyst transfers from January 2014 to December 2017. Live birth rates for the above categories were also calculated from January 2014 to December 2016. Results were compared. SPSS version 23 was used for analysis.

Main results and the role of chance: Age was normally distributed and comparable between fresh day 5 and frozen day 5 transfer (p = 0.095). It was also comparable between fresh day 6 and frozen day 6 transfers (p = 0.622). The number of embryos transferred per cycle was significantly more in the fresh compared to the frozen embryo transfers irrespective if the blastocyst was day 5 (1.6 ± 0.5 vs 1.3 ± 0.4, p = 0.000) or day 6 (1.8 ± 0.5 vs 1.3 ± 0.48, p = 0.000) respectively. Pregnancy rates were significantly higher for frozen embryo transfers compared to fresh transfers irrespective if the blastocyst was day 5 (65.8% vs 62.9%, p = 0.048) or day 6 (48.5% vs 35.2%, p = 0.011). Similarly, better outcome was seen in the clinical pregnancy rates for frozen compared to fresh transfers. Clinical pregnancy rates for day 5 frozen vs fresh (61.8% vs 58.1 %, p = 0.027) and those for day 6 transfers (45.1% vs 34.1%, p = 0.039) irrespectively. Live birth rate was significantly higher with frozen embryos compared to fresh embryos irrespective if the blastocyst was day 5 or 6. Live birth rate for day 5 frozen vs fresh blastocyst (51.1% vs 49.2%). Live birth rates for day 6 transfer frozen vs fresh (33.8% vs 31.1%).

Limitations, reasons for caution: Embryo quality was not analysed to check if there is any difference between the grade of the frozen vs the fresh embryos which could have attributed to the better pregnancy outcome in the frozen group.

Wider implications of the findings: This is the first large prospective study to compare outcome of frozen blastocyst with fresh blastocyst transfer relating to the day of blastocyst development. The results are very reassuring suggesting a possible benefit of freeze all policy at the blastocyst stage and transfer embryos on a frozen cycle.

Trial registration number: Not applicable.

P-227 Is Day 3 (D3) Laser Assisted Hatching Better than Day 1 (D1) Laser Assisted Hatching in PGS/PGD Cycles?

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Study question: The goal of this study was to evaluate whether there is a difference in the blastocyst development rate between embryos that were hatched using a laser on D1 versus D3.

Summary answer: There was no statistically significant difference in the blastocyst rate between the 2PN hatched on D1 versus cleavage stage embryos hatched on D3.

What is known already: D3 assisted hatching of some patients' embryos in non-PGS/PGD cycles is standard in embryology practice. However, due to an increase in blastocyst biopsy cycles, our lab adopted the policy to hatch on D3 to enhance the trophectoderm hatching potential on day 5 for easier biopsy.

Study design, size, duration: This study was carried out between May and October 2017 at Stanford Fertility and Reproductive Health, USA. Total of 261 2PN from 24 patients were prospectively randomized to be hatched on D1 or D3.

Participants/materials, setting, methods: Total of 130 2PN on D1 and 131 embryos on D3 were hatched using a 1460 nm, 300 mW diode laser. The hatching was performed on the outer edge of the zona pellucida using only a single 470-µs pulse. The embryos were cultured in the same single step medium from day 1 to day 6.

Main results and the role of chance: There was no significant difference in the blastocyst rates between the two groups (p > 0.05). There were a total of

123 and 122 embryos ≥5 cell grade 2 on D3 in the cohorts that were hatched on D1 and D3, respectively. Blastocyst rate was 66.9% (87 total blastocysts, with 37 on day 5) and 61% (80 total blastocysts, with 41 on day 5) with a grade ≥3BB in the D1 and D3 cohorts, respectively. Fisher's exact test with two tails was used.

Limitations, reasons for caution: This was a pilot study. IVF and ICSI were not equal as 20% 2PN that were hatched on D1 and 22% embryos that were hatched on D3 came from IVF. The study is limited to laser assisted hatching only.

Wider implications of the findings: By hatching on D1, embryos for PGS/PGD cases can be cultured uninterrupted in the incubator until the blastocyst stage to harness the full potential of single step culture media. This is especially useful for clinics that do not use time-lapse technologies.

Trial registration number: NA.

P-228 Oocytes cryoconservation and reproductive outcome: evaluation of embryo metabolomiic profiles

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Study question: Does oocyte cryopreservation change the metabolism of the embryo obtained?

Summary answer: Oocyte cryopreservation determines a greater activation of embryo metabolism.

What is known already: The oocytes vitrification is successfully applied to maximize the safety and efficacy of ovarian stimulation cycles in an IVF treatment, proving to be an effective approach also for the preserving female fertility.Oocyte vitrification showed a fertilization and implantation rates comparable to those of fresh oocytes, even if it is lack the possible effects of vitrification on metabolic status of the developed embryos. Recent data prove that the culture media of embryos that were positive or negative for successful implantation showed specific metabolomic signatures. However,there is some weak evidence that metabolomics technologies studying culture media of embryo developed from vitrified oocytes.

Study design, size, duration: Spent embryo culture media (ECM) were collected on day 3 before transfer from April 2017 to November 2017

Participants/materials, setting, methods: The control group was the ECM obtained from fresh oocyte (n = 9).The study group was composed of culture media of embryos developed from vitrified oocytes (n = 7). The oocyte vitrification-thawing was conducted in accord to the manufacturer's instructions. After the embryos were removed from the culture, the spent culture media were collected and we analyzed metabolomic profile by ^{1}H-NMR. Embryo quality was assessed by Gardner morphological classification. The data were analyzed using the unpaired Student's t-test (p-value≤0.05)

Main results and the role of chance: The IVF outcomes (fertilization, embryo quality and implantation rate) were statistically significant less in study group compared to control group. Our ^{1}H-NMR analysis evidenced that in culture media of embryos developed from vitrified oocytes the levels of formate, albumin, H-Cα lactate, H-Cβ lactate, glucose and endotoxin were lower in comparison to embryo obtained from fresh oocyte. The reduced embryo quality observed in study group can be certainly correlated to the different levels of these molecules.

Limitations, reasons for caution: Study limitation is the low number of samples analyzed.

Wider implications of the findings: Our study showed that quantitative and qualitative modifications of metabolites were linked to embryo development. We can suggest to integrate embryo analysis through the study of metabolomic parameters on culture medium. Detecting embryo with greater capacity for development and implantation will increase the oocyte cryopreservation success.

Trial registration number: None.

P-229 Can the distribution of morphokinetic parameters help predict pregnancy viability in women undergoing In-Vitro Fertilization/Single Embryo Transfer (IVF/SET)? A time lapse (TL) study

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Study question: Is there an association between the distribution of early- and/or late-morphokinetic parameters (as assessed by TL-microscopy) and pregnancy viability following IVF/SET?

Summary answer: Neither early- nor late-morphokinetic parameters predicted pregnancy viability in IVF/SET cycles. However, most embryos successfully implanting reached early blastulation within a defined time range.

What is known already: TL-monitoring is a novel tool widely used to predict an embryo's developmental and implantation potential. Several morphokinetic parameters, either static and/or interval, in early and/or late stages of embryonic development have been assessed as predictors of reproductive outcomes and appear promising. However, it remains unclear whether blastocysts resulting in successful pregnancies differ in all or some of these morphokinetic parameters from those of morphologically normal embryos that fail to implant or establish a viable pregnancy.

Study design, size, duration: Retrospective cohort of 191 women undergoing IVF/SET at a major academic center between 9/2013 and 4/2016. Women with embryos cultured to the blastocyst stage in a TL-monitored incubator and selected for fresh SET were eligible. Embryos resulting in viable pregnancies (progressing to ≥20 weeks gestation) were compared to those from unsuccessful transfers in regards to the distribution of certain early- and late-morphokinetic parameters.

Participants/materials, setting, methods: The primary outcome was time-period from pronuclei fading (tPNf) to early-blastulation (P_{SB}). Secondary outcomes were time-periods from i) tPNf to 1st-cytokinesis (P_1), ii) 2- to 3-cells (P_2), iii) 3- to 4-cells (P_3), iv) 4- to 5-cells (P_4), and v) 5- to 8-cells (P_8).

Statistics: Mann-Whitney U-, χ^2-tests, multiple logistic-regression to calculate odds ratio (OR), 95% confidence interval (95%CI) controlling for potential confounders (maternal age, body mass index (BMI), basal-FSH, fertilization method).

Main results and the role of chance: Groups did not differ in baseline characteristics (basal FSH, AMH, antral follicle counts). In 58.1% of cycles ICSI was utilized.

Following IVF/SET, 58.1% (111/191) of the patients conceived a viable pregnancy. When all embryos were included in the analysis and morphokinetic parameters from viable-pregnancies were compared to those from unsuccessful transfers in regard to P_{SB} distribution, no difference was noted [median (95% range): 68.78(59.78-77.81) vs. 69.26(59.28-79.81), p = 0.43; for viable pregnancy vs. unsuccessful SETs, respectively). Similarly, none of the remaining morphokinetic parameters' distributions differed between groups [median (95% range): 2.53(1.93-3.24) vs. 2.50(1.83-3.34), p = 0.42; 10.97 (2.94-12.67) vs. 10.97(2.11-13.40), p = 0.81; 0.69(0.00-8.51) vs. 0.58(0.00-8.32) hours, p = 0.18; 11.62(0.68-14.81) vs. 11.58(2.20-14.71), p = 0.72; and 4.34(0.83-18.55) vs. 4.67(1.28-15.93) hours, p = 0.71] for P_1, P_2, P_3, P_4 and P_8, respectively].

However, for the vast majority (80%) of embryos resulting in viable pregnancies, the time to reach early blastulation ranged between 61.96-75.72 hours. When P_{SB} remained in that range, the odds of a viable pregnancy were increased by 166% overP_{SB} durations slower or faster than that [adjusted OR (95%CI): 2.66 (1.17, 6.04); p = 0.02]. No other time period affected the odds of achieving a viable pregnancy.

Limitations, reasons for caution: The study was limited by its retrospective design. Moreover, only morphokinetic parameters thought to predict blastocyst formation were studied and algorithms taking into consideration standard morphologic criteria and/or genetic composition of embryo were not included.

Wider implications of the findings: Among women undergoing IVF/SET, no single morphokinetic parameter predicted pregnancy. However, the odds for a viable pregnancy were significantly higher among embryos that reached early blastulation within a defined time range. Additional studies taking into consideration morphologic criteria and ploidy status are needed to develop algorithms helpful in embryo selection.

Trial registration number: Not applicable.

P-230 Simple embryo selection for vitrified-warmed blastocyst transfer cycles: blastocyst derived from 4-cell embryo at 40-42 hours after insemination has the potential to improve clinical outcomes

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Study question: Does blastocyst derived from 4-cell embryo at 40-42 hours after insemination (Bla-4c) has the potential to improve clinical outcomes in vitrified-warmed blastocyst transfer cycles?

Summary answer: Bla-4c has significantly higher clinical outcomes compared to the blastocyst derived from non-4-cell embryo at 40-42 hours after insemination (Bla-n4c).

What is known already: The number of cells is the morphological marker strongly associated with implantation potential. Optimal results are achieved when 4-cell embryos are transferred on day 2. Implantation potential decreases when the number of cells is other than four. Developments of extended embryo culture and cryopreservation techniques have allowed IVF programs to offer patients the chances of embryo transfer without ovum-pick-up.

Study design, size, duration: A total of 880 vitrified-warmed blastocyst transfer cycles were retrospectively analyzed from June 2014 to September 2017. The cycles with frozen sperm, surgical retrieved sperm, genetic diagnosis, oocyte donation, poor responder, and advanced maternal age (≥38 years) were excluded.

Participants/materials, setting, methods: Cycles were divided into 2 groups based on the origin of blastocyst (Bla-4c, n = 336 vs. Bla-n4c, n = 544). 4-cell stage embryos were separated from non-4-cell stage embryos at 40-42 hours after insemination and culture them to blastocyst stage. Vitrification was performed for cryopreservation of blastocyst. The quality of warmed blastocyst was evaluated by Gardner and Schoolcraft's classification. The number of embryos transferred was less than or equal to two.

Main results and the role of chance: There were no differences between Bla-4c and Bla-n4c regarding female age (33.1 ± 2.3 vs. 33.4 ± 2.5, p = 0.136), the number of embryos transferred (1.5 ± 0.5 vs. 1.5 ± 0.5, p = 0.499), and abortion rate (14.4% vs. 18.5%, p = 0.354). However, Bla-4c achieved significantly higher rates of survival (99.2% vs. 97.5%, p = 0.022), good quality (≥BB) embryos (66.1% vs. 47.1%, p = 0.000), biochemical pregnancy (47.9% vs. 41.0%, p = 0.044), clinical pregnancy (39.3% vs. 28.9%, p = 0.001), ongoing pregnancy (33.6% vs. 23.5%, p = 0.001), and implantation (31.5% vs. 24.1%, p = 0.004) than those of Bla-n4c.

Limitations, reasons for caution: This study was based on static observation. The assessment of 4-cell embryo development was done at given time point (40-42 hours after insemination). Further studies are required prospectively to confirm our findings with respect to the rates of live birth outcomes.

Wider implications of the findings: Pre-selection of 4-cell embryo at 40-42 hours after insemination can eliminate inappropriately developed embryo and increase the chance to transfer appropriately developed embryos to the patients. Bla-4c has the potential to improve clinical outcomes in vitrified-warmed blastocyst transfer cycles.

Trial registration number: not applicable.

P-231 Non-invasive biomarkers for embryo quality in sterile patients

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Study question: To improve reproductive prognosis in sterile patients subjected to IVF, especially in endometriosis, by means of measuring miRNAs in CCs (Cumulus Cells) through quantitative RT-PCR.

Summary answer: For the first time, miRNAs expression has been measured in CCs endometriosis patients, where snU6 has been found downregulated compared to control group.

What is known already: Considering the need of selecting embryos with the highest implantation potential, morphological selection could be improved with the use of new methodologies. Among them, cumulus cells transcriptomics is a less invasive technique, since the analysis is not performed to the embryo but to cells removed prior to ICSI procedure. To our knowledge, there is no previous study of miRNAs expression in CCs of endometriosis patients. However, there are previous results of CCs miRNA profile sequencing. To notice is report by Tong et al., who using RNA-seq and validation by RT-qPCR identified hsa-miR-16-5p, hsa-miR-26a-5p and hsa-miR-101-3p in CCs.

Study design, size, duration: This preliminary work retrospectively analyzed miRNA expression of single-oocyte CCs from 34 patients undergoing ICSI and single embryo transfer, from 2015 until 2016. Exclusion criteria were Polycystic Ovary Syndrome, severe male factor and patients without Known Implantation Data (KID). Only CCs corresponding to the transferred embryo were included. Four clinical groups were defined according to endometriosis and KID. Control group was KID+ without endometriosis.

Participants/materials, setting, methods: From 34 total patients, 6 suffered from endometriosis and 28 did not. From endometriosis, 2 were KID+ and 4 KID-. From non-endometriosis group, 15 were KID+ (control group) and 13 KID-. The expression of 6 miRNA plus presumptive normalizer (snU6) was measured in CCs by rt-qPCR: hsa-miR-101-5p, hsa-miR-223-5p, hsa-miR-16-5p, hsa-miR-26b-5p, hsa-miR-124-3p, hsa-miR-335-5p. They were measured from microdrops where CCs were contained when total RNA was isolated. RefFinder software was used to select normalizer.

Main results and the role of chance: miRNAs of study were quantified below detection limit in blank culture media where CCs had been collected and stored, so they did not interfere in our results. 4 miRNAs and snU6 were successfully detected, while 2 did not (miRNA-101-5p and miRNA-124-3p). RefFinder output pointed at miR-26b-5p as better normalizer than snU6. The robustness of miR-26b-5p expression in our samples could be liked to its target, which is a cell cycle gene. When comparing miRNAs fold-change values between KID+ and KID- groups, no statistical differences were found. However, when we compared miRNAs expression data in endometriosis vs. non-endometriosis patients, snU6 was statistically significant ($p<0,05$). Allegra and colleagues are the unique reference of CCs transcriptome in endometriosis context, where they found genes related to inflammation, such as TNF-α, down-regulated in endometriosis. Interestingly, snU6 serum levels have been significantly correlated with established serum markers of inflammation, such as TNF-α, suggesting snU6 is specifically downregulated in inflammatory diseases (Benz et al., 2013). Surprisingly, in our study snU6 levels were lower in CCs of endometriosis patients, pointing that snU6 is also downregulated here. In line with that, exacerbated inflammation has been postulated to play an important role in endometriosis (Stilley et al., 2012).

Limitations, reasons for caution: Among limitations is reduced sample size. This is a frequent situation in IVF units performing less than 500 cycles per year, since samples are considerable reduced when non-KID cases are discarded. Another limitation is the methodology for selecting miRNAs of study.

Wider implications of the findings: For future insights, we consider using single-cell technology in CCs with a larger number of patients, including endometriosis. It is important to continue trying to improve reproductive chances of

these type of patients. Also, it would be interesting to validate miR-26-5p as normalizer gene for RT-qPCR in CCs.

Trial registration number: not-applicable

P-232 Single embryo transfers in frozen-thawed cycles may help to lower twin pregnancies and complications when compared to double embryo transfers without compromising outcomes

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Study question: Is the cumulative live birth rate of subsequent frozen-thawed cycles similar to that of double embryo transfers?

Summary answer: The cumulative pregnancy rate of patients with a potential OHSS risk is not compromised when good or top-quality blastocysts are transferred in FET cycles.

What is known already: IVF success has generally been measured based on live birth rates after a single episode of treatment resulting in the transfer of a first fresh or frozen thawed embryo. This fails to capture the real chance of having a baby after a number of complete cycles-each involving the replacement of remaining frozen-thawed embryos. Therefore, cumulative rate of live birth is more important since it better summarizes the chance of a live birth over an entire treatment period.

Study design, size, duration: This retrospective cohort study includes patients whose blastocysts were "all-frozen" between January 2015 and January 2016. The cumulative pregnancy rate was calculated for all patients whose live birth data was available until October 2017. For comparison, a patient group under 35 who have received a double blastocyst in a FET cycle in the same period were included in the analysis.

Participants/materials, setting, methods: The cumulative pregnancy rate was calculated by dividing the total number of births by the number of fresh cycles. The mean female age in the elective single embryo transfer (eSET; n = 486) group and in the double embryo transfer (DET; n = 457) group was 29.7 and 30.3, respectively.

Main results and the role of chance: The live birth data was available for 427 out of 486 patients who came to our clinic for a FET cycle and whose blastocysts were frozen because of a potential OHSS risk. 45.2% of first FET cycles (n = 193) resulted in a live birth. From the patients who did not get pregnant in their first FET and came for a second FET (n = 225) 39 resulted in a live birth (17.3%). When outcomes were calculated cumulatively, with a relative increase of 20.1%, the cumulative live birth rate of the eSET group has been calculated as 54.3%. The live birth rate of the DET group which has been transferred in the same period, was 55.1%. The statistical comparison of the live births of both groups gives a non-significant p value. Moreover, the multiple pregnancy rate was 1.2% and 38.5% in the cumulative eSET and DET groups, respectively ($p<0.0001$). When term pregnancies were analyzed, 84% of singleton pregnancies were found to be delivered after the 37th week of pregnancy whereas only 29.4% of twin pregnancies achieved term ($p<0.0001$).

Limitations, reasons for caution: Cumulative pregnancy outcomes are calculated as the number of pregnancies per initiated cycle, therefore the rate vary depending on patients who come forward to use their remaining frozen embryos over time.

Wider implications of the findings: In recent years, improvements in laboratory conditions and embryo-endometrium synchronization have resulted in higher pregnancy rates with the transfer of blastocyst-stage embryos in FET cycles. Therefore, a cumulative eSET strategy is advisable with the additional benefit of reducing multiple pregnancy risks and consequently neonatal complications.

Trial registration number: Not applicable.

P-234 Can assisted reproduction technologies decide unintentionally the sex of the babies?

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Study question: The main objective is evaluating the possible effect of assisted reproductive technologies (ARTs) on the sex ratio of the offspring.

Summary answer: We found statistical significance in sex ratio when transferring embryos at blastocyst stage, while there is no correlation when embryos are transferred at cleavage stage.

What is known already: Sex ratio calculated as male proportion is 1.7 times higher than female under natural conception. However, at birth, this sex ratio diminishes to 1.05 caused by abortion and other reasons. Under in vitro conditions this proportion could be modified. Studies using preimplantation genetic diagnosis (PGD) shown sex ratio at cleavage stage embryos is lower than those at blastocyst stage. In addition, sex ratio at birth was significantly higher in IVF compared to ICSI embryos. Recently, a group of researchers found correlation between male's BMI during treatment and higher probability of giving male singletons.

Study design, size, duration: This retrospective study included all live birth from IVF cycles between 2005 and 2017. During this period 399 babies were born from IVF or ICSI fresh and frozen cycles.

Participants/materials, setting, methods: A total of 333 patients were included in our study. All the analysis was carried out with Statistical Package for the Social Science software (SPSS version 21.0). Pearson χ^2 test was used to compare the distribution of categorical variables in different groups. We compare sex ratio at birth with the day of embryo transfer (Cleavage-stage or blastocyst-stage) in singletons and twins, the ART employed (conventional IVF or ICSI) and embryos from fresh or frozen cycle.

Main results and the role of chance: When we compare the sex ratio with the day of embryo transfer we found statistically significant differences towards the males when we transfer the embryos at blastocyst stage (2.57). At cleavage-stage the ratio at birth is maintained at 1.08 towards males, very similar to natural conditions (1.05). However, we did not find any association between sex ratio and ART employed nor the fresh/ frozen groups.

Our results suggested that the day of embryo transfer is directly related with the sex ratio at birth towards males. Blastocyst embryos are selected because male embryos grow faster than female embryos. Furthermore, there is a higher mortality in female embryos that may be caused by inactivation of one of the X chromosomes. Our findings agree to other investigators results. The transfer of Blastocyst-stage embryos seems to be directly related with sex ratio at birth both singletons and twins.

Limitations, reasons for caution: This study had also limitations. The number of cases included in IVF group is lower than ICSI group. Furthermore, we do not know if our twins are monozygotic or dizygotic twins.

Wider implications of the findings: Since ARTs landscape is increasingly moving to day 5 transfer, this result should be considered, so we could be unintentionally selecting embryos towards male children. In that line, blastocyst selection criteria may be revised.

Trial registration number: Not applicable.

P-235 Exploring the use of mid-infrared spectroscopy to detect development and implantation biomarkers in spent culture media of human embryos

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Study question: Can the mid-infrared spectra of spent embryo culture media predict the ability of an embryo to develop into blastocyst and implant?

Summary answer: Distinct spectra features have been observed in the metabolomic profile of media of embryos that developed into good quality blastocyst compared to those that arrested.

What is known already: There is great interest in detecting a biomarker with enough sensitivity and specificity to predict embryo development and implantation potential in order to select the best embryo from a cohort, for a single embryo transfer, reducing time to pregnancy and the economic and psychological burden of unsuccessful attempts. Approaches like single metabolite quantification by mean of high performance liquid chromatography and less

informative near-infrared spectroscopy haven't provided a reliable predictor. Mid-infrared spectroscopy is a powerful tool that provides richer spectra.

Study design, size, duration: Spent embryo culture media from embryos undergoing extended culture to the blastocyst stage was collected, frozen and subsequently used for the mid-infrared spectroscopy (MIR) analysis. A cohort of 101 embryos were analyzed in this pilot study. Data regarding embryo quality and development was collected retrospectively.

Participants/materials, setting, methods: Embryos were cultured from day 1-3 in 30 microliters drops of sequential media (G1Plus, Vitrolife, Sweden), with an oil overlay, at 37 °C, 6% CO_2, 5% O_2, and 90% humidity. Embryos were transferred to another culture dish by careful and individual pipetting and the media was collected within an hour and frozen at -20 °C. Sample processing and optimization of MIR spectral acquisition conditions, quality control indicators (ICQ) as the signal to noise ratio.

Main results and the role of chance: The processed MIR spectrum (e.g. the 2nd derivative) was analyzed and retrospectively evaluated for its association with embryo quality and development to blastocyst and embryo implantation. The high specificity and sensitivity of the technique is evidenced by statistically significant differences of diverse ratios of spectral bands between spent culture of embryos that developed poorly with those that produced good quality blastocysts.

Limitations, reasons for caution: The potential confounding factor of variables that can influence the analysis outcome, such as media type, volume of the culture drops, culture conditions, among others, has to be identified.

Wider implications of the findings: The informative MIR spectra shows potential to be used as a predictor of embryo development to blastocyst stage, and eventually, for the selection of the fittest embryo with highest implantation potential within a cohort.

Trial registration number: not applicable.

P-236 Increased anti-oxidative capacity of follicular fluid HDL is a positive predictor of embryo quality in modified natural cycle IVF

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Study question: Is anti-oxidative function of follicular fluid (FF) high density lipoproteins (HDL) associated with embryo quality in modified natural cycle in vitro fertilization (MNC-IVF)?

Summary answer: A higher FF-HDL anti-oxidative function is associated with increased development of a top quality embryo on day two after fertilization.

What is known already: HDL are the main cholesterol carriers in FF. Thus far, FF-HDL have been mainly studied in the context of steroid hormone synthesis.

Study design, size, duration: For the present cross sectional study, between August 2013 and April 2017 FF and plasma samples were collected during 561 consecutive MNC-IVF cycles of 249 consenting patients in a single academic infertility treatment center.

Participants/materials, setting, methods: The anti-oxidative function of HDL, measured as the capacity to prevent native LDL oxidation in vitro, was determined in (i) 19 matched plasma and FF samples from randomly selected patients and (ii) FF from 375 MNC-IVF cycles, where one oocyte was retrieved and minimum FF blood contamination was present. The association between HDL anti-oxidative function and oocyte and embryo quality as well as occurrence of a viable pregnancy were assessed statistically by general estimating equations.

Main results and the role of chance: The anti-oxidative function of FF-HDL was significantly higher than that of matching plasma-HDL (84 [79.2-87.1] versus 67.2 [63.8-70.1]; p <0.001); more than 80% of the total FF anti-oxidative capacity was attributable to the presence of HDL. A higher FF-HDL anti-oxidative function was associated with higher odds of development of top quality embryos, association that persisted after adjustment for BMI and smoking (OR 2.04, 95%CI 1.18-3.53, p = 0.011). However, the anti-oxidative function of FF-HDL was not associated with the occurrence of an ongoing pregnancy.

Limitations, reasons for caution: The present study did not find an association between the anti-oxidative function of FF-HDL and ongoing pregnancy, possibly as a consequence of low power. Only patients from a single center participated.

Wider implications of the findings: More research on the oxidative stress/anti-oxidant balance of FF in relation to oocyte development and fertility outcomes is warranted. Further, definition of lifestyle interventions to increase the anti-oxidative capacity of FF-HDL holds potential to increase the success rates of IVF procedures and of natural conceptions.

Trial registration number: Nederlands Trial Register number NTR4409.

P-237 Cleavage stage versus blastocyst stage embryo transfer in oocyte donation cycles

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Study question: Is there a difference in implantation and clinical pregnancy rates between day 3 cleavage stage and day 5 or blastocyst stage embryo transfer in frozen-thaw oocyte donation cycles.

Summary answer: Embryo transfers on day 5 are associated with significant improvement on implantation and clinical pregnancy rates in oocyte donation cycles.

What is known already: During the last few years a shift has occurred for transfer of embryos at the blastocyst stage. It has been shown that embryo transfer at the blastocyst stage following COH /ET has been associated with improved implantation rates and clinical pregnancy rates as compared to cleavage stage transfers. So far, there is no study evaluating this notion in oocyte donation cycles.

Study design, size, duration: This is a retrospective evaluation of all transfers performed after oocyte donation in our center during the period between January 2017 to December 2017. Oocyte donors is a unique populations in terms that they represent a good prognosis group, more homogeneous as compared to infertile patients. The study was approved by the ethics committee of the IASO maternity hospital and the scientific board of the IOLIFE fertility center.

Participants/materials, setting, methods: 303 oocyte donation cycles performed. From January 2017 to December 2017.

Donor ovarian stimulation was performed using a short GnRHantagonist protocol with rFSH. ICSI was performed in all donors. All embryos were cryopreserved either on cleavage or blastocyst stage.

Recipient endometrial preparation was standardized. Progesterone was initiated 3days prior to cleavage stage embryo transfers and 5-6 days prior to blastocyst stage transfers.Statistical analysis was done using SPSS, v25. A p value < 0.05 was considered significant.

Main results and the role of chance: Three hundred and one (301) frozen-thaw transfers from oocyte donation cycles where finally included in the study. There were 171 day 3 transfers out of which there were 92 clinical pregnancies (53.8%). In 123 day 5 transfers there were 75 clinical pregnancies (60.9%) In 7 day 6 transfers ,4 clinical pregnancies were reported (57%).

Overall there was no difference between the two groups in terms of the age of the donors, day 2 FSH, antral follicle count, number of MII oocytes and number of good quality embryos. There was no difference between the groups in terms of type of catheter use and documented difficulty during the transfer. Embryo transfers performed on day 3 were compared to transfers performed on blastocyst stage in terms of clinical and ongoing pregnancy rates. Day 5 and day 6 transfers were evaluated in the same group. Overall embryo transfers at the blastocyst stage were associated with significantly improved pregnancy rates

(60.7%) as compared to cleavage stage transfers (53,8%) p = 0.012. The risk of multiple pregnancies (mainly twins) was similar in the two groups (data not shown)

Limitations, reasons for caution: There is no complete information on the obstetrical outcome on a significant proportion of those pregnancies.

Wider implications of the findings: These findings suggest that in oocyte donation cycles embryo transfer at the blastocyst stage is associated with a significant improvement in pregnancy rates and should be adopted as a standard practice.

Trial registration number: Not applicable.

P-238 Can metabolomics provide information about blastocyst development?

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Study question: Is blastocyst´s culture media metabolic profile correlated with other embryo selection criteria?

Summary answer: Metabolic profile is not correlated to implantation, nor to women's age or morfokinetic parameters but may be linked with expansion degree and cryoresistance of blastocyst.

What is known already: With the aim of improving Assisted Reproductive Techniques (ARTs) outcome, new methods for embryo selection have appeared in the last few years. One of them is metabolomics of embryo culture media. Up to now, metabolic profile is not able to predict clinical outcome, so there is controversy about its applicability to clinical context. Nevertheless, it is true that metabolomics provide valuable information about embryo metabolism. It has been demonstrated that embryo at blastocyst stage changes its metabolism to glycolysis, which generates lactate. Furthermore, lipids metabolism has been studied in other animal species, and it has been related to cryoresistance.

Study design, size, duration: This study included 48 blastocysts from 38 patients, whose transfer ended in Know Implantation Data (KID). All patients performed an IVF treatment with fresh transfer at the blastocyst stage between September of 2016 until March of 2017.

Participants/materials, setting, methods: Embryos culture media (Continuous Single Complete, Irvine) were stored after transfer in day 5 was performed. All embryos were cultured in MIRI-TL (ESCO- Medical Ò) incubator, so that morphokinetical data was available for study. Culture media was analysed by Magnetic Nuclear Resonance (MNR). Spectrum obtained were processed and analysed by Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS-DA) using MATLAB. T-student test was performed using SPSS 15.0 v and significance was set as 0,05.

Main results and the role of chance: The 48 spectrum of culture media were significative separated in two groups based on its composition. More specifically, three regions of spectrum: lactate region (0.075 ± 0.003 in group 1 vs 0.05 ± 0.01 in group 2), lipids HDL+LDL+VLDL region (0.15 ± 0.03 in group 1 vs 0.72 ± 0.30 in group 2) and mobile lipids (0.09 ± 0.03 in group 1 vs. 0.82 ± 0.40 in group 2). Although the difference was not significant, blastocyst from group 1 tended to be more expanded than blastocyst from group 2 (p-value>0,05). No correlation was found between the two groups and KID, nor with patient's age and morphokinetic parameters (p-values>0,05).

With these results, metabolic profile of embryos cultured until blastocyst stage may not be related with implantation status. Nevertheless, two groups of blastocysts were differentiated in terms of its metabolism, particularly in lactate and lipids content of their culture media. This differentiation is independent from patient's age and morphokinetic parameters measured until day 5.

Limitations, reasons for caution: To confirm these preliminary results, it would be necessary to increase the number of culture media samples.

Wider implications of the findings: Metabolic differences found could be linked with grade of blastocyst expansion. Lactate differences between two groups refer to glucose intake in glycolysis, since results indicate there is more lactate production in more expanded blastocyst. Furthermore, we found less lipid content in more expanded blastocyst, which could be related to cryoresistance.

Trial registration number: Not applicable

POSTER VIEWING

Endometriosis, endometrium and fallopian tube, and benign disorders of the endometrium and fallopian tube

P-239 Preimplantation genetic screening in endometriosis and non-endometriosis patients in a single institution

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Study question: Is there any difference in between prevalence of aneuploidy embryos in endometriosis and non-endometriosis patients using preimplantation genetic screening?

Summary answer: No.

What is known already: Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity and it leads to chronic inflammation with oxidative stress, and genetic variations which eventually decrease pregnancy rates and live birth rates. Among these various factors, meiotic spindle alterations have been reported disturbing on chromosomal segregation and organization in endometriosis patients which could cause aneuploidy. As ART increases striving for higher pregnancy rate, it is believed that majority of aneuploidy embryos might cause lower live birth rates. Therefore people were recommended for PGS patients who are considered to be at high risk of having aneuploidy embryos.

Study design, size, duration: Cross sectional study.

Retrospective chart reviews.

Patients who have undergone IVF and PGS from August, 2014 to July, 2017.

PGS was recommended to couples who have at least one of these criteria; woman's age ≥37 years old, ≥ one parent has chromosomal mosaicism, spontaneous abortion ≥ 2 times, implantation failure ≥ 2 times.

Patients who were diagnosed with endometriosis were 19 people and who were not diagnosed with endometriosis were 93 people.

Participants/materials, setting, methods: Eighty two embryos from 19 patients with endometriosis and 371 embryos from 93 non-endometriosis women were enrolled. All of them were indicated for IVF with PGS. A cleavage-stage embryo biopsy was performed on Day 3 of embryo development by extruding one blastomere from embryos reaching at least the six-cell stage, and samples were taken for analysis on the same day.

Main results and the role of chance: Aneuploidy rates in between the two groups according to age groups in both endometriosis and non-endometriosis groups consistently tended to increase when they were calculated according to embryos. Among patients less than 35 years old, the aneuploidy rates were 52.6 %, 72.9 %, and among patients equal to greater than 41 years old, the aneuploidy rates were 89.6 %, 87.7 % in both endometriosis and non-endometriosis groups.

We saw whether age, abnormal karyotype, history of recurrent pregnancy loss, history of recurrent implantation failure, serum AMH, number of retrieved oocytes have significant impact on abnormal PGS. Among these factors, other than history of recurrent pregnancy loss and number of retrieved oocytes, they were significantly related to aneuploidy embryos that they were set for confounding factors and associations in between presence of endometriosis and aneuploidy embryos were re-analyzed. Presence of endometriosis was not significantly associated with abnormal PGS result (Exp(B) = 0.766, 95% CI = 0.401-1.465, *P*-value = 0.420).

Limitations, reasons for caution: The number of cases of endometriosis was small and endometriosis stages are missing. Also, we cannot say non-endometriosis patients do not have endometriosis. Lastly, PGS was done in

blastocyst staged embryos that we cannot say that abnormal chromosomal segregation and organization of embryos were thoroughly analyzed.

Wider implications of the findings: Although the sample size is small, we certainly are sure that the results of this study would be meaningful in recognizing that the aneuploidy rates in between the endometriosis and non-endometriosis groups are not much different in certain groups of Korean population.

Trial registration number: not applicable.

P-240 Factors affect the outcome of IVF and the recurrence of the endometrial disease of patients with atypical endometrial hyperplasia (AEH) and early well-differentiated endometrial adenocarcinoma (EC)

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Study question: When to initiate IVF is safe and effective for patients with atypical endometrium hyperplasia(AEH) and early well-differentiated endometrial adenocarcinoma (EC) after fertility-sparing treatment?

Summary answer: It is safe and effective to start IVF immediately after achieving first complete response (CR) for patients with AEH and early EC.

What is known already: The available data support the relative safety and efficacy of progestin therapy and allow fertility-sparing patients to achieve their fertility goals in a short window of disease resolution.More and more women with AEH and EC after fertility-sparing treatment have to receive IVF treatment. Although some literatures report that IVF is safe to these patients, the number of the cases is small. The factors that affect the outcome of IVF and the recurrence of the disease are still not clear.

Study design, size, duration: A total of 60 patients with AEH and early well-differentiated EC after fertility-sparing treatment were included. All these patients received IVF treatment in Reproductive medical center of Third hospital,Peking university between May 2011 and May 2017. Data of the results was retrospectively analysis.

Participants/materials, setting, methods: According to the time to start IVF after the first time to CR, 60 patients were divided into within 3 months (group A) and after 3 months (group B).The implantation rate, clinical pregnancy rate,spontaneous abortion rate and recurrence rate were compared between the two groups.

Main results and the role of chance: A total of 60 patients were included, 45 with AEH and 15 with EC. The follow-up time from the first CR was 39.6 ± 26.9 months. Ninety-five ovum pickup cycles were performed. There were 67 fresh embryo transfer cycles and 54 FET cycles. A total of 36 patients achieved 47 pregnancies, and 25 patients gave 32 babies. The pregnancy rate per cycle in fresh cycle was 38.8%(26/67),and the pregnancy rate per cycle in FET cycle was 25.93%(14/54). The pregnancy rate of fresh cycles in Group A and B was 39.1% and 38.6%, respectively(P = 0.969).In FET cycles,the pregnancy rate was 50% and 24% in Group A and B,respectively (P = 0.583). The total recurrence rate was 30% (18/60) during the follow-up period. It was significantly higher in the group B than the group A (17.1% vs 48%, P = 0.022).

Limitations, reasons for caution: This study is nonrandomized,as the proportion of such patients in IVF is low, and the number of cases was relatively small, so some statistical results may be limited by the smal sample size.

Wider implications of the findings: From the results of the paper, the recurrence of the endometrial disease is associated with the initiation of IVF after the first CR, and the outcome of IVF is not related to the starting time of IVF. It is recommended that IVF should be started as soon as possible.

Trial registration number: none.

P-240 Factors affect the outcome of IVF and the recurrence of the endometrial disease of patients with atypical endometrial hyperplasia (AEH) and early well-differentiated endometrial adenocarcinoma(EC)

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Study question: When to initiate IVF is safe and effective for patients with atypical endometrium hyperplasia(AEH) and early well-differentiated endometrial adenocarcinoma (EC) after fertility-sparing treatment?

Summary answer: It is safe and effective to start IVF immediately after achieving first complete response (CR) for patients with AEH and early EC.

What is known already: The available data support the relative safety and efficacy of progestin therapy and allow fertility-sparing patients to achieve their fertility goals in a short window of disease resolution.More and more women with AEH and EC after fertility-sparing treatment have to receive IVF treatment. Although some literatures report that IVF is safe to these patients, the number of the cases is small. The factors that affect the outcome of IVF and the recurrence of the disease are still not clear.

Study design, size, duration: A total of 60 patients with AEH and early well-differentiated EC after fertility-sparing treatment were included. All these patients received IVF treatment in Reproductive medical center of Third hospital,Peking university between May 2011 and May 2017. Data of the results was retrospectively analysis.

Participants/materials, setting, methods: According to the time to start IVF after the first time to CR, 60 patients were divided into within 3 months (group A) and after 3 months (group B).The implantation rate, clinical pregnancy rate,spontaneous abortion rate and recurrence rate were compared between the two groups.

Main results and the role of chance: A total of 60 patients were included, 45 with AEH and 15 with EC. The follow-up time from the first CR was 39.6 ± 26.9 months. Ninety-five ovum pickup cycles were performed. There were 67 fresh embryo transfer cycles and 54 FET cycles. A total of 36 patients achieved 47 pregnancies, and 25 patients gave 32 babies. The pregnancy rate per cycle in fresh cycle was 38.8%(26/67),and the pregnancy rate per cycle in FET cycle was 25.93%(14/54). The pregnancy rate of fresh cycles in Group A and B was 39.1% and 38.6%, respectively(P = 0.969).In FET cycles,the pregnancy rate was 50% and 24% in Group A and B,respectively(P = 0.583). The total recurrence rate was 30% (18/60) during the follow-up period. It was significantly higher in the group B than the group A (17.1% vs 48%, P = 0.022).

Limitations, reasons for caution: This study is nonrandomized,as the proportion of such patients in IVF is low, and the number of cases was relatively small, so some statistical results may be limited by the smal sample size.

Wider implications of the findings: From the results of the paper, the recurrence of the endometrial disease is associated with the initiation of IVF after the first CR, and the outcome of IVF is not related to the starting time of IVF. It is recommended that IVF should be started as soon as possible.

Trial registration number: none.

P-241 Chronic endometritis: Prevalence using hysteroscopy and CD138 immunohistochemistry and impact on reproductive outcome in patients with previous IVF/ICSI failure

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Study question: To evaluate the prevalence of CE in women with previous IVF/ICSI failure using hysteroscopy and CD138 immunohistochemistry and whether subsequent antibiotic treatment positively impacts implantation.

Summary answer: In women with previous IVF/ICSI failure the diagnosis and treatment of chronic endometritis CE, significantly improves implantation and pregnancy rates in subsequent attempts.

What is known already: A healthy receptive endometrium is essential for embryo implantation.Recently, the relationship between chronic endometritis (CE) and infertility-related conditions such as previous implantation failure has emerged as an area of inquiry. CE is diagnosed by endometrial biopsy, and the presence of plasma cells in the endometrial stroma is the generally accepted histological diagnostic criterion for CE.Recently, an immunohistochemical (IHC) stain capable of detecting CD38 and CD138 plasma cellspecific surface antigens was introduced for the confirmation of the presence of plasma cells inside the endometrium.The hysteroscopic evaluation of endometrial inflammatory disease showed a good sensitivity for detecting CE.

Study design, size, duration: Design: prospective cohort study.

145 women aged between 25 and 38 years with history of previous IVF/ICSI failure included in the study during three years.

Participants/materials, setting, methods: women underwent hysteroscopy and endometrial sampling for CD138 immunohistochemistry. Women diagnosed with CE were treated with antibiotics until follicular phase biopsy showed no CE. Patients were placed in two groups based on presence or absence of CE (group A and B, respectively). Primary outcome was sensitivity, specificity and accuracy of hysteroscopy in relation to immunohistochemistry (as a gold standard) in diagnosis of CE. Secondary outcomes were implantation and pregnancy rates in the post-treatment IVF/ICSI attempt.

Main results and the role of chance: Primary outcome was sensitivity, specificity and accuracy of hysteroscopy in relation to immunohistochemistry (as a gold standard) in diagnosis of CE. Secondary outcomes were implantation and pregnancy rates in the post-treatment IVF/ICSI attempt.

Prevalence of CE was 23.4% (34/145) using hysteroscopy and 19.3% (28/145) using immunohistochemistry (golden standard) showing 78.6%, 89.7%, and 87.5% sensitivity, specificity and accuracy respectively.

Implantation rate in subsequent cycles was significantly higher in group A vs. B (35.16% vs 16.43%, $p = 0.001$*). Group A also showed higher pregnancy rate compared to group B (57.14% vs 34.18%, $p = 0.076$*).

Limitations, reasons for caution: larger number of study cases is needed.

Wider implications of the findings: In women with previous IVF/ICSI failure the diagnosis and treatment of CE significantly improves implantation and pregnancy rates in subsequent attempts therefor hysteroscopy and endometrial sampling should be considered in evaluation of the endometrium in infertile women and specially before starting assisted reproductive technique ART.

Trial registration number:

- Informed consent was obtained from all participants.
- The study was approved by Alexandria University ethical committee.
- The study was performed at Alexandria University, Egypt.

P-242 Naturally occurring endometriosis in the cynomolgus macaque: effects of analgesics on pain-related behavior and brain activation

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Study question: Can pain and brain activation be observed in cynomolgus macaques with endometriosis?

Summary answer: Macaques with endometriosis demonstrate marked abdominopelvic pressure hypersensitivity and thalamic and insula activation, which were reduced with morphine and dienogest treatment.

What is known already: The mechanism of abdominopelvic pain associated with endometriosis is poorly understood, as reflected by the lack of effective treatments that offer significant pain relief for endometriosis patients. While more preclinical studies are needed, one potential barrier to translating preclinical findings to clinical application is that rodents, a species with a reproductive system that is distinct from that of humans, are most frequently used as the species to model clinical endometriosis. By contrast, the cynomolgus macaque is anatomically and phylogenetically closer to humans than rodents.

Study design, size, duration: This was an observational, preclinical study utilizing cynomolgus macaques (Macaca fascicularis) diagnosed with naturally occurring endometriosis. During abdominal pressure pain assessment, macaques were restrained and awake and during brain imaging, macaques were anesthetized with propofol. Following acute dosing with morphine, meloxicam and acetaminophen, macaques were treated for eight weeks with dienogest.

Participants/materials, setting, methods: Five macaques with endometriosis (10-18 yo) and three healthy controls (10 yo) were used. Responsiveness to pressure in the abdominopelvic area was measured using a pressure algometer and the effects of morphine, meloxicam, acetaminophen and dienogest on pressure sensitivity were determined. Non-noxious abdominopelvic pressure-induced brain activation was visualized with functional magnetic resonance imaging (fMRI; 3.0 T MRI Signa HDxt (GE)) and the effects of drug treatment on brain activation were observed with fMRI.

Main results and the role of chance: Macaques with endometriosis displayed significantly decreased abdominopelvic pressure thresholds compared to healthy macaques ($P < 0.05$), suggestive of pressure hypersensitivity. Morphine ($P < 0.05$) but not meloxicam or acetaminophen acutely ameliorated pressure hypersensitivity. Dienogest treatment ameliorated pressure hypersensitivity beginning on the second week of treatment ($P < 0.05$) which persisted for at least four weeks following termination of dienogest treatment ($P < 0.01$). Abdominal pressure also induced bilateral activation of the insular cortex and thalamus, which was reduced with an acute dose of morphine and eight weeks of dienogest treatment. Interestingly, while the mean endometrioma volume tended to decrease over time in dienogest-treated macaques, this was not statistically significant compared to the pre-treatment volume ($P = 0.09$).

Limitations, reasons for caution: The current study utilized a small sample size with possibly differing stages of endometriosis for each macaque. Whether it is possible to measure 'spontaneous pain" in the macaques with endometriosis, whether through behavior or brain activity, has yet to be determined.

Wider implications of the findings: The current macaque findings suggest that endometriosis patients could have significant pain-related brain activation. If so, then brain activation in the macaque could be utilized as a preclinical biomarker against which to develop treatments that not only reduce symptoms but modify the underlying disease.

Trial registration number: N/A.

P-243 Antifertility effectiveness of a novel copper-containing intrauterine device material and its influence on the endometrial environment in rats

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Study question: This study was designed to investigate the antifertility effectiveness of a novel copper-containing intrauterine device (Cu-IUDs) material and its effect on the endometrial environment in rats.

Summary answer: This study demonstrates that micro-Cu/LDPE/MVQ exhibits satisfactory contraceptive efficacy and causes fewer side effects than Cu.

What is known already: Cu-IUDs have excellent contraceptive efficacy, the existing Cu-IUDs are associated with undesirable side effects, such as menorrhagia, intermenstrual bleeding, pelvic pain, and spotting.

Study design, size, duration: One hundred sixty healthy female rats were randomly divided into four groups of 40 each: (a) a sham-operated control group (SO group); (b) the Cu/LDPE/MVQ group; (c) the LDPE/MVQ group; and (d) the Cu group.

Participants/materials, setting, methods: Twenty rats in each group were mated with male rats of proven fertility, from 30 days after insertion, and the antifertility rates (ATs) were observed at Day 12 of pregnancy. The pathological changes and factors associated with bleeding, pain, and inflammation in the endometrium of the remaining rats in each group were then investigated 90 days after insertion, and the surface condition of the implants was analyzed 90 days after insertion.

Main results and the role of chance: The contraceptive effectiveness was 100% in both the bulk Cu group and the micro-Cu/LDPE/MVQ group, and that in the LDPE/MVQ group was 30%. On day 90 after insertion, histopathological observation and the ultrastructural changes of the endometrium showed that the damage caused by bulk Cu was much more severe than that caused by the Cu/LDPE/MVQ microcomposite and that the surface of the Cu/LDPE/MVQ microcomposite was much smoother than that of the bulk Cu. Furthermore, compared with the sham-operated control group, the

concentrations of tissue plasminogen activator and prostaglandin E2 were significantly increased 90 days after insertion in all experimental groups except for the LDPE/MVQ group ($P < 0.05$), and the parameters in the Cu/LDPE/MVQ group were significantly lower than those in the Cu group ($P < 0.05$). In addition, the expression levels of matrix metalloproteinase 9, tissue inhibitor of metalloproteinase 1, plasminogen inhibitor 1, CD34, vascular endothelial growth factor, substance P, and substance P receptor in the endometrium in all experimental groups were significantly lower than those in the Cu group 90 days after insertion ($P < 0.05$).

Limitations, reasons for caution: We chose rats for the antifertility effectiveness and safety. We did not use other animals to show that the novel copper-containing composite are the most promising materials.

Wider implications of the findings: The micro-Cu/LDPE/MVQ may be useful in intrauterine contraceptive devices.

Trial registration number: not applicable.

P-244 Impact of long term pretreatment with GnRH agonist before standard GnRH agonist protocol for IVF/ICSI on cumulative live birth rates of infertile women with adenomyosis

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Study question: Can long term pretreatment with GnRH agonist before standard GnRH agonist protocol improve cumulative live birth rates (CLBRs) for infertile women diagnosed as adenomyosis compared with standard agonist protocol alone?

Summary answer: CLBRs per IVF/ICSI ovarian stimulation cycle were lower in long term GnRH agonist pretreatment group than without pretreatment group for infertile women with adenomyosis.

What is known already: GnRH agonist can cause hypoestrogenism therefore reduce uterine size in adenomyosis. Long term use of GnRH agonist is shown to improve pregnancy outcomes in women with adenomyosis in frozen embryo transfer cycle. However, it is unknown whether long term pretreatment with GnRH agonist before standard GnRH agonist protocol for IVF/ICSI can improve CLBRs of infertile women with adenomyosis.

Study design, size, duration: This is a retrospective cohort study; A total of 232 ovarian stimulation cycles and 201 women diagnosed as adenomyosis who underwent ovarian stimulation for IVF/ICSI with standard GnRH agonist protocol with or without long term GnRH agonist pretreatment at our hospital from January 2010 to March 2017 were identified and reviewed.

Participants/materials, setting, methods: Infertile women with adenomyosis who attempted ovarian stimulation for IVF or ICSI were categorized into two groups (standard GnRH agonist protocol with or without long term pretreatment with GnRH agonist). CLBR was defined as the first live birth per ovarian stimulation cycle including fresh and frozen cycles and evaluated by group. Binary logistic regression was used to assess the association between controlled ovarian hyperstimulation (COH) protocol and cumulative live birth after adjusting for confounding factors.

Main results and the role of chance: Oocyte retrieval was cancelled in 14 cycles. Baseline characteristics including female and male age, female BMI, gravidity and times of previous miscarriage, basal FSH, proportion of patients complicated with endometriosis as well as insemination method were similar in the two groups. Stimulation outcome including number of retrieved oocytes and mature oocytes, number of transferred embryos, high quality embryos and frozen embryos were similar in the two groups. Cumulative live birth rates were lower in standard GnRH agonist protocol with long term GnRH agonist pretreatment than without pretreatment (32.4% vs 46.6%, $P = 0.044$). The CLBRs is associated with female age (OR 0.923, 95% CI 0.852-0.994, $P = 0.035$), standard GnRH agonist protocol without long term pretreatment (OR 2.299, 95% CI 1.124-4.705, $P = 0.023$) and number of high quality embryos (OR 1.833, 95% CI 1.508-2.227, $P<0.001$) after adjusted for basal FSH, proportion of patients complicated with endometriosis.

Limitations, reasons for caution: As a retrospective study, our analysis depended on previously recorded data; therefore, certain variables such as serum CA125 level could not be collected.

Wider implications of the findings: Long term pretreatment of GnRH agonist may be not necessary for infertile women with adenomyosis who undergo IVF/ICSI with standard GnRH agonist protocol.

Trial registration number: not applicable.

P-245 Adenomyosis down regulates endometrial L-selectin ligands at the mid-secretory phase during menstrual cycle

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Study question: Does adenomyosis impair L-selectin ligand (LSL) expression in human endometrium?

Summary answer: Adenomyosis decreases LSL expression in the luminal epithelium of the endometria at the mid-secretory phase during the menstrual cycle.

What is known already: The relationship between adenomyosis and infertility is still unclear, but severe adenomyosis has a negative impact on pregnancy rate in clinical observation. One of the possible pathophysiology is impaired endometrial receptivity. LSL is a biomarker for endometrial receptivity. Defects in LSL expression leading to implantation failure were reported in women with endometriosis, PCOS and unexplained infertility. However, LSL expression in adenomyosis is still unknown.

Study design, size, duration: This is a retrospective, experimental study. Forty-two endometrial samples from reproductive-aged women with adenomyosis underwent hysterectomy were obtained at Cathay General Hospital, Taipei, Taiwan from Aug 2008 to July 2009. Twelve samples were collected from the proliferative phase, 10 from the early-secretory phase, 9 from the mid-secretory phase, and 11 from the late-secretory phase. Another 11 endometrial samples were collected from menopausal women as controls.

Participants/materials, setting, methods: The inclusion criteria of the participants were: (1) $35 < age < 50$ years; (2) regular menstrual cycle; (3) $BMI < 28$; (4) no hormone therapy at least 2 months before surgery; (5) no known endometrial pathologies. Immunohistochemistry with MECA-79 Ab, western blotting and RT-PCR were performed to evaluate LSL expression. The intensity of immunostaining was analyzed by HSCORE. Non-parametric Kruskal-Wallis one-way analysis of variance with multiple comparisons was performed to examine differences among phases.

Main results and the role of chance: In luminal epithelium, MECA-79 reactivity increased from the proliferative to late-secretory phase but decreased at the mid-secretory phase. The mean HSCOREs among the proliferative, early-secretory and late-secretory phase were significant differences ($P<0.05$). Five LSL genes were detected in adenomyotic endometria: PODXL, EMCN, CD300LG, GLYCAM1, and CD34. The mRNA expression of LSL genes occurred differentially among phases. Especially, PODXL differed significantly among phases ($P<0.05$). Comparison with the mRNA expression in the proliferative and mid-secretory phase, all the LSL genes totally increased in the mid-secretory phase then that in the proliferative phase. The mRNA expressions of CHST2 and CHST4 genes, which involved in the generation of LSL epitopes and LSL activity, were expressed without significant differences among phases.

Limitations, reasons for caution: The small number of samples could limit the power of the study. The heterogeneity of the samples could impact the results. Finally, because of ethical considerations, normal endometrial samples cannot be obtained from healthy women as controls.

Wider implications of the findings: Adenomyosis may cause abnormalities in the production of LSL, which may contribute to impaired endometrial receptivity and implantation failure. Further studies via in vitro and in vivo are required to determine the mechanisms related to the decrease of LSL expression in adenomyosis.

Trial registration number: None.

P-246 Defecation associated (dys)functions after surgical resection of (recto)sigmoid infiltration by deep infiltrating endometriosis

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Study question: Do the defecation associated outcomes in patients after surgical resection of (recto)sigmoid infiltration due to Deep Infiltrating Endometriosis (DIE) resemble the outcomes of healthy women?

Summary answer: Constipation, fecal incontinence (FI), Irritable Bowel Syndrome (IBS) and their symptoms have a significantly higher prevalence in patients after (recto)sigmoid resection of DIE than healthy women.

What is known already: Patients with DIE are known to experience fecal problems, such as constipation and FI. It is also known that patients with DIE tend to have more IBS related problems and hypertonia of the pelvic floor muscles. Further, vaginal delivery is known to influence fecal problems. (Recto)sigmoid resection performed for DIE has been reported to improve the defecation associated outcomes.

Study design, size, duration: For this cross-sectional study we included 181 adult women who had been treated with a (recto)sigmoid resection for DIE between January 2005 and December 2015. Of these women 132 filled in the questionnaire. The data of the control group, i.e. 680 women from the general Dutch population, were collected in 2015, and we used them retrospectively. Patients and controls with comorbidities which are known to influence defecation and fecal continence were excluded (n = 2 and n = 119 respectively). Afterwards, 130 patients were age-matched with female controls from the Dutch population in a 1:2 ratio. The afore described steps resulted in a data base which consisted of 130 patients and 260 controls.

Participants/materials, setting, methods: All patients and controls completed the validated Groningen Defecation and Fecal Continence questionnaire (DeFeC). Constipation, FI and IBS were defined according to the Rome IV criteria.

Main results and the role of chance: The prevalence of constipation, FI and IBS in the patients was significantly higher than in the control group (50.8% versus 26.2%, p<0.001, 15.4% versus 5.0%, p = 0.001, and 14.6% versus 5.4%, p = 0.002, respectively). Patients also used laxatives and other forms of conservative treatment more often (OR = 2.780, p<0.001). In addition, constipated patients experienced the co-existence of FI more often than their healthy counterparts (9.2% versus 1.5%, p<0.001). Surprisingly, women who have had less vaginal deliveries did suffer more from FI (p = 0.011). Furthermore, multivariate analysis showed that women who underwent (recto)sigmoid resection for DIE were more likely to be constipated or fecally incontinent (respectively p<0.001 and p = 0.002), independently of history of vaginal birth.

Limitations, reasons for caution: In this cross-sectional study we included only patients who had already undergone surgery; the occurrence of these symptoms before surgery is therefore unknown. Moreover, functional tests could indicate the pathophysiological factors behind the increased fecal problems.

Wider implications of the findings: The prevalence of fecal problems is significantly higher in patients who underwent (recto)sigmoid resection for DIE than in healthy controls. Further research is important to improve the complaints of patients.

Trial registration number: METc 2014/516

P-247 The human fallopian tube vs endometrium: can differences in function and steroid hormone receptor expression be explained by differential hormone responsiveness?

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Study question: Are there spatial and temporal differences in cellular proliferation and steroid hormone receptor expression in the human fallopian tube (FT), as there are in the endometrium?

Summary answer: The premenopausal FT does not show cyclical changes in cellular proliferation, with a similar steroid hormone receptor expression pattern to the quiescent postmenopausal endometrium.

What is known already: The human FT epithelium exists as a continuum of the endometrium. Both tissues share the same embryological origin. The endometrium is widely accepted as the main target organ for the ovarian steroid hormones, and undergoes a well-described monthly cycle of proliferation, differentiation, shedding, and regeneration throughout a woman's reproductive life. Hormonal regulation is exerted through the cognate ovarian steroid hormone receptors (ERa, ERb, PR, and AR), which are expressed in endometrial cells. Although the steroid hormone dependent cyclical changes are well described in the endometrium, the effect of the same hormones on the FT epithelium has not been comprehensively studied.

Study design, size, duration: A prospective observational study, analysing matched full thickness human endometrial and FT samples, from 38 healthy women undergoing hysterectomy for benign conditions, including 28 samples from premenopausal women, and 10 from postmenopausal women. Full thickness samples were obtained to allow investigation of the three compartments of the endometrium: the luminal epithelium, the functionalis, and the basalis. The FT samples were taken from both the isthmic and the fimbrial areas.

Participants/materials, setting, methods: Matched endometrium and FT samples were analysed by immunohistochemistry, for proliferative marker Ki-67, and steroid hormone receptors AR and PR (n = 38). Gene expression of AR and PR was examined by qPCR in matched endometrial and FT samples (n = 5). Primary human endometrial and FT epithelial cells were treated with E2 and DHT in short term cultures, and the expression of Ki-67, AR, and PR analysed by IHC and qPCR (n = 7).

Main results and the role of chance: The FT displayed low levels of proliferation throughout the menstrual cycle in all compartments, whereas the premenopausal endometrium was highly proliferative, particularly the functionalis (p = 0.03).

Interestingly, AR expression was high in the FT, and was weak in the matched premenopausal endometrium (p = 0.004). There was no significant difference in AR expression between the FT and the postmenopausal endometrium. PR expression in the FT and endometrium was not significantly different. AR and PR expression showed no correlation in the FT.

As both AR and PR are thought to be regulated by oestrogen in the endometrium, the matched FT and endometrial cell cultures with E2 and DHT in vitro, to explore the steroid hormone receptor response to ovarian hormones. In the FT, E2 treatment increased AR protein expression in the glands, and increased AR mRNA expression. There was no effect of E2 treatment on PR expression in the FT. DHT treatment increased protein AR expression in the FT glandular epithelium, and increased AR mRNA expression in the FT. There was no effect of DHT treatment on PR protein expression in the FT or endometrium, but there was an increase in PR mRNA expression in the FT.

Limitations, reasons for caution: This is a descriptive study with short-term culture of primary human endometrial and tubal epithelial cells in vitro, with a small sample size of matched endometrium and FT samples.

Wider implications of the findings: The FT does not undergo cyclical proliferation, and shares similarities in steroid hormone receptor expression with the postmenopausal endometrium. These findings contribute towards understanding the physiology of the FT. Further research in this area will enable research into disorders of the FT, such as ectopic pregnancy, infertility, and FT cancer.

Trial registration number: This work was funded by the ITM/Department of Women's Health at the University of Liverpool. All authors have to declare.

P-248 Comparison of IVF results in cases of severe endometriosis: after or without previous surgery

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Study question: Does surgery before in-vitro fertilization (IVF) improve the results of assisted reproductive technologies (ART) in patients with severe endometriosis?

Summary answer: There is evidence that IVF without previous surgical treatment results in a decreased delivery rate per cycle and per patient.

What is known already: Whether to operate severe endometriosis or not to improve ART results remains a debate. There is still no international consensus about the management of infertile patients with severe endometriosis. A recent french study demonstrated among a 110 patients cohort that IVF results were lower without previous surgery. But some others studies provided opposite conclusions.

Study design, size, duration: A 190 patients cohort was analysed (retrospective analysis on prospective recorded datas). All patients presented severe endometriosis with rectovaginal nodule and/or multiple endometriomas and/or major adhesions. The decision to operate or to move directly to IVF was taken by trained laparoscopic surgeons according to the expected chance of pregnancy after surgery and the potential risk of surgery.

Participants/materials, setting, methods: We could include 41 patients in the no surgery group (group NS) and 149 patients in the surgery group (group SU). All data were prospectively recorded. Main outcomes were clinical delivery rates per cycle (fresh+frozen transfers) and crude and actuarial cumulative rates to obtain the first delivery. A complementary analysis was done for attempts to obtain a second delivery. All outcomes are provided in terms of delivery.

Main results and the role of chance: The delivery rate per attempt to obtain the first child was 18.6 % in group NS versus 28.6 % in the group SU ($p = 0.051$).

The crude delivery rates after 5 attempts were respectively 43.9 % versus 54.4 % ($p = 0.23$).

The actuarial delivery rates after 5 attempts were respectively 52.2 % and 71.3 % ($p = 0.021$).

The delivery rate per attempt to obtain a second child was 75 % in the NS group versus 32.3 % in the SU group ($p = 0.09$).

A step by step analysis of IVF results among the attempts to obtain the first delivery shows a difference in defavour of the non operated group for the risk to collect no oocyte (10.3 % versus 4.6 %- $p = 0.003$), the mean number of oocytes (5.8 versus 7.4 – $p<0.008$) and the risk for miscarriage (35 % versus 17% -$p = 0.04$).

Limitations, reasons for caution: Retrospective study, subjective decision to operate or not and unicentric study.

Wider implications of the findings: This study suggests that operating severe endometriosis improves IFV results. On the other hand, if surgery is considered to be too risky, first-line IVF results seem to be acceptable. Surgery should thus be the first step because it is the only way to judge on operability.

Trial registration number: No object.

P-249 Aberrant ER stress induction in response to progesterone enhances endometriotic stromal cell invasiveness

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Study question: Whether endoplasmic reticulum (ER) stress is involved in the regulation of human endometrial cell invasiveness through AKT/mTOR pathway, and is this associated with the altered invasiveness in endometriotic cells?

Summary answer: Abnormal ER stress induction in response to progesterone was observed in endometriotic stromal cells, which led to the alteration of invasiveness via AKT/mTOR pathway.

What is known already: ER stress is a common cellular stress response, which is known to reduce invasiveness in some cell types including ovarian and breast cancer cells through inhibiting AKT/mTOR pathway. Recent studies have shown that ER stress is suppressed by estrogen in human endometrial cells, which suggests that estrogen may promote **endometrial cell invasiveness** by down-regulating ER stress. Therefore, aberrant ER stress in response to progesterone may contribute to the altered invasiveness found in endometriotic tissues.

Study design, size, duration: Normal endometrial stromal cells (NESCs) and endometriotic cyst stromal cells (ECSCs) were cultured with tunicamycin to induce ER stress. To evaluate the effects of estrogen and progesterone on ER stress, AKT/mTOR pathway and invasion, NESCs and ECSCs were cultured with estrogen and/or progesterone. In addition, to evaluate the cycle-dependent induction of ER stress and invasion, normal endometrial and endometriotic tissues were collected according to menstrual cycle.

Participants/materials, setting, methods: The expression level of glucose-regulated protein 78 (GRP78) was measured by Western blot to evaluate ER stress induction. As ER stress-mediated C/EBP homologous protein (CHOP)/tribbles homologue 3 (TRIB3) pathway is a negative regulator of AKT, CHOP and TRIB3 expression was measured to evaluate the effects of ER stress on AKT/mTOR pathway. In addition, cell invasiveness was evaluated by measuring the expression of invasion associated proteins (MMP2 and MMP9) and by performing invasion assay.

Main results and the role of chance: The expression levels of GRP78, CHOP and TRIB3 protein were markedly increased in NESCs treated with tunicamycin compared with control cells, which was accompanied by decreased AKT, mTOR activity and cellular invasiveness. Similarly, progesterone treatment significantly increased CRP78, CHOP, TRIB3 expression in NESCs. Subsequently, cellular invasiveness decreased through the inhibition of AKT and mTOR activity. This progesterone-induced decrease in cellular invasiveness was reversed by the inhibition of ER stress using either mifepristone (progesterone receptor modulator) or salubrinal (ER stress inhibitor). These results suggest that progesterone increases ER stress, which then is directly involved in regulation of NESC invasion. In contrast, progesterone had no significant effects on CRP78, CHOP, TRIB3 expression, AKT, mTOR activity and cellular invasiveness in ECSCs. Furthermore, in contrast to normal eutopic endometrium, endometriotic tissues showed constant CHOP, TRIB3, MMP2 and MMP9 expression throughout the menstrual cycle.

Limitations, reasons for caution: Only primary human endometrial and endometriotic stromal cell cultures were used in this study.

Wider implications of the findings: Our results reveal new insights into the pathogenesis of ovarian endometriotic cyst, and it may also allow the development of new therapeutic strategies based on the modulation of endometriotic cell invasiveness.

Trial registration number: not applicable.

P-250 The new strategy of dydrogesterone protocol as progestin primed ovarian stimulation is effective for patients with ovarian endometriosis undergoing in vitro fertilization

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Study question: Is dydrogesterone (DYG) regimen as progestin primed ovarian stimulation (PPOS) effective for patients with endometriosis during controlled ovarian stimulation(COS), compared to GnRH antagonist protocol?

Summary answer: Higher number of oocytes could be retrieved in DYG protocol than in GnRH antagonist protocol. The pregnancy rates were comparable between the two groups.

What is known already: A Large number of women with endometriosis eventually seek IVF/ ICSI to achieve pregnancy, but endometriosis is an estrogen-dependent chronic inflammatory condition that affects the pregnancy rate compared to women with other causes of infertility.Some progestin have been used for endometriosis therapy for more than 40 years, the use of progesterone may improve the embryo quality of women with endometriosis. Recently, PPOS using DYG was reported as very convenient regimen for patients during COS.

Study design, size, duration: This was prospective controlled study of 133 women with endometriosis (aged <41) undergoing COS for IVF/ICSI and frozen embryo transfer (FET) at our private infertility clinic from June 2016 through

October 2017. The patients were allocated alternately into two groups, one with DYG protocol (n = 68) and the other with GnRH antagonist protocol (n = 65).

Participants/materials, setting, methods: In DYG protocol, dydrogesterone (20 mg/day) was administered simultaneously with HMG (150-225IU) beginning on day 2 or 3. Ovulation was triggered with GnRH agonist and HCG (1000IU) when the leading follicle matured. All high quality embryos were cryopreserved for later transfer. The primary outcome measure was the number of retrieved oocytes. The secondary measure was the clinical pregnancy rate. Statistical analysis was performed using unpaired t-test and chi-square contingency.

Main results and the role of chance: Patient characteristics: the age, body mass index, basal hormone profile, duration of infertility, and AMH level were similar in the two groups. None of the patients experienced a premature LH surge in both groups. No significant difference was found between the two groups for the HMG dose and the stimulation duration of HMG. In DYG group, the number (mean±SD) of retrieved oocytes (10.71 ± 6.97 vs. 7.45 ± 4.71, P<0.01), and the number of mature oocytes (8.68 ± 5.74 vs. 6.18 ± 4.12, P<0.01) were significantly higher than in GnRH antagonist group. Other parameters such as fertilization rate, viable embryo rate, and the cycle cancellation rate due to no viable embryos were similar (P> 0.05). During the follow-up period of FET, a total of 120 patients completed 182 FET cycles, and single embryo transfer was performed in 93.6% of the patients. No significant difference was found in the clinical pregnancy rate (DYG group; 43.3% vs. GnRH antagonist group; 38.8%, P = 0.541), respectively (difference 4.48%; 95% confidence interval (CI): -9.86 – 18.8%), and ongoing pregnancy rate (DYG group; 38.1% vs. GnRH antagonist group; 31.8%, P = 0.369), respectively (difference 6.38%; 95% CI: -7.52 – 20.3%).

Limitations, reasons for caution: Patients affected of all stage of endometriosis have been included and the number of patients was small, because of single-center setting. A large sample size is needed to draw a firm conclusion.

Wider implications of the findings: This study shows that DYG regimen is feasible to improve the number of oocyte collected, and DYG has the advantages of oral administration, user convenience and cost reduction, so possibly providing a new choice for women with endometriosis undergoing IVF/ICSI treatment in combination with FET.

Trial registration number: Not applicable.

P-251 The expression of microRNA in uterine leiomyoma cell: submucosal versus subserosal leiomyoma

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Study question: To compare the expression of microRNA (miR)s in leiomyoma cells according to their location in the uterus.

Summary answer: The expression profile of miR in leiomyoma cells was different according to their location in the uterus.

What is known already: The expression profile of miR in uterine leiomyoma cells is different from that of normal uterine myometrial cell. Although the impact of uterine leiomyoma on uterine receptivity to embryos has been known to vary according to their location in the uterus, the difference of miR expression in submucosal versus subserosal leiomyoma cells is still rare known.

Study design, size, duration: To analysis of expression of miR and target gene, 18 of matched sample of leiomyoma and myometrium tissue were obtained from 9 of women with subserosal leiomyoma and 9 of women with submucosal leiomyoma by pelviscopic myomectomy.

Participants/materials, setting, methods: The relative expressions of miRs related leiomyoma and the candidate target genes in leiomyoma cells were compared to the related myometrial cells by RT-PCR. The target gene expression was analyzed after in vitro culture of leiomyoma cells with transfection of miR analogues. All experiments were repeated more than 3 times with more than 3 different portion of samples.

Main results and the role of chance: The relative expressions of miR-29 (0.633-fold, P = 0.031) in leiomyoma cells were downregulated compared to the myometrial cells and the relative expressions of estrogen receptor β (2.121-fold, P<0.001) and matrix metalloproteinase-1 (1.923-fold, P<0.001) were upregulated in leiomyoma cells compared to myometrial cells. After transfection with mimic of miR-29 into leiomyoma cells in-vitro-cultured, the expression of estrogen receptor β and matrix metalloproteinase-1 were downregulated (0.215-fold and 0.105-fold, P<0.001 in all). After transfection with mimic and inhibitor of miR-29 into leiomyoma cells in-vitro-cultured, the expression of estrogen receptor β and matrix metalloproteinase-1 were down- (0.215-fold and 0.105-fold, P<0.001 in all) and up-regulated (8.354-fold and 4.721-fold, P<0.001 in all). The expressions of miR-29 in submucosal leiomyoma relative to myometrium was upregulated compared to the subserosal leiomyoma relative to myometrium (1.213 ± 0.562 vs. 0.412 ± 0.214, P<0.001). The expression of estrogen receptor β (1.515 ± 0.467 vs. 2.124 ± 0.870, P = 0.041) and matrix metalloproteinase-1 (1.484 ± 0.230 vs. 2.221 ± 1.021, P = 0.021) in submucosal leiomyoma cells were downregulated compared to subserosal leiomyoma cells.

Limitations, reasons for caution: To fully understanding the regulatory roles of miRs on leiomyoma growth, further extended study with the other miRs related to leiomyoma should be necessary.

Wider implications of the findings: The difference in profiles of miR expression between submucosal and subserosal leiomyoma may implicate that miRs relate the growth of leiomyoma to alter the biomechanics of the uterus.

Trial registration number: Not available/This study was supported by grants of Ministry of Education, Republic of Korea (2016R1D1A1A02937287).

P-252 Comparison of uterine natural killer (uNK) cells count in the peri-implantation endometrium between natural cycles and hormone replacement therapy cycles

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Study question: Is there any difference in uNK cells count between natural cycles and hormonal replacement therapy (HRT) cycles in women undergoing IVF-ET treatment around the time of implantation?

Summary answer: There is no significant difference in uNK cells count between natural cycles and HRT cycles in the peri-implantation period.

What is known already: Our previous study, using a standardized method for uNK cells counting, has established a reference range for uNK cells count in natural cycles. However, the number of uNK cells may be influenced by the hormone level and the findings may not apply to HRT cycles in women undergoing IVF-ET treatment.

Study design, size, duration: A total of 163 women from two IVF centres participated in the study, including 55 women in natural cycles and 108 women in HRT cycles. All endometrial biopsies were collected precisely on the putative day of blastocyst transfer, that is seven days after LH surge (LH+7) of the natural cycles or five days after initiation of progesterone (P+5) of the HRT cycles.

Participants/materials, setting, methods: Endometrial sections were immunostained for CD56 to identify uNK cells. Image capture and cell counting were performed by a standardised protocol agreed and published by the participating centres. Results were expressed as percentage of positive uNK cells/total stromal cells.

Main results and the role of chance: There is no significant difference (p>0.05) in uNK cells count between natural cycles (median 2.28%, range 0.99-4.78%) and HRT cycles (median 2.55%, range 0.69-5.02%) in women undergoing IVF-ET treatment on the putative day of blastocyst transfer. Using our established reference range from 1.2% to 4.5% for uNK cell percentage, there is no significant difference in high uNK cell rate between natural cycles (9.1%, 5/55) and HRT cycles (10.2%, 11/108).

Limitations, reasons for caution: The prognostic value of uNK cells measurement has yet to be confirmed.

Wider implications of the findings: Observations on uNK cells in natural cycles may be applicable to those in HRT cycles. Further studies may be considered whether the same conclusion applies to other receptivity markers.

Trial registration number: non.

P-253 The effect and mechanisms of GM-CSF on endometrial regeneration

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Study question: Does granulocyte macrophage colony-stimulating factor (GM-CSF) play a role in endometrium and can it be used as a new application for treating thin endometrium?

Summary answer: GM-CSF might play a key role in endometrium and could provide new ideas for improving endometrial condition.

What is known already: GM-CSF is a cytokine normally expressed in the female reproductive tract and has key roles in embryo implantation and subsequent development. GM-CSF receptor in human endometrium suggests that epithelial-derived GM-CSF may influence various endometrial biological activities and local inflammatory response. However, the role and mechanism of GM-CSF on the endometrium have not been investigated.

Study design, size, duration: A total of 57 female ICR mice (aged 8 weeks) were used to generate thin endometrium model and assess the effect of GM-CSF. Human endometrial tissues were obtained from patients who underwent hysterectomy for uterine myomas, and patients who underwent hysteroscopy and endometrial biopsy for infertility examination.

Participants/materials, setting, methods: We established thin endometrium mice model by uterine perfusion with 20 μL 90% ethanol. GM-CSF were intraperitoneal injected and then the endometrial thickness by HE staining, Ki67 expression by immunohistochemistry and the number of embryo implantation after mating were evaluated, comparing to the control group. The effects of GM-CSF on primary cultured human endometrial glandular and stromal cells were examined by BrdU assay, transwell assay, followed by exploring protein signaling pathway by Western Blot.

Main results and the role of chance: 90% ethanol caused distinct endometrial injury in mice, with observably thinner endometrial thickness, decreased expression of Ki67 in endometrial glandular cells, and reduced number of embryo implantation. Compared with the saline group, GM-CSF injection was significantly beneficial for rescuing the thickness of impaired mice endometrium, improving the expression of Ki67 in endometrial glandular cells, and increasing the number of embryo implantation. GM-CSF significantly promoted the proliferation of primary cultured human endometrial glandular cells and the migration of stromal cells. GM-CSF significantly activated p-Akt and increased the expression of p70S6K and c-Jun protein, which could be blocked by LY294002, the PI3K/Akt pathway inhibitor.Our study found that GM-CSF is beneficial for regeneration of injured endometrium in mice, and can promote the proliferation of primary human endometrial glandular cells and the migration of endometrial stromal cells. The effect of GM-CSF on endometrial glandular cell proliferation is via activating the PI3K/Akt signaling pathway.

Limitations, reasons for caution: I.p. injection of GM-CSF in mice can not totally simulate human uterine perfusion.

Wider implications of the findings: We found that GM-CSF can improve endometrial regeneration and it has potential application in clinical therapies to improve reproduction. It provides new ideas for the treatment of infertile patients, who have difficulty in endometrial proliferation, with thin endometrium or severe intrauterine adhesion.

Trial registration number: Not applicable.

P-254 Treatment outcomes in women with endometrioma at time of IVF/ICSI treatment. An analysis of 4416 fresh and frozen treatment cycles using mainly SET

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Study question: Do IVF/ICSI outcomes differ between patients with presence of ovarian endometrioma versus patients with infertility from other causes?

Summary answer: Women with endometrioma had a significantly reduced ovarian response compared to controls and required higher gonadotropin stimulation doses; however, treatments resulted in similar cumulative live-birth.

What is known already: Single-embryo transfer (SET) with subsequent frozen-embryo transfer was shown to result in similar cumulative live-birth rates compared to double-embryo transfer in patients <36 years. Some studies suggested a negative effect of endometrioma on oocyte yield and number of embryos cryopreserved, which maintain live-birth rate in the fresh cycles although cumulative live-birth rate might be reduced.

Study design, size, duration: Single-center, retrospective cohort study of patients treated between January 2009 - December 2013. A total of 2651 patients underwent 4416 treatment cycles (2426 fresh and 1990 frozen). In 66 cases, the women presented with endometrioma at time of IVF/ICSI treatment (cases) and they were compared to infertile women without endometriosis (controls N = 2585).

Participants/materials, setting, methods: Seventy percent of patients underwent IVF/ICSI followed by SET and single FET in consecutive frozen-thawed cycles. Primary outcome measures: Oocyte and embryo yield, ovarian sensitivity index and live birth rate in fresh and frozen cycles as well as cumulative. Multivariable GEE models were applied including all subsequent treatment cycles, controlling for age, stimulation protocol and IVF/ICSI.

Main results and the role of chance: Women with endometrioma were similar to controls as regards to age (33.56 vs. 34.05 years, p = 0.31) or BMI (p = 0.97), however, they required higher gonadotropin doses for stimulation (3081 vs. 2370 IU FSH; p = 0.03) and their treatments resulted in reduced oocyte yields (7.64 vs. 9.54 retrieved oocytes, p = 0.0055) and number of frozen embryos (1.86 vs. 2.51; p = 0.0042). In fresh or frozen cycles, live-birth rates were similar between women with endometrioma vs. controls (25.0% vs. 25.5% per OPU, respectively, p = 0.94, and 27.5% vs. 28.4% per ET, respectively, p = 0.78, and FET per ET 25.0% vs. 26.2%, respectively, p = 0.86). The cumulative live-birth per OPU did not differ between the groups (38.4% vs. 34.7%, respectively, p = 0.39).

Limitations, reasons for caution: Retrospective character of the study. Endometrioma might be un- or misdiagnosed.

Wider implications of the findings: Elective SET and consecutive frozen-thawed cycles can be encouraged also in patients with endometrioma. Women with endometrioma had a reduced ovarian sensitivity to gonadotropins resulting in lower oocyte yields and less frozen embryos but similar live-birth rates per ET when compared to controls.

Trial registration number: Not applicable.

P-255 Impact of endometrial thickness on IVF/ICSI outcome

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Study question: To determine any existing correlation among endometrial lining thickness (ELT) and pregnancy out come in patient's undergone IVF/ICSI cycle.

Summary answer: ELT was found to be an independent positive prognostic factor for clinical pregnancy outcome.

What is known already: Receptivity of endometrium is a pre-requisite for initiation of a thriving pregnancy during IVF/ICSI treatment. Endometrium proliferates under hormonal influence especially estrogen and progesterone, involved in the control of endometrial thickness measured through trans-vaginal ultrasonic scanning (TVS).

Study design, size, duration: A retrospective cohort study was conducted on the available clinical data of 1500 patients, since from January 2015 to December 2017 at the Lahore institute of fertility and endocrinology, Hameed Latif Hospital Lahore, Pakistan.

Participants/materials, setting, methods: Fifteen hundreds cycles were divided into three groups based on ELT on the hCG day; First Group with ELT < 7-8 mm, Second group 2 ELT 8-14 mm and third group ELT > 14 mm.

A logistic regression analysis was conducted using a stepwise procedure to identify the variables which were significantly associated with the outcome of clinical pregnancy. Furthermore, ROC (Receiver-operating Curve analysis) and Chi-square test were used to validate the whole model.

Main results and the role of chance: Our primary outcome is that thicker endometrial lining associated with positive beta-hCG and clinical pregnancy. First group had significantly decreased pregnancy rate, embryo implantation and live birth rates than 2nd and 3rd group ($p < 0.001$). However, no significant difference was found among spontaneous abortion and miscarriage rate in between the three groups. A higher clinical pregnancy rate was observed when ELT > 15 mm.

Limitations, reasons for caution: not applicable.

Wider implications of the findings: the ranges of endometrium defines in the abstract. the endometrium range divided into three categories.

Trial registration number: not applicable.

P-256 Influence of the microRNA let-7d on epithelial-to-mesenchmal transition in endometriosis

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Study question: Does the microRNA let-7d have an impact on epithelial-to-mesenchymal transition in endometriosis?

Summary answer: Let-7d differentially affects the expression of mesenchymal markers in epithelial endometriotic and endometrial stroma cells.

What is known already: MicroRNAs are small noncoding RNAs that regulate gene expression at the posttranscriptional level. A dysregulation of miRNAs is observed in endometriosis, and may be functionally linked to the pathogenetic process. Let-7d is implicated in regulating EMT in malignancies. Let-7d is downregulated in the serum of endometriosis patients during the proliferative phase.

Study design, size, duration: In vitro study on the endometriotic epithelial cell line 12Z and the endometrial stroma cell line ST-T1b.

Participants/materials, setting, methods: Cells were transiently transfected with let-7d precursors to study the effects of miRNA upregulation in vitro. The impact of let-7d on cell viability and cell cycle progression was studied by MTT assay and flow cytometry, respectively, whereas invasiveness was assayed in matrigel invasion chambers. Predicted targets of let-7d were identified by microRNA.org database analysis, and target regulation was confirmed by quantitative real-time PCR and Western Blotting.

Main results and the role of chance: Let-7d upregulation had no significant effect on cell viability, whereas cell cycle analysis revealed a shift to the gap phases (G1/G2/M) in let-7d-transfected St-T1b cells. Data in the invasion assay were highly variable. A differential effect of let-7d upregulation was observed in endometrial stroma cells and endometriotic epithelial cells: In St-T1b cells, let-7d induced downregulation of the mesenchymal markers fibronectin and N-cadherin ($n>3$, $p<0.05$), whereas a panel of mesenchymal markers (Fibronectin, Snail1, Snail2, Vimentin, Twist and ZEB2) was upregulated upon let-7d transfection in 12Z cells ($n>3$, $p<0.05$).

Limitations, reasons for caution: This is an in vitro study based on a transient transfection approach in immortalized cell line models. Additional targets may be involved in the phenotypic changes.

Wider implications of the findings: Let-7d differentially affects the expression of mesenchymal markers in endometrial stroma and epithelial endometriotic cells, and may be implicated in regulating EMT as a contributing factor to locally invasive growth of endometriotic cells. Further exploration of this hypothesis in primary cells and animal models is worthwhile.

Trial registration number: Not applicable.

P-257 Caesalpinia sappan induces apoptosis of ectopic endometrial cells through inhibition of pyruvate dehydrogenase kinase

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Study question: We thought that if we returned the Warburg-like metabolic reprogrammed metabolism of endometriosis to normal metabolism, we could induce apoptosis of endometriosis.

Summary answer: The expression of TGF-β, PDK-1, PDK-2, PDK-4 and lactate production were increased in 12Z cells. Activated oxidative phosphorylation decreased mitochondrial membrane potential and increased ROS.

What is known already: Endometriosis has similar characteristics to cancer. It has been reported lactate production was elevated in endometriosis patients, and previous studies have also suggested that Warburg-like metabolic reprogramming may be associated with endometriosis. Heartwood of *Caesalpinia sappan(CS)* have been a medicinal herb used for improving blood circulation, accelerating hemostasis, removing of extravasated blood and reducing swelling. Especially, heartwood of CS was used for treating gynecological symptoms including algomenorrhea and amenorrhea. Recently, it has been reported that treatment of cisplatin with CS components arrests cell cycle and increases apoptosis in colon cancer. However, the effect of CS on endometriosis was not studied.

Study design, size, duration: Heartwood of CS was crushed and extracted with distilled water. The extract was filtered and concentrated using a rotary evaporator and lypophilized using a freeze dryer to yield of powder. The powder was dissolved in DMSO to prepare a stock solution and was diluted with culture medium prior to use in the in vitro experiments. Immortalized normal human endometrial (HES cells) and endometriotic epithelial cells (12Z) were used.

Participants/materials, setting, methods: The cytotoxicity of CS was examined using a MTT assay and lactate production of 12Z cells were measured with fluorometric assay kit. LDH activity was determed by measuring the decrease of absorbance at 340 nm. Also, in vitro pyruvate dehydrogenase kinase assay was done. Total RNA and protein was isolated from HES and 12Z cells and measured by RT-PCR and western blot analysis. Apoptotic cells treated with CS in12Z cells were examined.

Main results and the role of chance: The lactate production of 12Z cells was further increased higher than that of HES cells. There was no change in LDHA expression in between HES and 12Z cells. However, the phosphorylation of PDH was increased in 12Z cells. TGF-β expression was higher in 12Z cells than in HES cells. 12Z cells were more sensitive to CS-induced cytotoxicity compared with HES cells. CS reduced lactate production but not inhibited activity nor expression of LDHA. CS inhibited the phosphorylation of PDH through suppressing PDKs expression. CS produced ROS production in endometriotic cells.). In addition, cell viability reduced by CS is reversed by NAC treatment. CS induced apoptosis in endometriotic cells. In previous studies, endometriosis was resistant to apoptosis. These anti-apoptotic cells develop into endometriosis of endometrial cells refluxed into the abdominal cavity. Experiments were conducted to determine whether apoptosis of endometriotic cells was induced by CS treatment. 12Z cells were identified by FACS in that apoptotic cells were increased by CS treatment. The apoptosis signals were also increased in cleaved caspase 3 and cleaved PARP, confirming the occurrence of apoptosis.

Limitations, reasons for caution: In this study, CS treatment did not inhibit the downstream of TGF-β signaling pathway, but decreased PDK expression. Therefore, it is necessary to elucidate the mechanism responsible for suppressing PDK expression by CS treatment. The dose of CS should be determined by further *in vivo* studies.

Wider implications of the findings: We suggest that CS can be a candidate for development of novel drug treating endometriosis through inhibiting aerobic glycolysis and inducing ROS-mitochondria mediated apoptotic cell death.

Trial registration number: not applicable.

P-258 Extended doxycycline treatment versus salpingectomy in the management of patients of hydrosalpinx undergoing IVF-ET

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Study question: Is treatment with doxycycline before and after oocyte retrieval as effective as salpingectomy in minimizing the detrimental effect of hydrosalpinx on the outcomes of IVF-ET?

Summary answer: Salpingectomy is more effective than extended doxycycline treatment before and after oocyte retrieval in improving the outcomes of IVF-ET in patients with hydrosalpinx undergoing IVF-ET.

What is known already: Several prospective and retrospective studies revealed that hydrosalpinx has a detrimental effect on the outcomes of IVF-ET. A Cochrane review revealed that laparoscopic salpingectomy improves the outcomes of IVF-ET. A small retrospective study revealed that extended doxycycline treatment before and after oocyte retrieval was as effective as salpingectomy in improving the outcomes of IVF-ET.

Study design, size, duration: A retrospective analysis of the outcomes of the fresh IVF-ET cycles for patients with hydrosalpinx who were treated with salpingectomy prior to IVF cycle (n = 260) or extended doxycycline treatment (n = 45) in Riyadh fertility and reproductive health center during the period between 2012 and 2017. Laparoscopic salpingectomy or ultrasound guided aspiration of hydrosalpingeal fluid were offered to patients. Patients who declined surgery and aspiration of hydrosalpingeal fluid received doxycycline treatment.

Participants/materials, setting, methods: In doxycycline group, doxycycline (100 mg/ 12 h) was started one week before anticipated oocyte retrieval and continued for one week after oocyte retrieval. In salpingectomy group, laparoscopic salpingectomy was performed two months before IVF-ET cycle. The first attempt IVF-ET cycles after treatment were included in the analysis. Patients with age ≥ 37 years, anti-Müllerian hormone (AMH) ≤ 0.66 ng/ml, antral follicle count (AFC) < 6 and previous IVF cycles were excluded from the study.

Main results and the role of chance: The implantation, clinical pregnancy, ongoing pregnancy and live birth rates were significantly higher in the salpingectomy group (20.91% Vs. 9.91%, P value = 0.007, 44.62% Vs. 20% P value = 0.002, 39.62% Vs. 17.78%,P value = 0.005 and 37.31%Vs. 15.56% P value = 0.005 respectively) The abortion rate was comparable between both groups (11.21% Vs. 11.11 %, P value = 0.993). No significant differences were detected between both groups in age, basal FSH, AFC, AMH level, retrieved oocytes, fertilization rate, quality of embryos transferred and number of embryos transferred. None of the patients in salpingectomy group had peri-operative complications.

Limitations, reasons for caution: The retrospective design and the small sample size are the main limitations of the study.

Wider implications of the findings: The data presented in the current study suggest that extended doxycycline treatment before and after oocyte retrieval is not effective in minimizing the detrimental effect of hydrosalpinx on the outcomes of IVF-ET. Further, larger well designed randomized controlled trials should be conducted to confirm the findings of this study.

Trial registration number: Not applicable.

P-259 Exploring the RNA landscape of uterine fluid extracellular vesicles

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Study question: Can RNA species present in uterine fluid (UF)-derived extracellular vesicles (EVs) represent a potential noninvasive proxy of respective endometrial tissue transcripts?

Summary answer: RNA species in UF-derived EVs mirror the transcriptome of endometrial tissue, can discriminate between the menstrual phases and are enriched in molecules involved in migration.

What is known already: The human endometrium is a highly dynamic tissue that is cyclically shed, repaired, regenerated and remodelled, primarily under the orchestration of ovarian-derived hormones. Studies addressing the dynamics of phase transition and tissue abnormal remodelling have been based so far on endometrial sampling analysis that is unfortunately associated with several problems. EVs of uterine origin has been recently identified. Selective packaging of RNA/miRNA profile into uterine EVs has been demonstrated using cell lines. Data on the comparative RNA profile between the endometrial tissues and the corresponding UF-derived EVs are very limited.

Study design, size, duration: Endometrial tissues and UF samples were simultaneously obtained from proven fertility women (n = 10). Endometrial tissues were obtained with the use of a pipelle while UFs were obtained with a balloon hysterosonosonography catheter to avoid vaginal contamination. Based on the histological dating, 5 samples were in proliferative and 5 in secretory phase of the cycle. EVs have been isolated by differential ultracentrifugations.

Participants/materials, setting, methods: For RNAseq analysis, libraries were synthesized using Lexogen 3'UTR mRNA kit, starting from total RNA. In average, 10 M reads of 75 nt length were produced by Illumina NextSeq 500 via SBS technology. Two parallel differential expression (DGE) analyses were performed comparing RNA presence in proliferative and secretory phase on correspondent tissues and EVs. RNAs in EVs were ranked according to the normalized cpm counts. The Webgestalt platform was used for enrichment analysis.

Main results and the role of chance: Biotype composition of RNA species for each sample recognized most of them as derived from protein coding genes (86% in tissues and 77% in EVs), but fractions of Mt_rRNA (6% in tissues and 15% in EVs) and lincRNA (5% in tissues and 6% in EVs) were also present. Considering as 'expressed' those genes that showed at least 1 cpm on at least 5 samples, 15268 genes defined tissue samples transcriptional profile, while only 4132 were found in UF-derived EVs. Even if 'expression' in EVs was generally more unstable, it correlated well with that observed in tissues, especially considering mean values (Spearman coefficient 0.81). DGE analysis could identify 139 genes up-regulated in UF-derived EVs in the secretory phase ($p<0.01$) and none up-regulated in the proliferative phase. Nine genes (TMEM37, TNS2, RIMKLB, PABPN1, ITGB8, ALDH1A3, SNHG25, DCDC2, MECOM) varied simultaneously in tissues and UF-derived EVs according to phases. Among the biological processes enriched in the 20% RNAs with the greatest difference in ranking of secretory versus proliferative phase, those involved in cell migration regulation were identified. Of 57 meta-signature genes recently identified as human receptivity transcriptomic biomarkers (Scie Rep 2017), 18 (31%) were found to be present in UF-derived EVs.

Limitations, reasons for caution: UF-EVs samples showed an overall greater variability in cpm means among samples compared to those found in corresponding endometrial tissues. Therefore, the definitive RNA profile should be evaluated in a greater number of samples.

Wider implications of the findings: UF-derived EVs might be used as non-invasive endometrial biopsy to predict endometrial normal functions and dysfunctions becoming an advancement in the molecular medicine era.

Trial registration number: not applicable.

P-260 Extended doxycycline treatment versus aspiration of hydrosalpingeal fluid at the time of oocyte retrieval in the management of patients with ultrasound visible hydrosalpinx undergoing IVF-ET

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Study question: Is treatment with doxycycline before and after oocyte retrieval as effective as ultrasound guided aspiration of hydrosalpingeal fluid in minimizing the detrimental effect of hydrosalpinx on the outcomes of IVF-ET?

Summary answer: Ultrasound guided aspiration of hydrosalpingeal fluid is more effective than extended doxycycline treatment in improving the outcomes of IVF-ET in patients with ultrasound visible hydrosalpinx.

What is known already: The negative impact of the hydrosalpinx on the outcomes of IVF-ET has been confirmed by several retrospective and prospective studies. Several randomized controlled trials revealed that ultrasound guided aspiration of hydrosalpingeal fluid is effective in improving the outcomes of IVF-ET. A small retrospective study revealed that extended doxycycline treatment before and after oocyte retrieval was effective in minimizing the detrimental effect of hydrosalpinx on the outcomes of IVF-ET. Till now, no studies have yet compared the effectiveness of ultrasound guided aspiration of hydrosalpingeal fluid with extended doxycycline treatment in the management of patients with ultrasound visible hydrosalpinx undergoing IVF-ET.

Study design, size, duration: A retrospective analysis of the outcomes of the IVF-ET cycles for patients with ultrasound visible hydrosalpinx who were treated with ultrasound guided aspiration of hydrosalpingeal fluid (n = 61) or extended doxycycline treatment (n = 36) in Riyadh fertility and reproductive health center during the period between 2012 and 2017. Laparoscopic salpingectomy or aspiration of hydrosalpingeal fluid were offered to patients. Patients who declined surgery and aspiration of hydrosalpingeal fluid received doxycycline treatment.

Participants/materials, setting, methods: In doxycycline group, doxycycline (100 mg/12 hours) was administered one week before the expected time of oocyte retrieval and continued for one week after oocyte retrieval. In aspiration group, aspiration of hydrosalpingeal fluid was performed under ultrasound guidance at the time of oocyte retrieval. The analysis included the first attempt IVF-ET cycles after treatment. Exclusion criteria were age $\geq$ 37 years, anti-Müllerian hormone (AMH) $\leq$ 0.66 ng/ml, and antral follicle count (AFC) < 6.

Main results and the role of chance: The implantation, clinical pregnancy and live birth rates were significantly higher in the aspiration group (16.35% Vs. 6.52 %, P value = 0.024, 36.07% Vs. 16.67% P value = 0.042, and 32.79% Vs. 13.89 %,P value = 0.039 respectively). The abortion rate was comparable between both groups (9.09% Vs. 16.67%, P value = 0.595). The age, basal FSH, AFC, AMH level, duration of infertility, percentage of patients with bilateral hydrosalpinx, retrieved oocytes, fertilization rate, quality of embryos transferred and number of embryos transferred were comparable between both groups. None of the patients in the aspiration group had flaring of infection or peritonitis.

Limitations, reasons for caution: The main limitations of the current study are the retrospective design and the small sample size. The results of this study should be confirmed by larger well designed randomized controlled trials.

Wider implications of the findings: Extended doxycycline treatment before and after oocyte retrieval should not be used in the management of patients with ultrasound visible hydrosalpinx who declined salpingectomy prior to IVF-ET. Ultrasound guided aspiration of hydrosalpingeal fluid is a simple, safe and effective option for those patients.

Trial registration number: Not applicable.

P-261 Fertility preservation outcomes in endometriosis patients: A Pilot Study

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Study question: Does endometriosis affect ART outcomes for fertility preservation?

Summary answer: The size of endometrioma inversely affects the number and quality of oocytes and embryos, the clinical pregnancy rate is not related to the endometriosis characteristics.

What is known already: Fertility preservation may be of interest for women with endometriosis, particularly whom with bilateral un-operated endometriomas and those who previously had excision of unilateral endometriomas and require surgery for a contralateral recurrence. However, we should consider the probability of low successful outcomes and complication of the ovarian stimulation and ovum pick up in endometriosis patients. the feasibility and potential benefits of fertility preservation procedures in women with endometriosis are warranted prior to implementing its use in routine clinical practice.

Study design, size, duration: This cross-sectional study has conducted since March 2017 to January 2018, 52 patients with endometriosis who were referred for fertility preservation via ART, have been studied. They underwent controlled ovarian stimulation and ART results including number and quality of oocytes and embryos and also clinical pregnancy rate were obtained. Endometriosis characteristics such as presence, size, laterality, the number of endometriomas, the coexistence of DIE and previous history of endometriosis surgery were registered.

Participants/materials, setting, methods: 57 controlled ovarian stimulation cycles with GnRH antagonist protocol have been studied, and ovarian puncture were performed, then ART results were followed. The number and quality of oocytes and embryos were assessed and the clinical pregnancy rate was calculated in patients who underwent embryo-transfer (18 patients). Multivariate regression analysis, bi variate correlations and Chi-square test were applied for evaluation of impressive factors on ART results. The level of significance was considered 0.05.

Main results and the role of chance: Controlled ovarian stimulation and ovum pick up were performed for 52 patients, 10 patients had repeated COH cycles and embryo cryopreservation has performed, except 5 patients who had Oocytes cryopreservation. COH cycles were canceled for 5 cases due to poor ovarian responses. 18 patients had 23 embryo transferred cycle. Age and BMI mean and SD of cases were 33.11 $\pm$ 4.72 and 23.87 $\pm$ 3.9 respectively. From 46 patients who have previously suffered from infertility, there was mild to moderate male factor in16 cases. There was an inverse relation between the size of endometrioma and number of oocytes(P-value = 0.011), the quality of oocytes (P-value = 0.002), the number of embryos (Pvalue = 0.004), the quality of embryos(Pvalue = 0.007). The number and quality of oocytes and embryos have related to age and AMH levels significantly. Although these effects were not significant for clinical pregnancy rate (26% per embryo transfer) and there is no statistical relationship between those ART outcomes and endometriosis characteristic such as DIE, laterality and number of endometrioma and previous surgery history.

Limitations, reasons for caution: The cases of the study are more severe than total endometriosis population due to referral nature of Avicenna infertility clinic and endometriosis clinic. Because of rare cases of fertility preservation in normal population, selection of a standard control group seems unavailable.

Wider implications of the findings: Age, AMH level and the size of endometrioma significantly affect the number and quality of oocytes and embryos in endometriosis patients who were referred for fertility preservation.Our intresting findings offer further study to find which endometriosis patients benefit from fertility preservation via ART.

Trial registration number: This study is not clinical trial.

P-262 The gametotoxic effects of the endometrioma content: insights from a parthenogenetic human model

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Study question: To evaluate the effect of exposure of human cryopreserved oocytes to endometriotic fluid using a validated experimental model of human parthenogenesis.

Summary answer: Exposure of human oocytes to endometriotic fluid has a negative effect on the morphology of deriving embryos/parthenotes mainly due to an excess of cellular fragmentation.

What is known already: Exposure to the content of endometriotic cysts may be harmful to the oocyte and may alter its subsequent developmental potential as an embryo due to the presence of several potentially toxic substances such as growth factors and interleukins, matrix metalloproteinases, catalytic iron and lipid peroxide. The great amount of free iron in endometriotic cysts is a matter of concern because of the risk of local production of the highly toxic reactive oxygen species. However, experimental data supporting these concerns are scanty both in humans and in animal models.

Study design, size, duration: A randomized in vitro study conducted between January 2016 and July 2016. A three-folds decrease in the rate of day 3 good quality parthenotes for exposed oocytes compared to unexposed ones was considered important (from 37% to 12%, based on previuos data). Considering a survival rate of 80% after warming, the total number of oocytes to be used for the study were 140 (type I and II errors equal to 0.05 and 0.20, respectively)

Participants/materials, setting, methods: Oocytes used for the study protocol were donated by consenting women aged <42 years. After thawing, oocytes belonging to every single patient were randomly (1:1) allocated to fertilization medium containing endometriotic fluid (exposed oocytes) or to the fertilization medium (control). After 3 minutes of culture, oocytes were rinsed and activated using ionomycin followed by 3-hours incubation in 2 mM 6-DMAP. Parthenotes were cultured until day 5 and embryological parameters were registered daily.

Main results and the role of chance: Twenty-three women donated a total of 147 vitrified oocytes (range 2-17). After warming, 121 intact oocytes were randomized in 13 sets of experiments. A total of 60 oocytes were randomized to the exposure group while 61 oocytes were randomized to the non exposure group. The day after treatment and activation, 42 parthenotes (70%) among exposed oocytes and 46 parthenotes (75%) among unexposed oocytes were observed (p = 0.51). The developmental rates on day 3 and day 5 did not significantly differ between the two groups. The RRs (95%CI) of exposed oocytes for activation, cleavage on day 3 and blastulation on day 5 were 0.93 (0.75-1.16), 0.88 (0.65-1.19) and 0.47 (0.19-1.15), respectively. Conversely, data on morphology tend to support a detrimental effect of endometrioma fluid exposure. The rate of day 3 good quality parthenotes was 22% (13/60) and 41% (25/61) in exposed and unexposed oocytes, respectively (RR = 0.53; 95%CI: 0.30-0.93, p = 0.031). A trend was observed also in the development of good quality-expanded blastocysts on day 5, 5% (3/60) and 13% (8/61) of oocytes, respectively (RR = 0.39, 95%CI: 0.11-1.37). A significantly higher proportion of parthenotes failing to develop to the blastocyst stage showed cellular fragmentation in the exposed compared to unexposed parthenotes (RR = 0.64, 95%CI: 1.04-2.57; p = 0.024).

Limitations, reasons for caution: We used a fixed experimental model that cannot mimic all the variability of every possible clinical conditions. Endometriotic fluids from three different patients were used; even though we did not observed differences based on the donor of the endometriotic fluid, we cannot exclude possible different biological effects.

Wider implications of the findings: Even if robust inferences cannot be drawn, because our findings were obtained in an experimental context, we believe that our study has some potential clinical implications and can be interpreted as a scientific support of the common dogma that ovarian endometriomas should not be punctured or aspirated during oocytes retrieval.

Trial registration number: not applicable.

P-263 High mobility group box-1 increases cell proliferation, expression of adhesion molecules, and secretion of cytokines in human endometrial stromal cell in endometriosis

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Study question: Does endogenous ligands such as High mobility group box-1 (HMGB-1) signaling may play a pathogenic role developing endometriosis?

Summary answer: HMGB-1 is important in establishment of endometriosis through inflammatory pathway, which leads increase of inflammatory cytokines and change adhesion molecules in ectopic endometrial stromal cell.

What is known already: Oxidative stress and chronic inflammation play important roles in the pathophysiology of endometriosis. A representative Damage-associated molecular pattern (DAMP), HMGB-1 has previously shown increased release from the endometrial stromal cell at oxidative stress. Moreover, recombinant HMGB-1(rHMGB-1) stimulated cell proliferation and increased expression of Toll like receptor 4(TLR4) in human endometrial stromal cell(HESC), suggesting altered pathologic endometrium according to oxidative stress and chronic, endogenous inflammation.

Study design, size, duration: In-vitro, experimental study using primary cell culture and molecular biologic methods.

Participants/materials, setting, methods: HESC culture was done using ectopic endometrium from the surgical specimen. HESCs were examined to see cell proliferation and HMGB-1 release according to H_2O_2 treatment. Then, HESCs were treated with rHMGB-1 by dose dependent fashion. The supernatants acquired during the rHMGB-1 treatment were examined to measure inflammatory cytokines such as TNF-a, IL-1b, IL-6 and IL-10. Adhesion molecule, angiogenic marker, DAMP receptors were measured by RT-PCR and western blotting from HESCs treated with rHMGB-1.

Main results and the role of chance: HESCs showed decreasing cell viability and increasing HMGB-1 release according to H_2O_2 treatment. Cell proliferation and invasion was significantly increased according to the treated dose of rHMGB-1. Expression of mRNA and the protein expression of TLR4, RAGE, VEGF, ICAM-1 increased significantly according to the treated rHMGB-1 concentration. E-Cadherin showed decrease in mRNA and protein by increase of rHMGB-1 treatment. Inflammatory cytokines from the supernatants of HESC during rHMGB-1 treatment showed significant increase.

Limitations, reasons for caution: Our study is limited in that we only performed in vitro analysis using HESCs. Therefore, in vivo studies are required.

Wider implications of the findings: Our results support that the progression of endometriosis may be guarded by oxidative stress and endogenous stimuli results in chronic inflammatory pathway.

Trial registration number: NRF-2012R1A1A1013167

P-264 Circulating Cf-DNA quantification according to the location of the endometriotic lesions

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Study question: Is there an association between the circulating Cf-DNA (CCf-DNA) and the location of the endometriotic lesions?

Summary answer: CCf-DNA concentrations was higher in the deep pelvic endometriosis compared with control and other types of endometriosis.

What is known already: Circulating Cf-DNA are present in the bloodstream and their quantification is already used as a biomarker for gynaecological disorders such as ovarian and endometrial cancer. To date, only one study reported the potential role of CCf-DNA as potential biomarker for minimal and mild endometriosis. However, no studies analyzed the CCf-DNA according to the location of the endometriotic lesions.

Study design, size, duration: This was an retrospective study using serum prospectively collected from non-pregnant patients from the Cochin Hospital who were between 18 and 41 years of age (mean ± sem: 31.5 ± 0.6 years), and who underwent surgery for symptomatic benign gynecological conditions between January 2011 and December 2013.

Participants/materials, setting, methods: Surgery was performed on 76 patients available preoperative MRIs. After a thorough surgical examination of the abdomino-pelvic cavity, 55 women with histologically proven endometriosis and/or adenomyosis were allocated to the endometriosis group and 21 symptomatic women without evidence of endometriosis with or without adenomyosis to the endometriosis free group. CCf-DNA was extracted from 200 µl of serum and quantified by qPCR using primers amplifying ALU115 sequences in both groups and according to surgical endometriosis phenotypes.

Main results and the role of chance: CCf-DNA concentrations were similar in the endometriosis free group with or without adenomyosis (245 ± 70 *vs* 200 ± 29 fg/µL respectively, p = 0.6). The concentration of CCf-DNA was significantly higher in the endometriosis group than in the control group (342 ± 66 *vs* 215 ± 38 fg/µL, p = 0.1). In addition, accordingly to the location of the endometriotic lesions, the CCf-DNA concentrations was higher in the deep pelvic endometriosis (359 ± 110 *vs* 215 ± 38 fg/µL) compared with control group, superficial peritoneal and ovarian endometriosis.

Limitations, reasons for caution: These findings must be validated in a large cohort of patients to judge of the relevance of the CCf-DNA quantification as potential biomarker of endometriosis.

Wider implications of the findings: These findings of significantly increased concentrations of CCf-DNA in serum of patients with endometriosis suggests that CCf-DNA might be a potential biomarker for developing non-invasive diagnostic test in endometriosis.

Trial registration number: Not applicable.

P-265 The use of home remedies and complementary health approaches in endometriosis

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Study question: How do endometriosis-affected women use complementary health approaches (CHA) and home remedies (HR)?

Summary answer: Persisting disease symptoms and dissatisfaction with medical support are key factors for the use of CHA and HR in women with endometriosis.

What is known already: Endometriosis is characterized by pain symptoms such as dysmenorrhea, dyspareunia and chronic pelvic pain. The physiology of nociception and pain syndromes is not yet fully understood, but it is assumed that somatic mechanisms interact with psychological factors and are further modified by individual differences in pain perception. Surgical treatment of endometriosis lesions followed by hormonal and/or analgesic therapies are typical approaches to alleviate pain symptoms. However, these conventional treatments are often associated with adverse effects and endometriosis pain symptoms may reoccur despite treatment.

Study design, size, duration: Retrospective cross-sectional data analysis of 574 women with endometriosis, recruited in hospitals and associated private practices and self-help groups in Switzerland, Germany and Austria between 2010 and 2016. Primary outcome measures were frequency and perceived efficacy of the use of CHA and HR. The secondary outcome was the analysis of confounders such as duration of disease, stage of disease, disease symptoms, as well as satisfaction with conventional health care.

Participants/materials, setting, methods: Included were women aged 18 to 59 with surgically confirmed endometriosis. Data was acquired with a questionnaire focusing on endometriosis in general, including questions on surgical and hormonal therapies. The use of CHA and HR was assessed. The pain was investigated with a modified version of the validated standard questionnaires such as the Brief Pain Inventory and Pain Disability Index. Outcome measures were compared for the hospital recruited and the self-help recruited group.

Main results and the role of chance: A total of 359 (62.5%) from 574 included women with confirmed diagnosis of endometriosis, applied any form of CHA/HR. The approaches "topical heat" (by 48.6%) and "repose" (by 43.0%) were applied most often, followed by "movement/massage" (by 25.4%), "homeopathy/phytotherapy" (by 22.8%) and "acupuncture/traditional Chinese Medicine (TCM)" (by 22.6%) of the women. Therapeutic effect was assigned high (80.3% for "topical heat", 70% for "repose", 69.2% for "movement/massage", 44.3% for acupuncture/TCM ans 42% for "homeopathy/ phytotherapy". Women suffering from chronic fatiguing disease symptoms selected more often alternative therapies (odds ratio 3.14, 95% confidence interval 1.39–7.11, p = 0.006) compared to women without these characteristics. In total 18.9% (109/574) of women felt well supported by their physician in dealing with endometriosis-associated pain, while 13.8% (79/574) did not at all. Women in the self-help group were significantly more frequently dissatisfied with the treatment provided by their physicians (p<0.001). Furthermore, women dissatisfied with the treatment provided by their physicians used significantly more often complementary approaches. Our study shows that the majority of women with endometriosis seek additional treatment strategies such as CHA/HR to deal with and/or cure symptoms of the disease, as they seem to provide a good symptom alleviation than hormonal therapies.

Limitations, reasons for caution: Data analysis was based on questionnaire information of a non-standardized questionnaire. Apart from reports on endometriosis-related surgery, no other medical records were reviewed. As we asked for lifetime use of medication, we cannot give any information on time relations between conventional and complementary approaches.

Wider implications of the findings: Women dissatisfied with medical support and persisting disease symptoms frequently used CHA and HR. Gaining control and actively participating in the treatment motivates them to seek additional options. Women's' needs should be respected while counselling and a more active role should be offered in developing individual treatment strategies.

Trial registration number: ClinicalTrials.gov NCT 02511626.

P-266 Fatigue – an underestimated symptom in endometriosis

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Study question: Is fatigue a frequent symptom of endometriosis which should be given more consideration?

Summary answer: Fatigue is an underestimated symptom of endometriosis as it affects numerous women with endometriosis, but is not widely considered clinically or discussed in literature.

What is known already: Along with pain, fatigue can be a symptom of endometriosis which may cause major distress and further reduce the quality of life of women with endometriosis. However, few studies with large sample sizes have investigated prevalences and confounders of fatigue as a symptom of endometriosis.

Study design, size, duration: The study was designed as a multi-center matched case-control study. Recruitment took place at hospitals and private practices in Switzerland, Germany, and Austria between 2010-2016. Data was collected from 1120 women, 560 of them with endometriosis.

Participants/materials, setting, methods: Age +/- 3 years and ethnic background were chosen as matching criteria. Endometriosis diagnosis of 560 women was surgically and histologically confirmed while 560 controls were selected by surgical exclusion or absence of symptoms. More women showed an advanced disease stage (61.9% rASRM III or IV). Materials included surgical and histological reports as well as data retrieved from a self-administered questionnaire. Variable relationships were established by regression analysis and associations were quantified as odds ratios.

Main results and the role of chance: Severe fatigue is a very frequent symptom in women diagnosed with endometriosis (50.7% versus 22.5% in control women, p<0.001). Fatigue in endometriosis is associated with insomnia (OR: 10.37, CI: 6.83-15.75, p<0.001), chronic pain (OR: 2.56, CI: 1.81-3.62, p<0.001), and occupational stress (OR: 1.53, CI: 1.10-2.13, p = 0.012), but it is independent of age, time since first diagnosis, and stage of the disease.

Limitations, reasons for caution: Women with asymptomatic endometriosis cannot be excluded in the control group which would lead to underestimation of our results. The data was retrieved from a self-administered questionnaire, therefore, answers are at risk for recall biases. The study's design allows no evaluation of causal effects.

Wider implications of the findings: Our study is the first to systematically investigate fatigue in endometriosis in a large study population. High fatigue prevalences demonstrate the need of addressing it when managing the disease. In addition to treating endometriosis, it would be beneficial to reduce insomnia, pain, and occupational stress to better manage fatigue.

Trial registration number: ClinicalTrials.gov, NCT 02511626

P-267 A specific serum cytokines profile determines disease phenotype in adenomyosis-affected women

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Study question: Is there a specific serum cytokines profile in adenomyosis women according to the adenomyosis phenotype, as compared to disease-free counterpart?

Summary answer: A decrease in serum interleukin (IL)23, IL31, IL25, IL33 levels was identified adenomyosis women with associated diffuse and focal form as compared to disease-free counterpart.

What is known already: Adenomyosis is a benign uterine disorder characterized by the presence of heterotopic endometrial stroma and glands deeply into the myometrium associated with hypertrophy of adjacent uterine smooth muscle cells. Different forms of adenomyosis can be distinguished: Diffuse adenomyosis (DIF-ADE), Focal adenomyosis (FOC-ADE) and the association of diffuse and focal lesions (DI/FOC-ADE). The origin of ectopic endometrial implants within the myometrium is still debated and could be multiple. Abnormal immune phenomena have been described trying to understand adenomyosis physiopathology. However, the immune imbalance in adenomyosis is still poorly understood.

Study design, size, duration: This cohort study, conducted in a tertiary care university hospital, included 80 women who have received a pelvic magnetic resonance imaging (MRI) performed by one senior radiologist during the preoperative work-up.

Participants/materials, setting, methods: According to MRI findings women were allocated to two groups: Adenomyosis (ADE) group (n = 60) and the control group of women without any criteria for ADE at MRI (n = 20). Women in the ADE group were further sub-divided according to adenomyosis phenotypes: DIF-ADE, FOC-ADE and FOC/DIF-ADE. For all women, blood samples were obtained prior to a surgical procedure and serum cytokine levels were assayed by multiplex immunoassay.

Main results and the role of chance: Serum levels of interleukin (IL)23 (237.77 pg/ml ± 70.97 in ADE-group versus 1855.04 ± 1411.33 in control-group, p = 0.019), IL25 (31.98 ± 8.54 vs. 222.08 ± 170.90 respectively, p = 0.006), IL31 (10.13 ± 3.83 vs. 91.51 ± 71.21 respectively, p = 0.034), IL33 (3.77 ± 1.23 vs. 17.86 ± 11.49 respectively, p = 0.016) and IL17F (16.29 ± 2.35 vs. 30.12 ± 8.29 respectively, p = 0.042) were significantly decreased in adenomyosis-affected women as compared to controls. In DIF/FOC –ADE group, a significant decrease of serum IL23, IL31, IL25, IL33 levels were identified as compared to controls.

Limitations, reasons for caution: Diagnostic of adenomyosis was based only on strict imaging criteria and no correlation with local adenomyotic lesions has been performed.

Wider implications of the findings: Our results may provide new clues for understanding the pathogenesis of adenomyosis, potentially associated with an immunotolerant process more pronounced in associated diffuse and focal forms of adenomyosis.

Trial registration number: None.

P-268 Comparison of effect of Silymarin and Cabergoline on experimental model of endometriosis

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Study question: What is the effects of Silymarin and Cabergoline on induced endometrial lesion in experimental model of endometriosis?

Summary answer: Silymarin and Cabergoline administration resulted in decreased size and hystopathological grade of the induced endometrial lesions in experimental endometriosis model of rat.

What is known already: Previous lines of evidence suggest that cabergoline (dopamine agonist) is effective in treatment of patients with endometriosis (reducing size and symptoms). But less is known regarding the effects of silymarin (a natural compound with antioxidant effects).

Study design, size, duration: Twenty four female Sprague-Dawley mature rats.

were used for an experimental study in the Avicenna Research Institute.

Participants/materials, setting, methods: Endometriosis was surgically induced in 24 rats.animals were randomized into three groups. Group I was administered 100 mg/kg Silymarin S.C, group II was given 0.5 mg/kg Cabergoline S.C and group III had no medication.The rats medicated 3 weeks. Then rats were sacrificed and size and histopathological grade of the endometriotic implants and biochemical parameters were evaluated.Serum and peritoneal levels of vascular endothelial growth factor (VEGF), total antioxidant capacity(TAC) and tumor necrosis (TNF)-α were compared between groups.

Main results and the role of chance: All the animals had comparable baseline characteristics. We found that the size of endometrial lesions decreased significantly in cabergoline ($p<0.001$) and silymarin ($p<0.001$) groups. The

histopathology grade was significantly lower in cabergoline ($p<0.001$) and silymarin ($p<0.001$) groups compared to controls. There was no significant difference between study groups regarding serum levels of VEGF, TNF-α and peritoneal levels of TAC. Those receiving silymarin had significantly higher TAC compared to control after 21 days of therapy ($p<0.001$).

Limitations, reasons for caution: The study was performed on animal subjects.Endometriosis studies in humans are challenging to perform as the measured effect of the treatment on the actual peritoneal lesion will necessitate surgical interventions. The second weakness of the current data is the knowledge regarding the administration route of the treatment for human patients.

Wider implications of the findings: cabergoline and silymarin administration resulted in decreased size and histopathologic grade of the induced endometrial lesions in animal model of rat. Silymarin appear to be a virtual novel therapeutic agent for treatment of endometriosis probably due to its potent anti-oxidative properties.

Trial registration number: Not applicable.

P-269 The effect of endometriosis on the antimüllerian hormone level in patients with infertility

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Study question: Among patients with infertility, is endometriosis associated with differences in baseline antimüllerian hormone (AMH) levels?

Summary answer: Patients with endometriosis, regardless of prior ovarian cystectomy, were observed to have lower AMH values and higher incidence of diminished ovarian reserve at infertility evaluation.

What is known already: Patients with moderate to severe endometriosis experience an accelerated decline in AMH levels following ovarian cystectomy which may lead to a decreased reproductive life span. It is unclear whether women with endometriosis have lower AMH levels at baseline. Conversely, polycystic ovarian syndrome is associated with higher AMH levels and a longer time to menopause compared to normo-ovulatory controls.

Study design, size, duration: This retrospective cohort study included three groups of women who presented for infertility evaluation at our tertiary care center from 04/27/2015 to 05/31/2017: 29 women with endometriosis and a history of ovarian cystectomy (EndoOC), 29 women with endometriosis without a history of ovarian cystectomy (Endo), and 240 women with male factor infertility (MFI; control group).

Participants/materials, setting, methods: AMH levels (ng/mL; using Ansh Labs Ultra-Sensitive assay) were measured as part of the routine infertility evaluation. Patients diagnosed with endometriosis were surgically staged per the revised American Society for Reproductive Medicine scoring system. Linear regression using robust standard errors and logistic regression models were adjusted *a priori* for age, body mass index, race, and smoking status to estimate adjusted beta coefficients (aβ) and adjusted odds ratios (aOR) with 95% confidence intervals (CI).

Main results and the role of chance: The mean ± standard deviation of age (years) and body mass index (BMI; kg/m^2) was similar across groups: EndoOC age = 33.4 ± 3.5, BMI = 25.2 ± 5.9; Endo: age = 33.9 ± 3.9, BMI = 25.8 ± 5.7; MFI: age = 34.0 ± 3.6, BMI = 25.4 ± 5.9. Compared to the MFI group (4.0 ± 3.1 ng/mL), a lower mean AMH level was observed in the EndoOC group (3.1 ± 2.9; aβ -1.12, 95% CI [-2.2, -0.05]) and in the Endo group (2.7 ± 2.3; aβ -1.30, 95% CI [-2.17, -0.43]). The mean AMH level was similar for the Endo group compared to the EndoOC group (aβ -0.09, 95% CI [-1.34, 1.16]). Women were more likely to have an AMH <1 in the EndoOC group (24.1%; aOR 3.53, 95% CI [1.22–9.25]) and in the Endo group (24.1%, aOR 2.71, 95% CI [1.00–7.40]), compared to women in the MFI group (10.8%). The proportion of women with an AMH <1 was similar for the Endo group compared to the EndoOC group (aOR 1.00, 95% CI [0.27 – 3.65]).

Limitations, reasons for caution: The generalizability of these results to women with endometriosis and normal fertility may be limited. Given our small sample, we have limited power to detect differences in AMH levels between different stages of endometriosis which may represent important disease heterogeneity.

Wider implications of the findings: Lower baseline AMH levels in infertility patients with endometriosis could suggest an increased rate of decline in ovarian reserve over time in this population. Early referral for fertility assessment and treatment in patients with known endometriosis may be prudent.

Trial registration number: not applicable.

P-270 Oral contraceptive use in women with pelvic pain and endometriosis: history of ineffectiveness or discontinuation due to side-effects

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Study question: To investigate the relationship between failure of combined oral contraceptive pill (COC) regimens due to ineffectiveness or side effects, and pelvic pain severity and quality-of-life in women with endometriosis.

Summary answer: Discontinuation of COC due to ineffectiveness for pain or due to side-effects, was associated with more severe pelvic pain and poorer quality-of-life in endometriosis.

What is known already: COCs are regarded as the first line of therapy in treating endometriosis associated chronic pelvic pain. Although several studies were performed to evaluate their effectiveness, many has reported patient discontinuation of treatment because of side effects intolerability or lack of symptomatic control without expanding this trend.

Study design, size, duration: Analysis of a prospective patient registry from a tertiary care referral center for patients with endometriosis and pelvic pain between December 2013 to and April 2015.

Participants/materials, setting, methods: 656 patients in reproductive age who were prospectively consented for the registry. Patients had to fill the online questionnaire which included eleven point numeric rating scale (0-10) for pain symptoms, Endometriosis Health Profile 30 "EHP-30", Patient Health Questionnaire "PHQ-9" for depression assessment, and Generalized Anxiety Disorder "GAD-7" questionnaire for anxiety assessment.

Main results and the role of chance: Prior cyclical COC use was reported by 362 (55.2%) women, of which 167 (25.5%) stated it was ineffective for their pain and 139 (21.2%) stated they discontinued due to side effects. Previous continuous COC was reported by 267 (40.7%) women, of which 110 (16.8%) stated it was ineffective and 80 (12.2%) stated they discontinued due to side effects. Worse chronic pelvic pain severity in the last 3 months was associated with a history of ineffectiveness of COC use whether cyclical ($p<0.008$) or continuous ($p<0.001$). Worse dysmenorrhea and poorer quality-of-life was present in women who reported a history of COC discontinuation due to side effects.

Limitations, reasons for caution: Retrospective analysis potentiates for selection and information bias. However, our data was collected prospectively through online patient questionnaire, thus it was dependent on the accuracy of subject's input.

Wider implications of the findings: Further research is needed to identify novel treatment approaches for women with endometriosis who are non-responders to COC.

Trial registration number: not applicable.

P-271 Reciprocal changes of H3K27ac and H3K27me3 at the promoter regions of the critical genes for endometrial decidulaization

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Study question: To capture the epigenetic dynamics and to understand its roles during decidualization, we conducted transcriptome and epigenome profiling for endometrial stromal cells and decidualized cells.

Summary answer: We discovered that the promoter regions of the genes critical for decidualization are often associated with the reciprocal changes of H3K27ac and H3K27me3 marks.

What is known already: Decidualization, the transformation of endometrial stromal cells (ESCs) into secretory decidual cells, is essential for embryo implantation and placental development, and is dependent on the postovulatory increases in progesterone and local cyclic AMP production levels in humans. The responsiveness of ESCs to the hormonal cues is thought to be potentiated by genome-wide chromatin remodeling followed by the coordinated action of decidua-specific transcriptional networks. However, information for such epigenetic alterations has been limited.

Study design, size, duration: Endometrial biopsies were obtained from the uterine fundus from two women of reproductive age without endometriosis who underwent laparoscopic cystectomy. The tissues were processed for primary endometrial stromal cell culture. The primary cells remained untreated (D0), or decidualized for 4 days (D4) and 8 days (D8) in the presence of 8-bromo-cAMP and progesterone. Genomic DNA, total RNA, and formaldehyde-fixed chromatin were prepared from cells.

Participants/materials, setting, methods: The resultant endometrial stromal (D0) and decidualized cells (D4, D8) from two donors were subjected to transcriptome and methylome profiling as well as chromatin immunoprecipitation followed by next-generation sequencing (ChIPseq) for H3K27ac (an active chromatin mark), and for H3K9me3 and H3K27me3 (repressive chromatin marks). DNA methylation, gene expression, and histone enrichment profiles were compared between untreated (D0) and decidualized (D4 and D8) cells to search for differential gene expression and epigenetic modifications upon decidualization.

Main results and the role of chance: Although the H3K27ac and H3K27me3 profiles have already been reported previously, our data for these histone modifications enabled us to have detected larger numbers of peaks and differentially enriched regions upon decidualization. Among the epigenetic modifications examined (DNA methylation, H3K27ac, H3K9me3, and H3K27me3), the H3K27ac patterns changed most dramatically, with a moderate correlation with gene expression changes, upon decidualization. We revealed that subsets of up- and down-regulated genes upon decidualization were associated with reciprocal changes of H3K27ac and H3K27me3 modifications at their promoter region and were enriched with genes essential for decidualization. The top 23 genes most extremely up-regulated were found to contain four genes (namely, WNT4, ZBTB16, PROK1, and GREB1) that have been shown to be essential for decidualization. The top 8 genes most extremely down-regulated also contained two genes, CRABP2 and PTHLH, whose down-regulation has been shown to be critical for decidualization. These results demonstrate a central role of epigenetic regulation in the coordinated gene expression changes required for decidualization. We minimized the role of chance in our results by obtaining and assessing datasets from two independent donors. Our dataset is useful to elucidate further the molecular mechanisms underlying decidualization.

Limitations, reasons for caution: It should be noted that all results were obtained through the analysis of in vitro decidualized cells from donors without abnormality in endometrium. It is important to obtain epigenome profiles of ESCs and decidualized cells from donors with endometriosis and compare such datasets with those obtained in this study.

Wider implications of the findings: siRNA knockdown screening for the genes with the reciprocal changes of H3K27ac and H3K27me3 at their promoter region will help identifying additional critical genes for decidualization. Such genes represent novel targets for the development of therapeutic drugs to recover the implantation and the pregnancy rates of recurrent miscarriages.

Trial registration number: not applicable.

P-272 A comparison of Toll-like receptors and cytokine profiles in the endometrium around the time of implantation between women with and without chronic endometritis

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Study question: Does the presence of chronic endometritis (CE) affect the expression of Toll-like receptors (TLR) and inflammatory cytokine profiles in endometrial tissues around the time of implantation?

Summary answer: The expression of TLR1, 2, 3, and 4 and Interferon gamma (IFN-γ) in the endometrial tissues of women with CE was higher than without CE.

What is known already: The prevalence of CE has been found to be higher in women with recurrent miscarriage and recurrent implantation failure. However, the exact mechanism whereby implantation is affected in women with CE is largely unknown. One earlier study showed the expression of TLR4 was increased in women with CE. Another study showed the alteration of cytokine profiles in CE patients. However, in these earlier studies, the validity of the observations has been questioned because the specimens were not precisely timed, and because it is well recognized that TLRs and cytokine profiles change throughout the cycle.

Study design, size, duration: This is a non-interventional observational study on the expression of 10 TLRs and 17 different inflammatory cytokines in precisely timed endometrial tissue collected from 83 women with reproductive failure.

Participants/materials, setting, methods: Endometrial tissue was collected from participants seeking treatment for infertility. All specimens were collected on day LH+7. Chronic endometritis was defined as plasma cell density above normal range established from a fertile control population (> 5.15 CD138+ plasma cells/0.1 mm^2, Liu Y *et al.*, in press). TLRs 1-10 were stained by conventional immunohistochemistry for each specific antibody. In addition, 17 different inflammatory cytokines were analysed with the Luminex technique (Millipore, Billerica, MA) following protein extraction.

Main results and the role of chance: Among 83 patients studied, 19 were found to have CE, and the other 64 did not have CE. There was no difference in age, duration of infertility, and the number of miscarriage between these two groups.

In women with CE, the endometrial expression of some TLRs was significantly altered. In particular, stronger expression of TLR 1, 2, 3, and 4 were observed in endometrial epithelial and glandular cells when compared with women without CE.

Among the 17 cytokines examined, the expression of IFN-γ in women with CE was 3.69 pg/mL, which was significantly higher ($p = 0.034$) than that of in women without CE, 3.47 pg/mL. However, there was no statistically significant difference found among the other 16 cytokines and chemokines (EGF, G-CSF, GM-CSF, IL-10, IL-12P40, IL-12P70, IL-13, IL-1α, IL-1β, IL-2, Il-4, IL-5, IL-6, MCP-1, MIP-1β and TNF-α) studied. The data indicate that altered amount of IFN-γ in human endometrium with CE may be a result of higher expression of TLR 1, 2 3, and 4.

Limitations, reasons for caution: The relative small sample size of this study precludes the analysis of the relationship between conception and pregnancy outcome.

Wider implications of the findings: It appears that adverse effect of CE on reproduction is mediated via the IFN-γ pathway. Further study should be conducted to explore how alteration of IFN-γ leads to implantation failure with the regulation of TLRs.

Trial registration number: Chinese Clinical Trials Registry Number: ChiCTR-IOC-16007882

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P-273 Ovarian Endometriosis: deciphering the underlying mechanisms using a genomic approaches

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Study question: Endometriosis has long been suspected of familial tendencies. Our goal is to identify genes involved in the pathogenesis of ovarian endometriosis by using genomic approach.

Summary answer: Whole exome sequencing (WES) on a large consanguineous Turkish family revealed a possible causative gene mutation segregating with ovarian endometriosis phenotype in the family.

What is known already: Endometriosis is a common gynecologic disease defined as the growth of endometrial tissue outside the uterine cavity. Laparoscopic visualization of endometriotic lesions remains the 'gold standard' in diagnostic tests. Though, only 70–75% of visually diagnosed lesions are confirmed histologically. Despite its high prevalence and incapacitating symptoms, the etiology of endometriosis remains unclear. A number of chromosomal loci have been found to be associated with endometriosis in selective populations; however genes that play a role in endometriosis remain to be identified.

Study design, size, duration: This study has been performed at the Strasbourg University, France in collaboration with the Bahceci Health Group, Istanbul, Turkey. A large, consanguineous Turkish family having six affected women in two generations as well as more than two unaffected women was available for the study. 19 well-defined individual ovarian endometriosis cases and 20 endometriosis free women were also included for further validation.

Participants/materials, setting, methods: Saliva samples, not pathological samples, have been used for the WES in order to avoid possible accumulation of mutations in the pathological samples. Genomic DNA was extracted from saliva according to the manufacturer's instructions (DNAGenotech, Ottowa, Canada). WES was performed on four affected and two non-affected samples by the Institute of Genetics and Molecular and Cellular Biology microarray and sequencing platform, member of the 'France Génomique programme'. Suspected mutations were confirmed via Sanger sequencing.

Main results and the role of chance: We identified a heterozygous 3'-UTR variation in an autosomal gene which is co-segregating with the ovarian endometriosis phenotype in the family. The gene in question is located on chromosome 19, belongs to protein family that regulates endometrial epithelial cell adhesion, trophoblast motility and invasion during implantation. The variation is predicted to be deleterious via different prediction tools. In order to validate the identified mutation and check for other possible mutations in the gene, all exons and exon/intron boundaries of the identified gene were amplified and sequenced in 19 ovarian endometriosis patients and 20 controls. The analysis is still on going.

Limitations, reasons for caution: Our study contains only a limited number of Turkish patients. Mutation screening should be continued on larger groups of ovarian endometriosis patients, including women of other ethnicities. Also for obvious ethical reasons, no in-vivo works were possible.

Wider implications of the findings: In long term, results will help us to define potential non-invasive test for ovarian endometriosis. Understanding the fundamental molecular mechanisms in ovarian endometriosis pathogenesis is the first step to develop novel therapeutic approaches, to eliminate endometriosis lesions and to prevent recurrence.

Trial registration number: Not applicable.

P-274 Assisted Reproductive Techniques (ART) and their possible effect on the progression of endometriosis symptoms

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Study question: Do women with endometriosis experience worsening in endometriosis symptoms (quality of life, pain, bowel symptoms) when undergoing controlled ovarian stimulation (COS) during assisted reproductive techniques (ART)?

Summary answer: This study did not find worsening in the abovementioned parameters during ART, which supports ART as a suitable therapeutic option for infertile women with endometriosis.

What is known already: 10-25% of women with endometriosis require ART to conceive. During COS levels of estrogen increases, hence in theory increasing the risk of progression of symptoms related to endometriosis. Anaf et al. identified four cases of rapidly growing sigmoid endometriosis during ovarian stimulation resulting in cessation of ART and bowel surgery. Ten similar cases have occurred in our department since 2007. Moreover, isolated cases with severe worsening of endometriosis during COS have been reported.

Study design, size, duration: Prospective cohort study carried out from February 2016 to October 2017 with a total of 177 women recruited. Participants were excluded during the study if they were lost to follow-up, or if egg retrieval was cancelled. This study is based on questionnaires containing the Endometriosis Health Profile (EHP-30), and pain and bowel habits were evaluated on the numerical rating scale (NRS). Questionnaires were administered before and after COS in one cycle of ART.

Participants/materials, setting, methods: Patients < 40 years old were recruited from three fertility clinics and the endometriosis unit, Aarhus University Hospital (AUH). Depending on endometriosis diagnosis (confirmed by previous laparoscopy, transvaginal ultrasound or magnetic resonance imaging) and ART, patients were assigned one of three groups (endometriosis with or without ART, or no endometriosis undergoing ART). According to power calculations each group should include 48 women. Median and mean changes in pain and quality of life scores are reported.

Main results and the role of chance: 52 women with endometriosis undergoing ART (+Endo/+ART), 50 not undergoing ART (+Endo/-ART), and 52 without endometriosis undergoing ART (-Endo/+ART), answered two questionnaires.

The three groups differed significantly on all pain parameters (general and worst non-menstrual pelvic pain, dyschezia, dyspareunia, and dysuria) at baseline, women without endometriosis had a significantly better score in all EHP-30® modules (pain, control and powerlessness, social support and emotional well-being) except self-image where the groups did not differ. Bowel parameters (constipation, diarrhea, nausea, vomiting and bloating) were comparable between the groups, except that +Endo/-ART indicated worse bloating.

Changes from 1st to 2nd questionnaire indicated that -Endo/+ART experienced a one NRS point greater worsening in the parameters 'tired" and 'general non-menstrual pelvic pain" compared to women with endometriosis ($p = 0.003$, and $p<0.001$). Regarding worst non-menstrual pelvic pain, the median changes were 1, 2.5, and 0 for +Endo/+ART, -Endo/+ART, and +Endo/-ART, respectively ($p<0.001$). Women with endometriosis, undergoing ART or not, experienced a slight improvement in EHP-30® modules during ART compared to -Endo/+ART who worsened in all modules except 'self-image". None of the three groups changed in bowel parameters except bloating where both ART groups experienced an increase of one NRS point compared to +Endo/-ART ($p = 0.020$).

Limitations, reasons for caution: Women with endometriosis were included regardless of stage, which caused disease heterogeneity. +Endo/-ART was recruited from a tertiary referral center. Hence, this group may have particularly severe endometriosis stages. In the group of women without endometriosis subclinical disease might have been present without the clinicians' knowledge.

Wider implications of the findings: Our results are in line with previous studies (Benaglia et al. and Coccia et al.) which further supports ART as a suitable therapeutic option for infertile women with endometriosis. Our two reference groups added new information by allowing differentiation between changes in parameters caused by ART and endometriosis individually.

Trial registration number: The Danish Data Protection Agency: Registration number 2012-58-006). ClinicalTrials.gov: NCT02762461.

P-275 Molecular characterization of PRM associated endometrial changes (PAEC) following mifepristone treatment

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Study question: Does endometrium displaying PAEC after mifepristone treatment have a characteristic gene and protein expression?

Summary answer: Molecules significantly altered in PAEC after mifepristone exposure are mainly involved in the structural architecture of tissue, which may explain the morphological features of PAEC.

What is known already: The non-physiological endometrial changes caused by the progesterone receptor modulator mifepristone, PAEC, have previously been morphologically described but not explored at molecular level in regards to both gene and protein expression.

Study design, size, duration: A comparative analysis of gene and protein expression between endometrium displaying PAEC (n = 7) and endometrium that did not develop PAEC (n = 6) after three months of mifepristone treatment was performed. The current observational cross-sectional study is part of a previous randomized, placebo-controlled clinical trial at Karolinska University Hospital in Stockholm, Sweden.

Participants/materials, setting, methods: Endometrial biopsies, obtained from pre-menopausal women treated with mifepristone prior to surgery due to uterine leiomyoma, were evaluated regarding occurrence of PAEC and a comparative analysis of molecular expression between endometrial samples displaying PAEC and non-PAEC endometrial samples was performed. Methods used include microarray analysis, real time PCR, Ingenuity Pathway Analysis and Proteomics Analysis with Mass Spectrometry and Immunohistochemistry.

Main results and the role of chance: Combinations of characteristic non-physiological features were observed in 53.8% of collected endometrial samples. We observed non-decidualized stroma and both inactive and secretory active glands as well as extensively apoptotic glands, mitotic glands and apoptotic degeneration and atrophy.

Microarray analysis showed 181 differentially regulated genes by a minimum of two-fold in PAEC samples compared to non-PAEC samples. 142 genes were upregulated and 39 were downregulated. Microarray findings were validated by real time PCR for nine genes, three of them, THY1 (p = 0.02, fold change (FC) = 3.18), ADAM12 (p = 0.04, FC = 3.28) and TN-C (p = 0.04, FC = 3.21) reported in endometrial function were differentially regulated with PAEC. The proliferation marker MKi67 was not statistically significantly altered between the groups.

Mass spectroscopic analysis showed 25 proteins upregulated and 5 downregulated in endometrium with PAEC compared with non-PAEC and most of the proteins including TUBA4A (p = 0.002), MYOF (p = 0.006), TPM4 (0.013), RRBP1 (p = 0.021) and CSRP1 (p = 0.001), DPYSL3 (p = 0.012), FMOD (p = 0.045) are involved in tissue morphology. Mass spectrometry findings were validated with immunohistochemistry and were found to be in accordance with mass spectrometry results, but none of the tested antibodies were statistically significant by immunohistochemistry.

Limitations, reasons for caution: Although most of the endometrial samples from the clinical trial were used for analysis in this study, the main limitation is the small sample size. 14 women were randomized to receive mifepristone in the pilot study.

Wider implications of the findings: Our study has generated new knowledge on the molecular profile of endometrium displaying PAEC after 3 months of mifepristone treatment due to symptomatic leiomyoma. This knowledge is of great importance as the application of progesterone receptor modulators (PRMs) for the medical management of benign gynecological conditions is increasing.

Trial registration number: www.clinicaltrials.gov:NCT00579475

P-276 Endometriosis pathogenesis: role played by the oxidative stress due to MTHFR mutations

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Study question: Can endometriosis in infertile patients be linked to the oxidative stress due to methylenetetrahydrofolate reductase (MTHFR) mutations?

Summary answer: A clear relationship has been established between MTHFR mutations and the pathogenesis of endometriosis. Treating patients to by-pass MTHFR mutations consequences improves ART outcomes.

What is known already: Endometriosis pathogenesis is unclear and its etiology is complex. Recent studies mention the role of oxidative stress implicated in the pathophysiology of endometriosis by causing a general inflammatory response in the peritoneal cavity (Augoulea, 2009).

MTHFR is a key enzyme involved in folate metabolism and in the genesis of major antioxidant molecules (gluthatione, hypotaurine). Oxidative stress can be induced by polymorphisms of MTHFR through the increased homocysteine level (Guo, 2016).

To our knowledge, no study in the literature analyzed the role played by MTHFR in the endometriosis genesis of infertile patients.

Study design, size, duration: From January 2016 to january 2018, we followed 30 infertile patients suffering from endometriosis and having had at least 1 ART (Assisted Reproductive Technologies) cycle failure.

At first, we compared the MTHFR mutations distribution in our population.

The patients carrying a MTHFR mutation were afterwards treated and we compared the pregnancy rates obtained before and after treatment.

Participants/materials, setting, methods: All the infertile patients involved in this study were diagnosed with endometriosis according to the ESHRE 2013 guidelines.

The presence of MTHFR C677T was determined from a venous blood sample, using real time PCR with the RealFast™ assay (ViennaLab Diagnostic GMBH, Vienna, Austria).

The infertile patients with recurrent ART failures (2 to 7) and carrying MTHFR mutations were treated with 5MTHF (5 Methylene Tetrahydrofolate), a treatment by-passing the problems linked to MTHFR impaired activity.

Main results and the role of chance: Among the endometriosis population of our study, 60% of the patients are carrying the MTHFR mutation (46.7% in a heterozygous state, 13.3% in a homozygous state). This proportion is significantly more important ($p<0.05$) than the proportion of patients carrying MTHFR mutations in the general population: 50.5% (Zappacosta, 2009).

Furthermore, after we treated infertile couples with endometriosis and recurrent ART failures (2-7) carrying MTHFR mutations, we significantly improved their ART outcomes (average ongoing pregnancy rate per cycle: 23.4% before treatment; 29.6% after treatment, $p<0.05$).

Limitations, reasons for caution: Our results needs to be confirmed on a larger population. The genetic analysis of the MTHFR gene needs to be enlarged to other mutations related to the one carbon cycle in order to screen all the patients in which fertility could be affected by oxidative stress due to MTHFR mutations.

Wider implications of the findings: Endometriosis can be explained by MTHFR mutations. The resulting oxidative stress impairs the fertility of the female patients.

Therefore, by improving the methylation and decreasing the oxidative stress, treating MTHFR mutation carriers improves the quality of the gametes and their ART outcomes.

Trial registration number: None.

P-277 Functional genomic meta-analysis identifies similarities between endometrial-related subfertilities

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Study question: Are there relevant enriched functions shared both within and between endometrial-related subfertilities as endometrial adenocarcinoma, endometriosis, Recurrent Implantation Failure (RIF) and Recurrent Pregnancy Loss (RPL)?

Summary answer: Specific enriched functions were common between all considered endometrial-related subfertilities. RIF and endometriosis were the most similar, and RPL differed from all others.

What is known already: Endometrial-related pathologies are complex, multifactorial conditions affecting female fertility. Prior studies described endometrial adenocarcinoma, endometriosis, RIF, and RPL through transcriptomics analysis using case vs control approaches to identify altered genes and functions. However, the underlying mechanisms linking these conditions to endometrial subfertility remain controversial due to small sample sizes and differing experimental designs. Functional meta-analysis techniques provide a more robust method to highlight the most important functions associated with a set of individual studies. The objective of this research was to identify enriched shared functions within and between endometrial-related subfertilities studies for robustly underlaying the common molecular basis.

Study design, size, duration: An in-silico study involving systematic review, transcriptomic analysis, and functionally integrative meta-analysis was applied to selected case vs control experiments associated with endometrial-related pathologies. From 613 datasets, we included 3 from endometrial adenocarcinoma, 2 from RIF, 2 from RPL, and 2 from eutopic endometrium of endometriosis. Functional meta-analysis techniques were employed to a) separately identify shared functions and pathways related to each single condition; and b) to determine altered functions common between endometrial-related conditions.

Participants/materials, setting, methods: Raw data were downloaded from Gene Expression Omnibus (GEO). Selected datasets were preprocessed, normalized using quantile method (Limma R-package), and explored through principal component and clustering analysis. Gene Set Enrichment (mdgsa R-package) was performed on differential expression analysis results (Limma R-package). Functional meta-analysis was performed using DerSimonian & Laird random-effects model to identify significant shared functions (False Discovery Rate (FDR) < 0.05). Functional databases consulted were Gene Ontology and Kyoto Encyclopedia of Genes and Genomes.

Main results and the role of chance: Functional meta-analysis allowed us to identify functional connections within studies related to each endometrial-related pathology. Functions such as cell junctions, p53 signaling pathway, endoplasmic reticulum, and cell adhesions (3.9412e-07<=FDR< = 0.00016) were associated with adenocarcinoma; proteasome complex, amine transport, apical part of cell, and chromosome-related functions (7.2107e-06<=FDR< = 0.04849) with RIF; DNA replication and ribonucleoprotein complex (0.00035<=FDR< = 0.04281) with RPL; and proteasome, mitochondria, and microtubule-related processes (0.00434<=FDR< = 0.04823) with endometriosis. Results from integrating all endometrial subfertilities (adenocarcinoma, RIF, RPL, endometriosis) identified 8 shared functions related to cell projections (0.00282<=FDR< = 0.03690), chromatin DNA binding (FDR = 0.0440), and organelle assembly (0.00053<=FDR< = 0.0336). Furthermore, when RPL, RIF, and endometriosis were integrated by pairs, RPL was the least similar, having only one common function with RIF (FDR = 0.0484) and 8 with endometriosis (1.0609e-05<=FDR< = 0.04390). RIF and endometriosis were the most similar, sharing 21 functions (1.1262e-08<=FDR< = 0.04823). Finally, those pathologies involving tissue growth (endometrial adenocarcinoma and endometriosis) were found to have a higher number of altered genes and functions than RIF or RPL.

Limitations, reasons for caution: Despite including good-quality GEO datasets, this study is limited by available endometrial-related subfertilities and the heterogeneity between studies. However, all datasets were transcriptomically analyzed using the same methodology and the applied meta-analysis incorporates the variability of each study, robustly integrating them at a functional rather than at a gene level.

Wider implications of the findings: Due to functional alteration similarities between endometriosis and RIF, there is evidence that eutopic endometrium is affected, supporting the controversial idea that endometriosis affects endometrial function. Shared detected functions between all considered endometrial subfertilities could enable development of a common method to diagnose all of them.

Trial registration number: not applicable.

P-278 Interventions for endometriosis related infertility: a systematic review and network meta-analysis

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Study question: What is the comparative effectiveness of different treatments for women with endometriosis associated subfertility?

Summary answer: More research is required to clarify the relative effectiveness of treatments for endometriosis-related infertility.

What is known already: Most recognised therapies have not been directly compared in randomised controlled trials, therefore there is no direct evidence to inform clinical decision-making, hence selection of the most effective treatment is difficult. NMA compares multiple treatments in one statistical model. It is possible to guide treatment decision-making through the provision of a hierarchy of effectiveness of the treatment options. In the setting of subfertility related to endometriosis, the ability to apply this model is invaluable, as it is an area where there are numerous intervention options, and it has the potential to promote selection of the optimal therapy.

Study design, size, duration: A systematic review and network meta-analysis of relevant randomised control trials (RCTs) was performed. We searched electronic databases including, MEDLINE, Embase and the Cochrane Central Register of Controlled Trials (CENTRAL), as well as reference lists to identify eligible studies.

Participants/materials, setting, methods: We included RCTs comparing any medical or surgical interventions to each other or placebo/ no treatment in couples with endometriosis associated subfertility. The primary effectiveness outcome is a composite of clinical pregnancy.

Main results and the role of chance: 4,252 titles/abstracts were identified through the literature search, of which we included 27 trials reporting on 2,195 women with endometriosis associated subfertility. We included 14 different interventions in NMA. The most frequent direct comparisons were surgical laparoscopy versus surgical laparoscopy plus gonadotrophin releasing hormone analogues (GnRHa) (7 studies, 686 women), surgical laparoscopy versus placebo (3 studies, 483 women), GnRHa versus danazol (4 studies, 127 women) and danazol versus placebo (2 studies, 99 women). All 27 studies reported on clinical pregnancy. In vitro fertilisation (IVF) and intrauterine insemination (IUI) could not be included in the NMA, as RCTs compared only different stimulation protocols for IVF and for IUI and none of the RCTs compared IUI or IVF versus no treatment, placebo or other interventions.

Network meta-analysis showed that compared to placebo, Lipiodol (OR 7.56, 95% CI 1.95-29.37) and surgical laparoscopy plus pentoxifylline (OR 3.44, 95 CI 1.08-10.93) resulted in more clinical pregnancies; GnRH-a (OR 1.54, 95% CI 0.93-2.56) and surgical laparoscopy OR 1.43, 95% CI 0.93-2.56) were likely to result in more clinical pregnancies. Dydrogesterone (OR 3.00, 95%CI 0.69-13.30), pentoxifylline (OR 1.98, 95%CI 0.55-7.17) and laparoscopy plus danazol (OR 1.72, 95%CI 0.34, 8.78) showed imprecise effect sizes.

Limitations, reasons for caution: The CIs in most interventions with large ORs were imprecise and therefore should be exercised with caution. The value of the NMA was limited as IVF and IUI could not be included. Live birth was reported in only 5 studies in the network meta-analysis.

Wider implications of the findings: The most important conclusion is that more RCTs are needed to clarify the relative effectiveness of treatments for endometriosis-related infertility, in particular RCTs comparing IVF or IUI to other treatments including surgical laparoscopy and lipiodol to other treatments.

Trial registration number: N/A.

P-279 The role of reproductive tract microbiome in infertility

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Study question: How the microbiome of the endometrium of asymptomatic women with a history of infertility influences IVF techniques?

Summary answer: The molecular investigation of endometrium tissue from infertile women for common microbes unravels "silent" infections that may affect on Assisted Reproductive Technologies (ART).

What is known already: The human microbiome has gained much attention recently for its role in health and disease. Sexually transmitted infections are a major concern to clinicians and researchers in the field of reproductive medicine especially if the inflammation concerns the endometrium. An untreated inflammation caused by those agents, can lead to serious consequences, including miscarriages, tubal obstruction, preterm birth, chronic pelvic pains and unexplained infertility. The most common used method for microbe detection is based on culture results of cervicovaginal secretions. However, this diagnostic method is insufficient since it is difficult to detect any kind of infection in the upper genital tract.

Study design, size, duration: The aim of our study is to use the endometrium as a study material for the molecular detection -using real time PCR- of *Chlamydia trachomatis*, *Ureoplasma sp*, *Mycoplasma hominis*, *Mycoplasma genitallium* and Herpes I/II. In order to subtract cervicovaginal secretions and to detect microbes directly from the endometrium, we used a pipelle suction to retrieve the material. In the present study, endometrium tissue samples of Greek women were collected from January 2013 till December 2017.

Participants/materials, setting, methods: The study group was a total of 358 Greek women of age 36.4 ± 4,8 (Mean ± St.Dev) with fertility problems. Endometrium samples were collected with endometrial biopsy under hysteroscopy with mild sedation of the women and were screened for the presence of *Chlamydia trachomatis*, *Ureoplasma sp*, *Mycoplasma hominis*, *Mycoplasma genitallium* and Herpes I/II by real time PCR. The procedure of sample collection trying to mimic the embryo transfer technique.

Main results and the role of chance: In the overall study group, the prevalence of *Chlamydia trachomatis* was 1.1%, *Ureoplasma sp* was 7.26%, *Mycoplasma hominis* was 0.83%, *Mycoplasma genitalium* was 0.27% and Herpes I/II was 0.27%. There is a prevalence of Ureoplasma sp infection in the upper genital tract of infertile women. These findings suggest that microbiome present in the upper genital tract and could be detected efficiently by molecular techniques (real time PCR).

Limitations, reasons for caution: The sample collection occurs under mild sedation of the women.

Wider implications of the findings: Our study suggests that there is a possible correlation with uterus microbiome and infertility. In addition, our results demonstrate that the investigation of endometrium tissue for infectious microbes by using real time PCR, could be used as an alternative technique, unraveling a "silent infection".

Trial registration number: Not applicable.

P-280 Effect of endometriosis on sperm motility and organization of the tail microtubules

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Study question: To evaluate in vitro the effect of peritoneal fluid (PF) from women with endometriosis on sperm motility and organization of the sperm flagellar structure.

Summary answer: In vitro exposure of sperm to endometriosis PF is associated with an early decrease of progressive motility and an alteration of the tail microtubules.

What is known already: Endometriosis is a hormone-dependent disease associated with infertility in 30% of patients. The peritoneal fluid (PF) of women with endometriosis decreases sperm function and interfere with the correct oocyte-sperm interaction. The clinical relevance of sperm motility is evident, but the molecular mechanisms involved in this process have not yet been fully understood.

Study design, size, duration: Experimental study. 36 patients were recruited for the study obtaining the sample of PF during surgery (18 patients with endometriosis and 18 with other benign ovarian cysts).

Participants/materials, setting, methods: Human donor sperm was incubated during 24 and 48 hours in absence (control group) or presence of PF diluted at 20% from women with (n = 18) and without endometriosis (n = 18). Sperm motility was assessed by optical microscopy and the organization of the sperm tail microtubules was evaluated by fluorescence microscopy (alpha-tubulin immunostaining) in all experimental conditions, evaluating three types of patron after staining: 1) continuous tail; 2) discontinuos tail; and 3) not stained tail.

Main results and the role of chance: Sperm motility decreased significantly along the time of culture in all conditions. The greatest difference was observed at 24 hours, being a higher decrease in endometriosis group compared to non-endometriosis group ($p = 0.004$) and control ($p = 0.001$). Regarding to the alpha-tubulin distribution, the greatest difference was observed at 48 hours of culture in all patrons: 1) continuous tail, $p = 0.008$; 2) discontinuos tail, $p = 0.002$; and 3) not stained tail, $p = 0.046$, comparing endometriosis versus non-endometriosis group.

Limitations, reasons for caution: The main limitation of our study is the introduction of a possible variability in the basal cell properties because of the selection of four different human sperm donors. Moreover the sample size must be increased in order to establish definitive conclusions.

Wider implications of the findings: Previous studies have described altered sperm motility comparing PF from endometriosis patients to healthy women. In our study, we compared the condition of endometriosis to other ovarian pathologies, showing that quality sperm is more affected by factors present in the endometriosis PF.

Trial registration number: NA.

P-281 Comparing the clinical efficacy of intrauterine cook balloon and contraceptive device in preventing adhesion reformation and improving pregnancy outcomes after hysteroscopic adhesiolysis in IVF/ICSI

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Study question: Are the intrauterine cook balloon effective for preventing adhesion reformation and improving pregnancy outcomes?

Summary answer: The long term intrauterine cook balloon placement for 2 months with hormone replacement therapy are efficacy in preventing adhesion reformation and improving clinical outcomes.

What is known already: There are many strategies now available to prevent adhesion recurrence and restoring endometrium. Previous studies have described that intrauterine cook balloon and IUD are of similar efficacy in the prevention of adhesion reformation. But both devices were removed only after 1 week. The recurrence of adhesion rate is 10-30%, and the study did not report the pregnancy outcomes following the treatment. Limited dates are available whether to increase days of cook balloon stents can decrease the recurrence of adhesion rate and remove the balloon in the FET cycle can promote endometrial proliferation and improve the clinical pregnancy rate.

Study design, size, duration: This retrospective analysis involved 566 infertile women with moderate to severe intrauterine adhesion and received the frozen thawed embryo transfer (FET) cycle at the Reproductive and Genetic Hospital of CITIC-Xiangya between October 2016 and July 2017.

Participants/materials, setting, methods: The patients in the cook balloon group were matched by 1:2 with IUD controls. At the end of the hysteroscopy procedure the patients were fitted with cook balloon or IUD for 2

months. In all cases hormone replacement therapy was commenced since the third day of menstrual cycle that the first hysteroscopy were carried out, until FET cycle which second-look hysteroscopy was carried out in the proliferative phase 2 months after the first surgery.

Main results and the role of chance: According to the inclusion criteria and exclusion criteria, there were 76 cook balloon patients and 152 IUD control patients.

The hysteroscopy outcomes suggested that there was no statistical different in adhesion grade(P = 0.647), American Fertility Society (AFS) score before operation(6.8 ± 1.31vs 6.58 ± 1.28, P = 0.233) in two groups. But compared to IUD group, the AFS score after operation(P = 0.00) and recurrence of adhesion(1.32%vs 20.4%, P = 0.00) were significantly decreased in cook balloon group. It is comparable to the endometrial thickness on embryo transfer (ET) day(9.65 ± 1.12vs 9,66 ± 1.66,P = 0.963), number of embryo transferred (1.30 ± 0.61vs 1.36 ± 0.58, P = 0.527), high quality embryo rate(55,71%vs 51.38%, P = 0.552) and cancellation rate(7.89% vs 5.26%, P = 0.435) between cook balloon group and IUD group. The clinical outcomes suggested that the implantation rate(32.32% vs 19.43%;P = 0.013), clinical pregnancy rate(44.28% vs 25%;P = 0.004), ongoing pregnancy rate(40% vs 20.83%;P = 0.003) in cook balloon group are higher than IUD group.

Limitations, reasons for caution: Lack the evaluating of the bacterial colonization or infection in the uterus after 2 months with cook balloon stent. Well-designed, adequately powered random controlled trials are needed.

Wider implications of the findings: Long term intrauterine cook balloon placement can improve the pregnancy outcomes of infertile women with moderate to severe intrauterine adhesion.

Trial registration number: none.

P-282 A long-term pretreatment with GnRH agonists prior to IVF/ICSI does not improve the clinical pregnancy rate in patients with endometriosis: a systematic review

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Study question: Does a long-term pituitary suppression with GnRH agonists prior to IVF/ICSI improve the clinical pregnancy rate (CPR) in patients with endometriosis?

Summary answer: In endometriosis, a long-term pituitary suppression does not improve the CPR, increases the stimulation duration and total gonadotropins dose, reducing the number of retrieved oocytes.

What is known already: The mechanisms of endometriosis-associated infertility have been debated for years and they are still unclear. Excluding altered pelvic anatomy, these mechanisms include: abnormal folliculogenesis, elevated oxidative stress, altered immune function. In IVF, these factors lead to poor oocyte quality, impaired fertilization and implantation. In order to increase pregnancy rates in patients with endometriosis, various approaches have been proposed. A meta-analysis (Sallam et al., 2006) demonstrated that a down-regulation for 3-6 months with GnRH agonists prior to IVF/ICSI increases the odds of clinical pregnancy by >4-fold. This meta-analysis was included in the ESHRE guidelines published in 2014 (grade of recommendation B).

Study design, size, duration: A systematic review, based on PubMed, ISI-Web, Cochrane CENTRAL, EMBASE, was conducted to verify the effectiveness of the long-term pretreatment with GnRH agonists in patients with endometriosis. According to *PICO* format, inclusion criteria were: *Population*, endometriosis patients; *Intervention*, long-term pretreatment with GnRH agonists; *Control*, no long-term pretreatment; *Outcome*, clinical pregnancy in patients undergoing IVF/ICSI. Secondary outcomes were: duration of stimulation, total dose of gonadotropins, oocytes retrieved, fertilization rate (FR), embryos transferred, ongoing pregnancy rate (OPR).

Participants/materials, setting, methods: A bibliographic search was undertaken from 2006 to 2017, yielding 54 studies. Three researches (A.M., S. G., A.V.) reviewed independently the studies, excluding 36 studies after the first screening (title and abstract) and 10 studies after the second screening. The Mantel-Haenszel method was used to calculate odds ratios (OR) and heterogeneity among studies (I^2). The results were expressed as OR with 95% confidence intervals (CI). Standardized mean differences (SMD) between groups were used for continuous outcomes.

Main results and the role of chance: Eight studies were included. Three were RCTs; the remaining studies were cohort/case-control.

In two studies (Söritsa et al., 2015, Decleer et al., 2016), stage I-II endometriosis was considered; in three studies (Ma et al., 2008; Tamura et al., 2014; van der Houwen et al., 2014), stage III-IV endometriosis; in two studies (Surrey et al., 2010; Rodriguez-tarrega et al., 2016) all stages. In one case (Gong et al., 2009), the endometriosis was not classified.

The female age was comparable among the studies.

CPR was not significantly different in women receiving the long-term pretreatment compared with the control group (OR 1.25, 95% CI 0.82 to 1.91, I^2 = 0%). No differences in FR (OR 0.83, 95% CI 0.51 to 1.35, I^2 = 0%), number of embryos transferred (t=-1.26, p = 0.21, SMD -0.19, 95%CI -0.49 to 0.11, I^2 = 64%) and OPR (OR 1.36, 95% CI 0.80 to 2.31, I^2 = 0%) were observed. The patients pretreated had a lower number of oocytes retrieved in comparison with control group (t = -2.53, p = 0.01, SMD -0.20, 95%CI -0.35 to -0.04, I^2 = 36%), such as a longer duration of stimulation (t = 2.49, p = 0.01, SMD 0.41, 95%CI 0.09 to 0.73, I^2 = 76%), and a higher total dose of gonadotropins (t = 2.40, p = 0.07, SMD 0.48, 95%CI 0.09 to 0.88, I^2 = 84%).

Limitations, reasons for caution: In selected studies, patients with different stages of endometriosis were included. The couples' basal characteristics (AFC, AMH, FSH, BMI, seminal parameters, previous surgery) were not indicated in all the studies. Although the duration of the pretreatment with GnRH agonists was comparable among the studies, the formulations and dosages were different.

Wider implications of the findings: This review indicates no improvement in terms of IVF/ICSI success when the endometriosis patients are pretreated with GnRH agonists. We believe that diagnosis of endometriosis is not a critical parameter in the choice of treatment protocol.

Further well-designed studies are needed to corroborate our results.

Trial registration number: Not applicable.

P-283 Impact of endometriosis on assisted reproduction cumulative outcome

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Study question: Is cumulative live birth rate (CLBR) impaired in endometriosis patients?

Summary answer: Cumulative birth rate is overall significantly lower in endometriosis patients, compared to idiopathic infertility, but the significance disappears when adjusted for number of oocyte retrieved.

What is known already: Endometriosis can cause adhesive disease that alters pelvic anatomy and may yield an inflammatory altered immune environment that has the potential to impact oocyte quality, embryogenesis and implantation.

Several studies, including systematic reviews and meta-analyses, have yielded conflicting results regarding the impact of endometriosis on ovarian reserve and IVF outcomes; some suggest comparable ART outcomes between women with and without endometriosis, whereas others suggest that the presence of endometriosis negatively affects ART success, but cumulative success rates have not yet been analyzed.

Study design, size, duration: This is a retrospective cohort study. 1672 patients diagnosed with idiopathic infertility and 387 endometriosis patients who underwent IVF between 2009 and 2012, were included.

Participants/materials, setting, methods: CLBR has been calculated by adding up births resulting from fresh embryotransfer and births from thawing embryo cycles, per number of oocyte retrievals.

CLBR was evaluated in idiopathic group and endometriosis group. A further analisys included stratification per age and per number of oocytes retrieved.

Patiens aged < 37 years and 38-45 years, with more than 8 oocytes retrieved were analyzed in the two groups.

Main results and the role of chance: Number of oocytes retrieved was overall statistically lower in endometriosis group (6.3 vs 8.1; p< 0.001), as well as CLBR (16.5 % vs 21.8; P< 0.001).

In patients younger than 37 years old, with more than 8 oocytes retrieved (respectively 13.2 and 11.9 on average, in idiopathic and endometriosis; p = ns), CLBR was comparable between the two groups, with a tendency towards worse results in endometriosis group (41.5 % and 32.9%, p = ns)

In patients aged 38-45 years old, with more than 8 oocytes retrieved (respectively 12.5 and 13.8 on average, in idiopathic and endometriosis; p = ns), CLBR was comparable between the two groups, with even a tendency towards better results in endometriosis group (23.6 % and 29.2%, p = ns).

Therefore, the cumulative outcomes of assisted reproduction seem to be impaired by endometriosis, mainly by reducing the number of oocytes retrieved. When the number of oocyted retrieved is the same, cumulative birth rate is not statistically reduced in endometriosis patients, not even in older patients.

Limitations, reasons for caution: The study groups are quite small. The endometriosis group includes all endometriosis stages.

Wider implications of the findings: Given the importance of the number of oocytes retrieved in endometriosis, ovarian reserve should be preserved from radical surgery and ovarian stimulation should be personalized and improved, in order to obtain a good number of oocytes.

Trial registration number: Not applicable.

P-284 The sensitivity of DNA damage response target ovarian gap junction proteins in granulosa cells in women with advanced endometriosis

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Study question: What is the impact of DNA damage response (DDR) on granulosa cells (GC) in women with advanced endometriosis?

Summary answer: Double strand breaks (DSBs) trigger ATM/ATR/Chk1 axis to activate DDR suppressing ovarian gap junction protein (GJP) expression in GCs in advanced endometriosis.

What is known already: Endometriosis is a disease associated with elevated reactive oxygen species (ROS), both in follicular environment and systemically. DSBs are the most critical type of DNA damage which activate signal transduction by ataxia telangiectasia mutated (ATM), ataxia telangiectasia (ATR) and Rad3-related proteins to promote DNA repair and arrest cell cycle. However, recent data document other factors besides DDR proteins responsible for detection of DSBs in mouse granulosa cell DNA. Attenuation of ovarian GJP (connexins (Cx)) involves decreased follicular development and growth retardation in oocytes. There is little information whether DDR target Cx proteins to alter follicular growth in advanced endometriosis.

Study design, size, duration: Experimental study; Samples of follicular fluid (FF) and GC were obtained from February 2016 to October 2017 from 48 infertile women, 14 with early endometriosis, 23 with advanced endometriosis and 11 with tubal or male factors of infertility as control group, who underwent ovarian stimulation for IVF at our Assisted Reproduction unit. FF was obtained during oocyte retrieval and MGC were isolated from the follicular fluids of each woman through a Histopaque gradient.

Participants/materials, setting, methods: GCs were exposed to FF to estimate ROS production by flow-cytometry. DNA damage was assessed by immunofluorescence to quantify histone-H2AX phosphorylation (γH2AX) foci. DDR (pATM, ATR, Chk1, GADD45G) and ovarian GJP expression (Cx 37, 43) were evaluated by immunoblot in MGCs. Effect of DDR on Cxs in endometrial epithelial cell line (RL95-2) was evaluated with addition of 10 mm ATM,ATR, Chk1,and GADD45 inhibitors by immunoblot. P <0.05 was considered to be significant as evaluated by Student's t-test.

Main results and the role of chance: GCs exposed to 50 µl/ml FF for up to 24 hours led to a significant increase in ROS production in both early and advanced endometriosis. No significant difference was observed between GCs in early endometriosis and controls in γH2AX foci assay; however, induction of DSBs was noted in advanced endometriosis showing more γH2AX foci (P<0.01). Increased levels of ROS and cell death induced by γH2AX accumulation result in the activation of DDR components in early and advanced endometriosis; pATM (NS, P<0.01), ATR (NS, P<0.01), Chk1 (NS, P<0.025), and GADD45G (P< 0.05, P<0.03). Moreover, a significant diminution was observed in Cx 37 (NS, P<0.01) and 43 (NS, P<0.03) protein expressions of GCs in early and advanced endometriosis. To evaluate the effect of DDR on Cxs *in vitro*, we observed addition of ATM inhibitor, ATR inhibitor and Chk1 inhibitor ameliorated the protein expression/s in the culture system considerably (P<0.001). However, a combination of all four inhibitors demonstrated no extra advantage. Taken together, the findings suggest that a reduction in both Cx protein expressions in advanced endometriosis after DSBs requires ATM/ATR/Chk1 activity, suggesting possible arrest in early pre-antral and antral follicles impeding folliculogenesis leading to production of incompetent oocytes.

Limitations, reasons for caution: Small sample size; Signals emitted from GCs is solely due to an increase in ROS was not evaluated by a ROS inhibitor either in early or advanced endometriosis. In addition, no positive control has been used for the experimental set-up/s.

Wider implications of the findings: DDR induced by increased ROS alter oocyte-granulosa cross talk to influence follicular growth and maturation in women with advanced endometriosis in GCs. Identification of other gap junctional proteins and elucidation of their physiological functions within the follicle must be research priorities of the future.

Trial registration number: Not applicable.

P-285 Systemic inflammation and coagulation status in stage 3-4 endometriosis

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Study question: Do the coagulation and inflammation status differ in women with surgically confirmed Stage III- IV endometriosis?

Summary answer: Neutrophil count and mean platelet volume are higher in women with stage-III/IV endometriosis and activated partial thromboplastin time is shortened compared to benign gynecologic pathologies.

What is known already: Local inflammatory process has a pivotal role in the pathogenesis of endometriosis. However, the role of systemic inflammation in the pathogenesis of endometriosis is unclear.

Study design, size, duration: This retrospective cohort study used data from a single medical center from 2012 to 2017.

Participants/materials, setting, methods: A total of 321 women who underwent surgery were evaluated. The case group (n: 220) included patients with a surgical diagnosis of stage III-IV endometriosis. The control group (n: 101) consisted of women with a surgical diagnosis of benign gynecologic pathology. The routine preoperative tests including complete blood count parameters, activated partial thromboplastin time, prothrombin time and International Normalized Ratio (INR) were obtained at a maximum of 1 month before surgery.

Main results and the role of chance: Mean platelet volume (MPV) and neutrophil count were higher in patients with endometriosis when compared to controls (8,63 ± 5,32 vs. 7,95 ± 1,45; p = 0,012) and (4,96 ± 2,58 vs 4,33 ± 1,34; p = 0,004) respectively. Although in the normal range, women with endometriosis had significantly shortened activated partial thromboplastin time (aPTT) (29,86 ± 3,19 vs 28,72 ± 2,69; p = 0,002) than controls. Conversely,

no difference was found for protrombin time, INR, platelet count, lymphocyte count between women with endometriosis and controls.

Limitations, reasons for caution: This study has some limitations: (1) the retrospective design of the study that could have influenced the interpretation of our findings and (2) the presence of only one type of controls, the surgical population. Therefore the results should be taken with caution.

Wider implications of the findings: Elevated neutrophil count and MPV and shortened aPTT in patients with Stage III-IV endometriosis may reflect an inflammatory and/or procoagulant systemic status in these patients. Further studies are warranted to confirm our findings and to assess the role of systemic inflammatory and coagulation markers in the pathophysiology of endometriosis.

Trial registration number: Not applicable.

P-286 Influence of peritoneal fluid from patients with endometriosis on sperm glycocalyx

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Study question: Can peritoneal fluid (PF) from women with endometriosis affect the redistribution of glycoconjugates in human sperm membrane?

Summary answer: Sperm cultured with PF from women with and without endometriosis presented different distribution of sugar residues in the glycocalyx.

What is known already: Endometriosis is a chronic gynaecological condition that affects around 10% of women of reproductive age and it is associated with infertility in 40% of the cases. Studies report that endometriosis significantly reduces oocyte quality, embryonic development and percentage of implantation. Also, it has been shown that PF from patients with endometriosis decreases sperm motility, acrosome reaction and the ability to penetrate the oocyte, hindering the correct interaction between oocyte and sperm. However, additional studies are necessary to know how the presence of endometriosis affects the disposition of glycocalyx sugars involved in sperm-oocyte recognition.

Study design, size, duration: We conducted a double-blind prospective study. A total of 30 PF were collected from patients with endometriosis (EP) (n = 15) and non-endometriosis (NEP) (n = 15) from November 2015 to September 2017. Semen samples were obtained from normozoospermic men (n = 5) as assessed according to World Health Organization criteria 2010.

Participants/materials, setting, methods: PF was collected by laparoscopy from EP and NEP. Sperm were capacitated by density gradients and cultured with 20% of PF during 0 h, 24 h, 48 h and 72 h (37°C and 5% CO_2). Controls were performed with commercial culture medium. Sperm cells were fixed in paraformaldehyde and carbohydrates were evaluated by fluorescent lectins: *Aleuria aurantia agglutinin* (AAA), *Concanavalin A* (ConA), *Peanut agglutinin* (PNA) and *Wheat germ agglutinin* (WGA). Results were observed by fluorescence microscopy and statistically evaluated.

Main results and the role of chance: Results showed significant differences in the location of certain sperm membrane carbohydrates depending on the PF. When we compared the results obtained in controls with cultures performed with PF from EP, significant differences ($p<0.05$) in the disposition of all sugars recognized by the AAA, ConA, WGA and PNA lectins at 24 h, 48 h and 72 h were found. On the other hand, these differences were reduced when comparing controls against FP cultures from NEP, since only significant differences were found in the distribution of carbohydrates identified by lectins ConA and AAA at 24 h and 48 h. In the same way, when contrasting the results obtained from the cultures using PF from patients with and without endometriosis, we found significant differences just in the location of the carbohydrates bound to the ConA and WGA lectins at 24 h. Therefore, these results allow mentioning that both the PF from EP and that of NEP influenced the distribution of sperm membrane sugars. However, as indicated by the results these glycocalyx differences are more marked when the sperm were cultured with PF of EP.

Limitations, reasons for caution: A relative small number of peritoneal fluids would be the main limitation of our study.

Wider implications of the findings: The high rate of significant differences found between the sugars glycocalyx location of sperm cultured with PF from EP and controls could negatively affect sperm-oocyte recognition and therefore be associated with infertility in endometriosis.

Trial registration number: Not applicable.

P-287 Role of TGF-b on endometriotic stem cells and its differentiation into cells with cancer phenotype

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Study question: Does high levels of TGF-b reduce the invasiveness of endometriotic stem cells (EndoSC) and their differentiation into cells with cancer phenotype?

Summary answer: Treatment with TGF-b reduced invasion of EndoSC at doses and positively alters the expression of genes that are highly expressed in pre-cancerous cells.

What is known already: In a previous study, high mRNA levels of TGF-b1 was observed in stem cells derived from endometriotic tissue. High levels of TGF-b is seen in peritoneal fluid (PF) of endometriosis patients and is positively correlated to the severity of endometriosis.

Study design, size, duration: Tissues from endometrioma were collected after the planned surgical excision of endometrioma from women (n = 16) underwent surgery for endometriosis.

Participants/materials, setting, methods: EndoSC were isolated from endometriotic tissue using stem cell markers CD73, CD90, and EpCAM. Spheroids (50 m) were generated from EndoSC and the effect of TGF-b (2 ng/ml; 250 ng/ml; with/without TGF-b inhibitor Ly2109761) on cell invasion was studied by invasion assay. Molecules involved in epithelial-mesenchymal transition (SNAI1) and 20 genes over expressed in pre-cancer cells (SDC1, SDC4, ESR1, CTNNB1, BMI1, ARIDA1 ect.) were studied by real time PCR.

Main results and the role of chance: Treatment with TGF-b reduced the invasion of EndoSC spheroids. Addition of TGF-b inhibitor Ly2109761 reversed this effect in a dose dependent manner with the up regulation of SDC -1 and -4 indicating a direct link with Syndecan family and cell invasion process. Analysis of gene expression data by heat-maps with supervised hierarchical cluster and principle component analysis showed few samples clustered together with similar expression of molecules (TGF-b, ESR1, CTNNB1, BMI1, ARIDA1 and SDC4) known to be involved in pre-malignant conditions.

Limitations, reasons for caution: This study is limited by a small sample size and results will need to be confirmed in a larger study.

Wider implications of the findings: Results from this study if confirmed in a larger sample may help in developing treatment strategies to reduce the risk of endometriosis-associated cancer by using TGF- b inhibitor.

Trial registration number: NA.

P-288 Wnt signaling as a target for the treatment of endometriosis

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Study question: We ultimately aim to assess Wnt signaling as a target for treatment of endometriotic lesions *in vivo* and *in vitro*.

Summary answer: We have shown that Wnt signaling is auto-activated in endometriotic lesions and appears to play a role in the development of endometriosis lesions.

What is known already: Endometriosis is a chronic condition defined by the presence of endometrial tissue outside the uterine cavity. It affects about 15% of women of reproductive age and can cause severe pain and infertility. The painful symptoms are only treated with hormonal treatments that achieve amenorrhea. Our lab has shown that Wnt signaling plays a role in the development of endometrial glands and in stromal cell proliferation and this pathway appears to be aberrantly activated in lesions of endometriosis patients.

Study design, size, duration: For *in vivo* experiments, we used 5 control and 5 mutant mice for each of the 3 time-points of disease development, and 10 mice for the *in vitro* mouse experiments.

For the *in vitro* patient study, we are in the process of obtaining endometriosis samples from 30 patients and treating them with different Wnt signaling inhibitors. We are measuring proliferation rates and investigating gene expression changes and expect to observe a reduction in proliferation following treatment.

Participants/materials, setting, methods: We are using the established auto-transplantation mouse model with a Wnt reporter strain to look at Wnt signaling activation *in vivo*, as well as a uterine-specific Porcupine conditional knock-out strain to study the role of Wnt signaling in endometriosis. We are comparing the *in vitro* proliferation of endometriotic cells in controls and mutants.

Peritoneal endometriosis lesions are obtained by physicians from the MUHC-RVH Department of OBGYN from women of reproductive age presenting with the condition.

Main results and the role of chance: We observed canonical WNT signaling activation in the lesions of reporter mice, but not in Porcupine knock-out mice, during the intermediate stages of the disease. Since these mutant mice do not express Wnt ligands in their reproductive tract, the activation of Wnt signaling must be due to uterine Wnt ligands, and not WNTs produced by other tissues. Additionally, mutant mice developed smaller lesions 3 and 6 weeks after the induction of endometriosis. This suggests that Wnt signaling is involved in endometriosis this mouse model. We are now looking to verify this *in vitro* in mouse endometriotic cells and patient samples.

Limitations, reasons for caution: Other mouse models exist, and some model the initiation of the disease more accurately, but the auto-transplantation model allows us to evaluate the development of endometriosis.

Wider implications of the findings: Understanding the function of Wnt signaling in the development and maintenance of endometriosis could lead to the identification of a new treatment to induce regression of the disease. The upstream signaling component Porcupine could be targeted to treat endometriosis, which our lab is currently testing *in vitro* and *in vivo*.

Trial registration number: not applicable.

P-289 Outcomes of conventional IVF versus ICSI in infertile women with endometriosis: a retrospective cohort study

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Study question: Are biological and clinical outcomes in infertile women with endometriosis better after ICSI than after conventional IVF?

Summary answer: Fertilization rate is significantly higher in ICSI cycles versus conventional IVF. Secondary outcomes are similar in the two groups.

What is known already: Endometriosis is a benign chronic gynaecological disease, defined as the presence of endometrial tissue outside the uterus. Prevalence is estimated to reach 15% of reproductive-aged women and 25-50% of infertile women. ART are commonly offered for managing endometriosis-related infertility. However it has been showed that peritoneal fluid from women with endometriosis inhibits the binding of spermatozoa to the zona-pellucida. One previous study concluded that ICSI is a better option than IVF in endometriosis because of a higher rate of fertilization leading to more embryos. There nonetheless remains a lack of knowledge concerning the optimal treatment between IVF and ICSI.

Study design, size, duration: Retrospective cohort study including 538 cycles (341 IVF and 197 ICSI) in infertile women with endometriosis performed between October 2012 and October 2017 at a tertiary care center (Cochin Hospital, APHP-Paris). Among the 538 cycles, fresh embryo transfer (FET) at Day 2/3 was performed for a total of 238 women (157 IVF and 81 ICSI).

Participants/materials, setting, methods: IVF and ICSI cycles were performed in women under 42 years having at least five cumulus-oocyte complexes (COC) after oocyte retrieval with ejaculated sperm. Primary outcome (fertilization rate) was compared between IVF and ICSI for the studied population and in FET subgroup. Secondary outcomes (number of embryo D2/D3, blastocyst formation) and clinical outcomes (implantation, miscarriage and ongoing pregnancy rates) were compared for FET subgroup. Statistical analysis was conducted using univariate and multivariate logistic regression models.

Main results and the role of chance: For the baseline characteristics between women undergoing IVF or ICSI, only AMH and AFC were significantly lower in ICSI group (p-values were respectively: 0.01 and 0.006). A significantly higher fertilization rate was reported in the studied population and FET subgroup (62.97 ± 22.12 % for IVF *versus* 69.11 ± 22.99% for ICSI $p = 0.001$ and 62.98 ± 22.16 for IVF *versus* 69.27 ± 19.30 for ICSI $p = 0.03$, respectively). No significant difference in the number of retrieved oocytes was found.

In the FET subgroup, no significant difference was shown when comparing the mean number of embryos at Day2/3 for IVF *versus* ICSI (7.2 ± 3.16 and 5.79 ± 2.77; $p = 0.074$), the mean number of embryos per transfer ($p = 0.825$) and blastocyst formation rate (42.0 ± 51.5% *versus* 36.0 ± 32 95%; $p = 0.85$). Clinical outcomes (implantation, miscarriage and ongoing pregnancy rates) were similar between the two groups.

Limitations, reasons for caution: Histological proof of endometriosis was not available for all the patients. Cumulative pregnancy rate was not evaluated because couples are still having frozen blastocysts not yet transferred.

Wider implications of the findings: These findings need to be confirmed by further prospective studies taking into account the stage of endometriosis. Yet, it brings a new insight in the complex task of dealing with infertile deep infiltrating endometriosis patients and might help physicians to choose between IVF and ICSI to optimize ART outcomes.

Trial registration number: No funding was sought for this study. Authors declare no competing interests.

P-290 Endometrial receptivity: Expression profile of candidate genes in endometriosis

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Study question: Are the genes involved in endometrial receptivity differentially expressed ineutopic compared to healthy endometrium? Does the hormonal hyperstimulation modulates thisprofile?

Summary answer: There is a different gene expression profile in tissues from healthy and eutopicendometrium. The in vitro hormonal hyperstimulation may affect this specific fingerprinting.

What is known already: Many pathologies affecting endometrial functionality should alterimplantation process and, as a consequence, cause infertility. Among others, endometriosis has anegative impact on embryo implantation and pregnancy rate and, for these reasons, it is consideredas one of the major causes of women infertility. Really, despite the great progress achieved byassisted reproductive techniques, implantation represents one of the most crucial stage. Moreover,several evidences suggest that controlled ovarian hyperstimulation may alters endometrialphysiology thus impairing receptivity, with possible negative effects on the embryo implantation.

Study design, size, duration: For this study, we have collected 6 endometrial tissue biopsies from healthy subjects and fromwomen with endometriosis, who underwent surgical interventions starting from January to October2017. Gene expression analysis of 24 genes involved in endometrial receptivity was carried out intissue biopsies and in primary HESC untreated or treated with FSH in combination with LH orhCG.

Participants/materials, setting, methods: The primary HESCs prepared from endometrium ofhealthy or endometriotic women, collected during

implantation window, were treated for 24 h withFSH in combination with LH or hCG, in order to reproduce hormonal hyperstimulation conditions.mRNA, extracted from both endometrial tissue and HESC was subjected to qRT-PCR in order toevaluate the expression levels of 24 selected genes involved in endometrial receptivity.

Main results and the role of chance: The investigated genes resulted differentially expressed inhealthy compared to eutopic endometrium, both in tissue and in stromal endometrial cells. Inaddition, we observed that in vitro hormonal stimulations significantly affect the gene expressionprofile of stromal endometrial cells. In particular, the expression of LIF and LIF receptor (LIFR)resulted differently modulated in healthy and eutopic endometrial tissue, whereas in HESC both LIFand its receptor have the same trend of expression. Noteworthy, the hormonal treatmentsignificantly reduced the expression of LIF in stromal cells, whereas significantly increased theexpression of its receptors. Among other genes, PAEP mRNA levels are significantly increased ineutopic compared to healthy tissue HESC, whereas its expression was extremely low in botheutopic and healthy HESCs, becoming undetectable upon hormonal treatment.

Limitations, reasons for caution: Different parameters may influence the response tohyperstimulation protocols, therefore larger study needs to be carried out in order to definitivelyvalidate these findings.

Wider implications of the findings: This approach allowed us to identify key genes whose expression is significantly modulated ineutopic compared to healthy HESC, also in response to hormonal hyperstimulation. This study, ifreplicated in a larger population, might contribute in understanding impact of the ovarianhyperstimulation protocols used in ART cycles.

Trial registration number: NONE.

P-291 Endometriosis is associated with a lower number of oocytes during IVF independently of serum AMH level

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Study question: Does the serum anti-mullerian hormone level (AMH) overestimate the oocytes yield in infertile patients with endometriosis undergoing in vitro fertilization (IVF)?

Summary answer: In infertile patients, endometriosis is an independent predictor of a lower number of oocytes retrieved during IVF after adjustment for serum AMH and other confounders.

What is known already: AMH is a member of TGF-ß superfamily recognized as a biomarker of ovarian response and as a reliable tool for controlled ovarian stimulation (COS) for IVF. Endometriosis is a hormonally-mediated inflammatory condition, being hypothesized that the members of TGF-ß family, including AMH, are increased, affecting its ability to reflect the ovarian reserve. However, data showing the predictive value of AMH for IVF outcome in endometriosis are limited.

Study design, size, duration: We performed a retrospective study which included 376 infertile female patients undergoing controlled ovarian stimulation for IVF between June 2016 –June 2017

Participants/materials, setting, methods: The study group included 94 patients with endometriosis and 282 patients with other causes of infertility. All the patients were evaluated and treated for infertility with IVF in a private outpatient clinic. Only patients with endometriosis, tubal causes, idiopathic infertility and male factor were included. Patients with polycystic ovary syndrome were excluded from the study. The following data were recorded: age, body mass index (BMI), AMH, gonadotrophins dose, number of oocytes retrieved during IVF protocol.

Main results and the role of chance: The mean age of the study group was 34.23 ± 3.5 years, mean BMI 22.1 ± 3.81 kg/m^2, mean AMH value 2.57 ± 0.78 ng/mL. The two groups of patients were similar in terms of age, BMI and gonadotrophins dose used during COS. Patients with endometriosis had significantly lower AMH serum level (2.13 ± 0.99 versus 2.95 ± 1.1 ng/mL, p<0.05) and obtained a lower number of oocytes during IVF (6.24 ± 4.6 versus 7.84 ± 4.9, p<0.005) in comparison with control group. After adjustment for confounders (including AMH level) in a model of multivariate linear regression, both endometriosis (beta=-0.117, p = 0.019) and AMH (beta = 0.519, p<0.001) independently predicted the number of oocytes retrieved during IVF.

Limitations, reasons for caution: The limitations of the study are its retrospective design and the lack of data about the subtypes of endometriosis. Moreover, our study did not include patients with an increased level of AMH (possibly as a consequence of inflammation) and our results can not be applied to these patients.

Wider implications of the findings: The results of our study suggest that patients with endometriosis could have decreased response to COS in comparison with patients with other causes of infertility with similar AMH level. Therefore, AMH serum level might not have the same predictive value in patients with endometriosis.

Trial registration number: NA.

P-292 Telomerase component, Dyskerin (DKC1), is expressed and hormonally regulated in healthy endometrium: implications for endometrial pathologies

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Study question: Is dyskerin expressed in the healthy endometrium, altered in proliferative endometrial conditions and is it regulated by ovarian steroid hormones?

Summary answer: Healthy human endometrium expresses dyskerin, significantly lower dyskerin protein levels are observed in endometrial cancer and the expression is regulated by oestrogen, progesterone and dihydrotestosterone (DHT).

What is known already: Telomeres are maintained and elongated by the specialized enzyme telomerase. Telomerase activity is important for endometrial epithelial proliferation, dynamically regulated by ovarian steroid hormones and is implicated in endometrial proliferative conditions such as endometriosis and endometrial cancer. Although two of the core subunits of telomerase holoenzyme, the RNA Component, hTERC and the catalytic component, hTERT have been extensively studied in human tissues including the human endometrium, the 3rd component, dyskerin protein is not well described. The effect of ovarian steroid hormones on dyskerin in human cells is not yet known.

Study design, size, duration: A prospective observational study, included endometrial samples collected from 240 women in total. They were 52 healthy premenopausal (29 proliferative phase; 23 secretory phase); 32 postmenopausal women; 29 women with endometriosis (18 eutopic secretory phase; 11 ectopic endometriotic samples). 109 women with endometrial cancer. A further 18 women using a Mirena IUCD were also recruited.

Participants/materials, setting, methods: Endometrial samples were analysed with immunohistochemistry and western blotting for dyskerin protein, qPCR for *DKC1* gene expression. Telomerase activity was measured by TRAP assay, hTERC mRNA levels with qPCR. The hormone receptor expression in the endometrial samples were measured using immunohistochemistry and a four-tiered Liverpool endometrial steroid quick score. Ki67 proliferative index was evaluated as the percentage of immunopositive cells. Endometrial cancer cell line, Ishikawa was used to examine in-vitro hormonal regulation.

Main results and the role of chance: Healthy human endometrium showed a dynamic spatio-temporal expression pattern of dyskerin. The expression of dyskerin protein and mRNA was significantly increased in healthy postmenopausal endometrium, compared with the premenopausal endometrium (P = 0.0022 and P = 0.0021 respectively). Secretory endometrium had the lowest *DKC1* mRNA expression levels compared with the postmenopausal endometrium (P = 0.01).

We did not see a significant difference in dyskerin protein or DKC1 mRNA levels in the eutopic secretory endometrium of women with endometriosis compared with secretory phase from healthy women.

This was in contrast to *hTERC* which was significantly upregulated in secretory endometrium of women with endometriosis compared with healthy secretory endometrium (p = 0.0199).

Dyskerin immunoscores were significantly low in endometrial cancer samples compared with the healthy postmenopausal endometrium (P = 0.0002).

DKC1 mRNA levels were up regulated by oestradiol (E2) and DHT and down regulated by progesterone *in vitro* in Ishikawa cells. Progesterone induced downregulation of *DKC1* was counteracted by E2. However, in our in vitro study, ovarian steroid hormones did not have an obvious effect on telomerase activity measured by TRAP assay.

Limitations, reasons for caution: This is an observational study. The sample size that we have used to evaluate *DKC1* mRNA and telomerase activity was relatively small.

Wider implications of the findings: The observed in vivo and in vitro data showed that dyskerin is hormonally regulated and its immunoexpression was significantly lower in endometrial cancer compared with healthy postmenopausal endometrial tissue. That suggests that dyskerin might be a new target in developing treatments for endometrial proliferative disorders such as endometrial cancer.

Trial registration number: not applicable

P-293 Interactions between GSTs genes polymorphisms and trace metals are associated with endometriosis

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Study question: Are there interactions between GSTs genes polymorphisms and trace metals for endometriosis.

Summary answer: Yes, null genotype of GSTs genes may modify the effect of blood zinc and lead for endometriosis.

What is known already: The women with null types GSTs genotypes may associate with risk of endometriosis. Besides blood metal levels such as lead and cadmium are also associated with risk of endometriosis.

Study design, size, duration: This is a cross-sectional study design. From 2008 through 2010, this study included 181 women who visited the infertility clinic at Taipei Medical University Hospital. 64 endometriosis women and 117 non-endometriosis women were included.

Participants/materials, setting, methods: Blood samples were taken from each woman. Trace metal levels in blood were measured by inductively coupled plasma mass spectrometry (ICP-MS). The genotypes for GSTM1 and GSTT1 genes were identified by PCR.Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using multiple logistic regression models adjusted for probable confounders.

Main results and the role of chance: In stratification analysis, the null type GSTM1 group is with an OR = 0.34 (3rd tertile vs. 1st tertile, 95% CI, 0.13–0.86) for a reversed association between zinc levels and endometriosis and OR = 2.22 (3rd tertile vs. 1st tertile, 95% CI, 1.05-4.73) for an association between lead levels and endometriosis. The null type GSTT1 group is with an OR = 0.26 (3rd tertile vs. 1st tertile, 95% CI, 0.09–0.75) for a reversed association between zinc levels and endometriosis. In present type GSTM1 and GSTT1 group are with non-significant OR for associations between both these metal levels and endometriosis.

Limitations, reasons for caution: Because of the hospital-based design, generalization of these results to the general Taiwanese population and to other ethnic groups needs caution.

Wider implications of the findings: The GST family genes are known to be involved in the metabolism of environmental chemical agents. Women with null type GSTM1 and GSTT1 genotypes may be sensitive to zinc and lead exposure for having endometriosis.

Trial registration number: not applicable

P-294 No treatment versus surgical management of endometrioma on the IVF outcomes

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Study question: Does surgery have a positive impact on the IVF outcome of patients with endometrioma?

Summary answer: Surgery for endometrioma showed to favor pregnancy rate and live birth rate per cycle, but this was not statiscally significant.

What is known already: Two meta-analyses have assessed the impact of endometrioma resection on IVF outcomes. One demonstrated no significant differences in clinical pregnancy rates, comparing surgical resection to no treatment. Other suggested that surgical management of endometrioma resulted in no benefits for a subsequent IVF cycle. Also, some studies suggested a decrease in ovarian reserve in women with endometrioma, and some studies suggested a further decrease in ovarian reserve after surgery.

Study design, size, duration: Retrospective case-control study, performed at Fertilitat - Reproductive Medicine Center, from 2001-2017, including 252 IVF cycles. Patients included were referred to IVF due to endometriosis. Group 1 was composed by patients who had laparoscopic excision of endometriomas by cystectomy or cauterization of the cyst. Group 2 was composed by patients who had an image of endometrioma confirmed by an ultrasound exam, and did not receive surgical treatment.

Participants/materials, setting, methods: We found 76 IVF cycles on group 1 and 176 IVF cycles on group 2. Number of oocytes retrieved, pregnancy rate and live birth rate was compared between groups. Summary data were presented as mean and standard deviation. Student's *t*-test or chi-square test were used. The null hypothesis was rejected when p <0.05

Main results and the role of chance: Group 1 was composed by 76 IVF cycles. Group 2, by 176 IVF cycles. The maternal age between group 1 and 2 was: 35.1 ± 3,5 *vs* 35.8 ± 3.7 year-old, p = 0,15. The number of oocytes retrieved between group 1 and 2 was: 5,5 ± 3,7 *vs*. 6,0 ± 5,1, p = 0,25. From 76 IVF cycles in Group 1, there were 47 embryo transfer cycles. From 176 IVF cycles in Group 2, there were 107 embryo transfer cycles. The number of embryo transferred per cycle was 2.0 ± 0.7 *vs* 2.1 ± 0.8 p = 0,66, between Group 1 and 2 respectively. The pregnancy rate between Group 1 and 2 was 21 (44,68%) *vs*. 41 (38,31%), p = 0,45. The live birth rate between Group 1 and 2 was 15 (31,91%) *vs* 29 (27,10%), p = 0,54.

Limitations, reasons for caution: Group 1 had fewer patients. On Group 1, there were different surgical techniques.

Wider implications of the findings: In patients with endometrioma, surgery seems to improve pregnancy and live birth rate, but this was not statiscally significant. It is important to assess the surgery risks of ovarian intervention, when current evidence suggests that women treated with surgery have similar cycle outcomes compared to those not treated.

Trial registration number: Not applicable.

P-295 Results from a case-control study comparing mean aneuploidy rates and pregnancy outcomes in women with and without endometriosis undergoing In Vitro Fertilization (IVF) cycles

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Study question: Do women with and without endometriosis undergoing IVF with Pre-implantation genetic test for aneuploidies (PGT-A) before blastocysts transfer differ for aneuploidy rates and reproductive outcomes?

Summary answer: Endometriosis patients do not differ from their age and MII obtained oocytes-matched healthy peers for mean aneuploidy rates and for pregnancy outcomes in IVF cycles.

What is known already: It has been estimated that 30-50% of women with endometriosis can't conceive naturally and affected IVF patients have worse outcomes than women without endometriosis, such as lower number of oocytes retreived, reduced fertilization, implantation and pregnancy rates.

Several pathways have been exploited to explain the endometriosis-related subfertility, both at ovarian than at the endometrial side. The evidence that peritoneal higher levels of reactive oxygen species and inflammatory cytokines can cause oocyte microtubule and chromosome instability has suggested that even the rate of embryo aneuploidy could be increased in these patients, contributing to the poorer IVF outcomes of endometriosis patients.

Study design, size, duration: This study retrospectively evaluated data of women producing at least one blastocyst subjected to PGT-A after Intracytoplasmic Sperm Injection from November 2008 to March 2017. To limit the potential bias of the analysis, a case-control study design was adopted. Age at pick-up, number of previous IVF failures and number of MII oocytes were chosen as matching criteria, since they are reported among the strongest success predictors in IVF. Every case was matched with two controls.

Participants/materials, setting, methods: Data available from the entire PGT-A population were reviewed and the study population was divided into two groups, according to the presence or the absence of endometriosis. Demographic (age, previous miscarriage, previous failed IVF) and IVF-related information (number of MII, injected and fertilized oocytes, of blastocysts, of euploid blastocysts, of single embryo transfers, of positive beta-Human chorionic gonadotropin, biochemical pregnancy, clinical pregnancy loss, miscarriage and live birth rates) were collected and compared between groups.

Main results and the role of chance: In this study, 249 women affected by endometriosis were matched with 498 women without the disease, according to maternal age at pick-up (38.1 ± 2.9 years), previous IVF failure (0.6 ± 0.8 cycles) and number of MII oocyte retrieved (7.1 ± 3.8 per pick-up). In our population, the percentage of fertilized oocytes per number of MII oocyte was significantly lower in women with endometriosis than in control groups (77 vs 74%).

Percentage of blastocysts obtained per fertilized oocytes (51.0 vs 53.3%), mean blastocyst rate per fertilized oocyte per cycle (56.5 ± 25.2% vs 58.3 ± 26.8%), percentage of euploid blastocysts per biopsied embryo (49.4 vs 50.1%) and mean euploidy rate per blastocyst per cycle (46.8 ± 38.7% vs 43.7 ± 37.3%) did not differ between control patients and women with endometriosis.

Moreover, no significantly differences in terms of percentage of positive pregnancy tests per Single Euploid Embryo Transfer (eSET) (52.4 vs 57.7%), percentage of Biochemical Pregnancy Loss per positive pregnancy test (11 vs 10.6%), percentage of miscarriages per clinical pregnancy (12.3 vs 12.9%) and percentage of ongoing implanted blastocysts or babies born per SET (41.0 vs 44.9%) were detected between group. According to Post-hoc power calculation, we had 80% power to detect a 10% difference in the mean euploidy rate per cycle between groups.

Limitations, reasons for caution: This is a first retrospective analysis performed in 3 IVF Italian centers undergoing PGT-a cycles and eSET. Prospective studies could allow better stratification of endometriosis type and stage. Moreover, only patients which obtained at least one blastocysts have been included in the final analysis.

Wider implications of the findings: Our preliminary data demonstrate that the rate of aneuploidy and the pregnancy outcomes are not statistically significantly different between women with endometriosis as compared with age-matched controls in the IVF population. Aneuploidy therefore could not be related to the poorer IVF outcomes of endometriosis patients.

Trial registration number: not applicable.

P-296 RCT of intra-uterine administration or subcutanious injection of GCSF (granulocyte colony-stimulating factor) before embryo-transfer on resistant thin endometrium in IVF

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Study question: Does intrauterine administration Or Subcutanious injection of G-CSF (granulocyte colony-stimulating factor) prior to Embryo Transfer in patients with resistant thin endometrium improve endometrial thickness and pregnancy rate in IVF?

Summary answer: Subcutanious injection OR Uterine perfusion with G-CSF represents a promising new tool for the currently mostly intractable problem of inadequate, thin endometrium.

What is known already: In 2009, GCSF was successfully used in patients with recurrent abortions (Scarpellini & Sbracia 2009),and later another study reported promising results by using GCSF in four women undergoing IVF who had demonstrated highly inadequate endometrium(Gleicher et al. 2011). Since then, several studies with controversial results have evaluated the effect of GCSF on endometrial thickness and IVF success (Kunicki et al. 2014, Li et al. 2014,Xu et al. 2015). The implantation and pregnancy rates were significantly higher (Scarpellini & Sbracia 2012,et al.2015), whereas did not affect endometrial thickness, implantation, or pregnancy rates (Barad et al. 2014, Li et al. 2014).

Study design, size, duration: The study group (n = 56) received either intrauterine infusion of 300 microgramme /1 ml of G-CSF, or subcutanious injection of GCSF and control group (n = 56) underwent either placebo-saline infusion or placebo subcutanious multivitamin injection before Embryo Transfer.

Participants/materials, setting, methods: The study group (n = 56) received either intrauterine infusion of 300 microgramme G-CSF, or SC injection of GCSF and control group (n = 56) underwent either placebo-saline infusion OR placebo subcutanious injection before ET.

G-CSF was administered per intrauterine catheter on the day of hCG administration. If the endometrium had not reached at least a 7-mm within 48 h, a second infusion was given following oocyte retrieval and same was for Subcutanious route.

Main results and the role of chance: The endometrial growth was significantly different within the two groups. An improvement was shown between the control and G-CSF groups. Endometrial expansion to minimal thickness occurred within approximately 48–72 hour from G-CSF infusion./ SC injection. In all the subjects at the time of infusion of G-CSF, endometrial thickness was 6.23 ± 1.45 mm, and,after infusion /SC injection it increased significantly to 8.46 ± 1.27 mm. The IR and PR were statistically significantly higher in the group that received intrauterine infusion of G-CSF/ SC injection (24% and 34%, respectively) as compared with the control group (11% and 16%, respectively).

Optimal endometrial thickness reflects an adequate maturation, which is a key factor for embryo implantation.

Limitations, reasons for caution: The main strengths of our study were its randomized,controlled design and to evaluate for the first time the efficacy of both systemic subcutaneous and local intrauterine GCSF administration on IVF success.

Further more RCT are required to establish the best route and dose of GCSF administration in IVF patients.

Wider implications of the findings: Uterine perfusion or SC injection with G-CSF represents a promising new tool for intractable problem of thin endometrium. Proliferative and secretory changes in the endometrial lining are the result of a complex intrauterine environment where sex steroid hormones and different local factors play an important role for endometrial thickening.

Trial registration number: NOT APPLICABLE.

P-297 Optimising for patients with endometriosis and reducing the time of the gynaecological examination with a new concept of a pelvic examination chair

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Study question: Is it possible to improve the pelvic examination and particularly for patients with pain due to endometriosis?

Summary answer: The intrusive examination process was compensated by improved comfort and respect for the patients' integrity which also significantly reduced the examination time.

What is known already: Most women with endometriosis have an unpleasant expectancy on gynaecological examinations. If an examination has been unpleasant, the patient will be hesitant to future pelvic examinations and vaginal ultrasounds. Awkward examination positions may cause psychological stress

for the patients with a subsequent risk of generating or magnifying a negative experience. The gynaecological chair with stirrups has had the same design for many years. One reason for the lack of development could be that the examination products are designed from the medical perspective. Human-oriented innovations for comfort irrespective of age and gender should be developed in collaboration with the patients.

Study design, size, duration: A prototype of a gender neutral pelvic examination chair was constructed without stirrups and with built-in heating in the soft upholster. The new technical solution provided improved integrity for the patient with the perineum only exposed during the examination procedure. The patients and the gynaecologists who performed the examination, answered 131 questionnaires concerning experiences after the examinations in the traditional examination chair and in the new prototype, respectively. The examination time was registered.

Participants/materials, setting, methods: The protypes of pelvic examination chairs were evaluated at outpatient gynaecological wards of two different university hospitals. The investigation was carried out by 13 gynaecologists who examined 131 patients in two subgroups with and without endometriosis. The questionnaires were filled out after the examinations were coded and collected feedback on an easy 5 graded scale on 9 questions.

Main results and the role of chance: The questionnaires demonstrated significance ($p<0.01$) in favour of the new concept on a majority of the aspects evaluated. The new pelvic examination chair with heating and without stirrups was rated significantly more comfortable and respectful for the patient's integrity. There were no differences between the groups in their attitude towards going through a pelvic examination, neither in any experience of pain during the examination. A question concerning the experience of examination without stirrups was rated 3.8 on the five graded scale. The gynaecologists were significantly more positive on all questions comparing the pelvic examination chairs with and without stirrups. Measuring the examination time demonstrated a significantly shorter procedure without stirrups. The time saved (average 1.6 minutes per patient) during a day on an outpatient gynecological clinic can permit that one patient more can be examined per day. The patients were not randomised to examination in the different pelvic examinations chair but the number of patiens and gynaecologists participating reduces the role of chance.

Limitations, reasons for caution: The questionnaires were coded but the investigation was for practical reasons not randomised. The patients were randomly invited to participate before the examination. There were no differences between the groups in the patients´ attitude towards going through a gynaecological examination, neither in any experience of pain during the examination.

Wider implications of the findings: The results in the present study demonstrated that minor adjustments of a gynaecological chair can generate a significantly more positive examination experience for the patients in a majority of the aspects investigated. This might have a positive impact on the drop-out rate from fertility treatments.

Trial registration number: not applicable.

P-298 IVF or ICSI: Which is better in women with endometriosis associated infertility requiring ART?

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Study question: Does IVF or ICSI improve reproductive outcomes in women with endometriosis associated infertility compared to infertile women with no endometriosis?

Summary answer: The findings of this study seem to suggest that there is no added benefit and it is potentially detrimental to recommend ICSI in women diagnosed with endometriosis associated infertility especially in moderate to severe (Stage III & IV) endometriosis.

What is known already: Endometriosis is associated with infertility. Between 25–50% of infertile women have endometriosis and 30 – 50% of women with endometriosis are infertile. Many of these women will have to undergo IVF. Abnormal folliculogenesis, impaired oocyte and embryo quality due to radical oxidative stress and imbalances in the levels of cytokines and interleukins have been described as potential contributors to infertility in women with endometriosis. We wondered if utilising ICSI may overcome these stressors and improve fertilisation rate and pregnancy rate in women with endometriosis associated infertility.

Study design, size, duration: A retrospective cohort study was performed and included 262 conventional IVF cycles and 260 ICSI cycles from 2006 – 2016 at the Fertility Clinic in London, Ontario, Canada.

Participants/materials, setting, methods: The study group included women with endometriosis associated infertility who were surgically staged according to the ASRM revised classification of endometriosis: 1996. They were classified as no endometriosis (control group), minimal to mild (Stage I & II) endometriosis or moderate to severe (Stage III & IV) endometriosis. Women aged > 40 years old, male factor infertility, donor gametes and gestational surrogates were excluded.

Linear and logistic regression analyses were performed to examine the association between stages of endometriosis and method of fertilisation on various reproductive outcomes. The primary outcome was live birth rate. The secondary outcomes included number of oocytes retrieved, percent of oocytes fertilised, number of embryos transferred, number of embryos that developed to blastocyst, biochemical pregnancy rate and clinical pregnancy rate. Outcomes were adjusted for age, BMI, FSH, and number of embryos transferred.

Main results and the role of chance: No statistically significant differences in reproductive outcomes were noted between women with no endometriosis and those with minimal to mild (stage I & II) endometriosis whether conventional IVF or ICSI was utilised. However, ICSI was associated with a 71% decrease in clinical pregnancy rate (OR = 0.29, $p = 0.046$) and approached statistical significance for a 69% decrease in live birth rate (OR = 0.31, $p = 0.0796$) for women with moderate to severe (stage III & IV) endometriosis.

Limitations, reasons for caution: The small sample size of this study may result in the possibility of observations occurring by chance alone.

Wider implications of the findings: Further study with a larger sample size will need to be done to further delineate the relationship between ICSI and endometriosis associated infertility. However, at this time caution should be practiced against recommending ICSI when there is no clear indication of failed fertilisation with conventional IVF or male factor infertility, especially in women diagnosed with endometriosis associated infertility.

Trial registration number: Not applicable.

POSTER VIEWING
ETHICS AND LAW

P-299 A comparative analysis of marketing materials used to recruit egg donors in Belgium, Spain and the United Kingdom

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Study question: How is egg donation framed in clinic marketing material used to recruit and/or inform potential egg donors in (Dutch speaking) Belgium, Spain and the UK?

Summary answer: In Belgium, egg donation (ED) was constructed as an engagement that required considerable investment and entailed clear risks in contrast to Spain and the UK.

What is known already: Across Europe, ED recruitment is performed in different ways. Some countries permit a range of advertising methods while others limit or completely prohibit any form of advertising relating to human bodily material (e.g. Belgium). Much of the existing research on recruitment of gamete donors comes from the US where market forces shape practice more directly. This paper focusses on Belgium, Spain and the UK - three countries that hold a stake in the growing global reproductive bio-economy and share features of technological innovation and expertise, but have adopted different regulatory positions in relation to the governance and marketing of ED.

Study design, size, duration: An interdisciplinary team of researchers (bioethics, political economy, sociology) conducted a content analysis (including high frequency words analysis) as well as a comparative thematic analysis to consider 'framing' of egg donation in the data. Interdisciplinary auditing was used to challenge constructed categories and the conceptual framework at several points in the analysis. The findings were compared with country laws and informed consent rules and the implications for informed consent were studied.

Participants/materials, setting, methods: In Belgium, all Dutch language websites of centres were included compared to around 20 clinic websites in both Spain and the UK. For the latter countries, maximum variation sampling was used taking into account geographical location, number of cycles, and sector (public/private). In Belgium, ED is almost entirely situated in the publicly funded system whereas in Spain and the UK it is mainly performed in the private sector.

Main results and the role of chance: In all three countries, ED recipients were presented as women whose fertility problems were no fault of their own, constructing a clear need for the donor to fulfil. Descriptions of medical profiles included 'early menopause' while natural menopause was absent. With regard to the act of donating, in Spain and the UK, words such as 'sharing', and 'helping' were considerably more frequently used compared to the Belgian data. Especially in Spain, ED was constructed as a form of solidarity between women nonetheless with a clear emphasis on the compensation. In Belgium, where clinic advertising is strictly regulated, ED was presented as requiring a considerable investment of time and energy from the donor. Potential egg donors in Belgium were repeatedly warned that the act was 'not straightforward' and 'something to reflect about very carefully'. The Belgian material also appeared to be more focussed on risks and side effects than the Spain and UK material. The data were analysed within the policy context of the countries. We will discuss the possible impact of the public/private sector setting and of the Belgian ban on advertising for the way ED is framed and the implications of the differences in marketing material for informed consent.

Limitations, reasons for caution: The results are limited to three countries, and to a (well considered) selection of clinics, therefore precluding generalisation to whole countries. Further research will be needed on the effects of recruitment discourses on potential donors in order to generate more general conclusions and recommendations.

Wider implications of the findings: These results can contribute to a more complete understanding of the recruitment of egg donors as a practice that depends on specific discourses and is embedded in particular policy contexts. The identification of problematic framing of marketing material is crucial in terms of safeguarding true informed consent of donors.

Trial registration number: Not applicable.

P-300 Attitudes and opinions towards gestational surrogacy among physicians working within reproductive medicine and obstetrics in Sweden

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Study question: What attitudes and opinions do physicians working within obstetrics and reproductive medicine in Sweden display towards gestational surrogacy?

Summary answer: The physicians were relative positive towards surrogacy being introduced in Sweden, however they had concerns about the risk of coercion or emotional pressure and exploitation.

What is known already: Gestational surrogacy is not allowed in Sweden, however about 50 children are born annually to Swedish citizens through cross-border surrogacy. Very sparse investigations suggest that there is no evidence of harm for the surrogate, her family or the child, but there is an ongoing debate about the ethical aspects and medical and psychological risk for the surrogate and for the child in the future.

Study design, size, duration: A cross sectional survey study was conducted in 2016. Questionnaires was sent to 141 physicians working within obstetrics and reproductive medicine.

Participants/materials, setting, methods: This was a Swedish nationwide study including all physicians working with assisted reproduction technology (ART) at both public and private IVF units as well as obstetricians heading maternity clinics and all delivery departments in the hospitals. The study-specific questionnaire measured attitudes and experiences in three domains: Attitudes towards surrogacy, Assessment of prospective surrogate mothers, and Obstetric and delivery care for surrogate mothers.

Main results and the role of chance: A total of 103 of the physicians returned the questionnaire yielding a response rate of 74%. While 63% of the physicians were positive or neutral towards altruistic surrogacy being introduced in Sweden, only 28% thought that it should be publically financed. The majority of the physicians agreed that surrogacy involves risk of exploitation of women's bodies (60%). They also expressed concerns about potential surrogate mothers not being able to fully understand the risks of entering pregnancy on behalf of someone else, and agreed that there was a need that a clear legal contract to be written stating the responsibilities of all involved. They also expressed concern that there is a risk that the commissioning couple might pay 'under the table' to the surrogate mother (82%). As concerns the attitudes of specific professional groups, ART-physicians were more positive towards legalization as well as public financing compared to those working within maternity and delivery care. Severe gestational problems, depression, and preeclampsia in potential surrogate mothers were all regarded as criteria for rejection.

Limitations, reasons for caution: The attitudes and opinions reported here are based on hypothetical situations. It is possible that physicians would think differently if surrogacy were to be allowed in Sweden. Due to the anonymous design we were not able to identify non-responders in order to assess the representativeness.

Wider implications of the findings: The study findings provide important knowledge about attitudes towards surrogacy among the ART-physician and obstetricians who would be the ones to implement surrogacy if it were to be allowed in Sweden. Further discussions about legalization of surrogacy should include views from a wide field of different medical professions and laymen.

Trial registration number: Not applicable.

P-301 Ethical evaluation of informing relatives of premature menopause (PM) patients

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Study question: As PM has genetic origins, is it morally right to require the PM patient to inform her relatives so as to receive ATRs?

Summary answer: In general, it is morally right and justifiable to require a PM patient to inform her relatives who are in reproductive age.

What is known already: Genetic science has enabled us to identify genetic causes of PM. Chromosome disorders and cases of advanced mutation of FMR1 (fragile X Syndrome) are known as the most prominent genetic causes of PM. Genetic techniques of Next-Generation Sequencing help growing identification of single-gene causes of PM in recent years.

There is an up to 50% PM risk for patient's relatives. Also, 6% of cases of sporadic PM and 13% of familial PM disease is a result of FMR1 Pre-mutation and Fragile-X Syndrome carriage. Therefore, informing relatives at risk of PM saves their natural reproduction and helps preventing unhealthy reproduction.

Study design, size, duration: Firstly, the cases available in the Avicenna ARTs Clinic over the last five years are studied. Secondly, based on the empirical data, ethical arguments for and against informing the PM patients' relatives are considered. Thirdly, we conclude with the moral stance of our research to the defence of a general rule of necessity of informing, as this would be conducive to a natural healthy reproduction and prevention of giving birth to children with severe illnesses.

Participants/materials, setting, methods: Experts in the area genetics, law and philosophy are involved in this study. Materials are taken from the data existing in the Clinic relating to the issue at hand, and also analytical substance derived from the related areas. All ethical requirements for research are met. The research is conducted on the basis of a combined method. That is, it is based both on empirical and analytical (moral) methods.

Main results and the role of chance: Notwithstanding the advantages PM patients gain from ARTs services, confidentiality of certain information may be violated. In this regard, there exist two obligations which may fly in the face of each other: 1. patients' or geneticists' obligation to let the patients' relatives know the possibility of PM and exigency of genetic screening; and 2. an obligation to keep information of child conceived through ARTs confidential.

The are two radical approach on this issue: 1. taking confidentiality as an absolute value and plan all other actions on this basis; and 2. profound significance of obligation to inform people on the basis of the principle of no harm and taking all measure on this basis.

Given the above, if PM patients do not inform their relative they may inflict harm on them, especially where the relatives could have taken measures to utilize the awareness of their genetic situation.

On the other hand, informing the relatives has to be morally set in tune with the confidentiality of information of children resulted from ARTs, as this may amount to disclosure of the conception methods of such children and in the end damage their quality of lives. How can we make a balance here?

Limitations, reasons for caution: As the study is taking place in a traditional and heavily religious context, cautions have to be taken on the airing and publishing of the results of the study.

Wider implications of the findings: Given the context of the study, implementation of the results of the study has to be well thought and cautiously initiated.

Trial registration number: n/a.

P-302 Reproductive Endocrinology Infertility (REI) specialists' utilization and attitudes towards expanded carrier screening for oocyte donors

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Study question: To report REIs' current utilization and attitudes towards ECS for OD in clinical practice.

Summary answer: Data suggests disagreement concerning the utilization and dissemination of ECS. Standardized guidelines are needed to clarify ECS application and provide guidance regarding ethical quandaries.

What is known already: ECS has become an increasingly important component of preconception and prenatal care, however its use for OD remain unclear globally. Currently, American Society for Reproductive Medicine (ASRM) advises that genetic screening should be performed based on the donor's ethnic background and family history, while European Society of Human Reproduction and Embryology (ESHRE) recommends it based on effectiveness and proportionality.1,2

Study design, size, duration: An IRB approved qualitative survey study from February to December 2017. A total of 83 responses were obtained.

Participants/materials, setting, methods: A 33-question qualitative survey was distributed through SurveyMonkey® to all Society for Reproductive Endocrine Infertility (SREI) members.

Main results and the role of chance: Of 83 surveys, 81 (97.6%) were completed. Based on current ASRM recommendations, 86.7% of REIs request donors to update their medical history, particularly after identifying a heritable condition. While 60.2% of respondents discuss ECS with recipients, only 26.5% of recipients request it. ECS for ODs is utilized by 51.5% prior to acceptance, while 54.7%. responded that ODs could also decline ECS or request information not be disclosed. However, 75% felt that OD agencies and sperm banks have a responsibility to obtain ECS on all their donors. From a practice standpoint, REI physicians (46.7%), nurses (12.0%), and geneticists (34.8%) counsel those with positive carrier status. Overall, 57.8% agreed that ECS should not be offered until the clinical significance is understood for rare diseases. Moreover, 30.6% believed that ECS could lead to a genetically selected population. In contrast, 58.1% felt screening should be performed regardless of clinical significance. Interestingly, only 20.5% excluded carriers with heritable conditions from their OD pool.

Limitations, reasons for caution: Limitations of this survey-based study include varying interpretation of the questions, ability to skip questions, and low response rate. The sensitive nature of the questions may have deterred individuals from replying honestly or at all, even considering the anonymous nature of the survey.

Wider implications of the findings: ECS is becoming customary practice for third party reproduction. However, varying responses have been reported concerning who/what to test, counseling patients, and notifying individuals of positive carrier status. Our data suggests the necessity of standardizing guidelines including the inclusion and exclusion of third party gamete donors with a positive carrier status especially as genetic testing advances and a wider array of genetic aberrations are discovered.

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Trial registration number: Not applicable.

P-303 The ethics of commercialization: a temporal analysis of newspaper coverage of IVF add-ons in the UK

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Study question: What changes have occurred in recent reporting on IVF add-on treatments in the UK?

Summary answer: During the past year, the public conversation has shifted from concerns about perceived low-rates of success to issues surrounding add-on costs and patient financial strain.

What is known already: Most media analyses of assisted reproductive technologies have focused on the North American context (Campbell 2011; Markens 2012; Orr et al. 2017). Although the UK provides a unique case where public debates about the cost of IVF treatments have been prominent, there have been few systematic media reviews to clearly identify temporal changes in how public concerns have been framed in recent years.

Study design, size, duration: We performed a search of all newspaper articles published in the last four years (2013-2017) using the Factiva database. The key words used were "IVF" and "Add on." We included all articles that were: 1) focused on IVF and add-ons and 2) published in the UK media. In total, we retrieved 93 articles. After further screening, we selected 67 records, as some were duplicates, while others were on topics outside of our inclusion criteria.

Participants/materials, setting, methods: Data were analyzed using content analysis based on similar work in health-related areas (Graneheim and Lundman 2004; Forman and Damschroder 2007). Codes were developed both deductively and inductively according to standard qualitative methods used in the social sciences (Strauss and Corbin 1997). Articles were then coded in order to analyze emerging themes.

Main results and the role of chance: This study aimed to explore recent changes in public debates on IVF add-ons. The term "add-ons" is usually referred to additional treatments offered – often at an additional cost – to IVF patients on top their main treatment. However, as fertility professionals have pointed out, even the term "add-on" itself can be misleading as it can sometimes include routine treatments. We found that the UK public debate significantly changed in the wake of a BBC Panorama special on IVF add-ons. Although findings suggest that the UK media's focus has always been on commercialization issues, newspaper articles published before the BBC special mainly talked about the costs paid by older women undergoing IVF given their low chances of conceiving. However, starting with the end of 2016, the public debate has focused particularly on add-ons and the lack of evidence for their effectiveness. Such treatments are now represented mostly and indiscriminately as a scientifically unjustifiable cost for patients, focusing on the lack of evidence of their efficiency, safety and cost-effectiveness, and assuming a tacit and uncritical understanding of what "sufficient" and "robust" evidence is. Additionally, we found that the most commonly critiqued add-ons are PGS and intralipid infusion.

Limitations, reasons for caution: This study is limited to media coverage and does not include surveys or interviews with members of the public.

Wider implications of the findings: Previous authors have shown that media coverage can influence behaviours and health service utilization (Howe et al., 2002; Leask et al. 2010). The ways in which news reports are framed can affect policy initiatives in public health as well as the general public's understanding of IVF add-ons.

Trial registration number: not applicable

POSTER VIEWING
FEMALE FERTILITY

P-304 The anogenital distance can be a response biomarker in patients undergoing controlled ovarian stimulation for IVF

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Study question: Is the length of the anogenital distance (AGD) a biomarker of ovarian reserve and response to controlled ovarian stimulation (COS)?

Summary answer: Shorter AGD is associated with presence of poor ovarian response.

What is known already: Organ development during prenatal life is influenced by the prevailing intrauterine environment, and it has been suggested that nutritional, environmental and toxic factors could affect ovarian reserve set prenatally. AGD is a biomarker of prenatal-hormonal environment and observational studies have shown an association between its length and reproductive parameters in both sexes.

Study design, size, duration: This was a prospective cohort study of 437 women treated with IVF/ICSI conducted in a tertiary-care university hospital between January and December 2016.

Participants/materials, setting, methods: Patients were divided into three groups: poor responders (<3 oocytes) (n = 50), normoresponders (4-15 oocytes) (n = 332) and high responders (>15 oocytes) (n = 55).Clinical characteristics, ovarian reserve markers, total doses of gonadotropins used and ovarian sensitivity index (OSI) were recorded. Patients with previous pregnancies, polycystic ovary syndrome (PCOS), endometriosis and previous ovarian or genital surgery were excluded. AGD_{AC} (anus-clitoris) and AGD_{AF} (anus-fourchette) were measured before proceeding to oocyte pick-up. Multiple linear regression and logistic regression analyses were used.

Main results and the role of chance: Baseline FSH, AMH, AFC and age were significantly different among the three groups of ovarian response, as were the units of gonadotropin used, and the ovarian sensitivity index (OSI) ($P< 0.001$). Both AGD_{AC} and AGD_{AF} measurements were positively correlated with AMH levels ($r = 0.38$ and $r = 0.21$; $P< 0.05$), AFC ($r = 0.41$ and $r = 0.20$; $P< 0.05$), the OSI ($r = 0.24$ and $r = 0.19$; $P< 0.05$) and the number of oocytes retrieved ($r = 0.29$ and $r = 0.28$, respectively; $P< 0.05$). Conversely, there was a negative correlation between both AGD measurements and the doses of gonadotropins used ($r = -0.19$ and $r = -0.15$; $P< 0.05$). The area under the curve (AUC) for prediction of poor response of AGD_{AC} was 0.70 (95%CI 0.66-0.75), which was comparable to the classic ovarian reserve markers, such as AFC and AMH. AGD_{AF} showed a significantly worse predictive capacity for poor ovarian response (AUC 0.60 [95% CI 0.55-0.60]) than AMH and AFC.

Limitations, reasons for caution: As this was an observational study, causal inference cannot be ruled out.

Wider implications of the findings: The findings of this study suggest that *in utero* exposure to certain hormonal environments could affect the ovarian reserve set prenatally.

Trial registration number: No.

P-305 Non steroidal anti-inflammatory drugs (NSAID) such as ibuprofen have only a limited effect on on the intrafollicular milieu of pre-ovulatory follicles

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Study question: What is the impact of NSAID, specifically ibuprofen, on the intrafollicular milieu of naturally matured pre-ovulatory follicles?

Summary answer: Ibuprofen slightly decreases cytokine and estradiol but not matrix metalloprotease concentrations in the fluid of naturally matured pre-ovulatory follicles.

What is known already: NSAID are not recommended around ovulation in women trying to conceive spontaneously. In contrast, in Natural Cycle IVF (NC-IVF) NSAIDs are used in daily routine to reduce the risk of premature ovulation. NSAID inhibit cyclooxygenases (COX2) that catalyse the synthesis of PGE2, a key prostaglandin in the inflammatory cascade of ovulation. Ibuprofen, at a dosage of 3×400 mg/day, is a frequently used analgesic in women.

Study design, size, duration: Prospective, within-subjects study, performed at a University based infertility centre between 2014 and 2017 and including 19 women undergoing NC-IVF. Patient age was 26-44 years; menstrual cycles were regular. If an early onset of an LH-surge (10-20 IU/L) was diagnosed the women were administered ibuprofen (3×400 mg/day), and follicle aspiration was performed 36 hours after HCG injection. If no LH-surge was detected no ibuprofen was taken.

Participants/materials, setting, methods: Follicular fluid was collected from the 19 women, undergoing one cycle with and one cycle without ibuprofen. The concentrations of follicular cytokines (IL-1β, IL-6, IL-8, IL-12, GM-CSF, TNFα, VEGF), prostaglandin E2, matrix metalloproteases-2 and -9 and hormones (LH, FSH, estradiol, total testosterone, AMH) were determined by

single or multiplexed immunoassays and compared in matched cycles with and without ibuprofen administration.

Main results and the role of chance: In the follicular fluid of ibuprofen treated women, IL-8 was significantly ($P<0.05$) decreased and IL-6 and PGE2 showed a trend towards reduced concentrations compared to controls (IL-8, 170 pg/mL vs. 218 pg/mL, $P<0.05$; IL-6, 2.2 pg/mL vs. 3.3 pg/mL; PGE2, 5.4 ng/mL vs. 6.3 ng/mL). Estradiol concentration was also lower in patients with Ibuprofen (2293nmol/L vs. 3047nmol/L, $P<0.05$). However, other cytokines, matrix metalloproteases and hormones, including IL-1β, TNFα, VEGF, MMP-2, MMP-9, LH, FSH, total testosterone and AMH, were unchanged between cycles with or without ibuprofen. Our study revealed that NSAIDs, specifically ibuprofen, had some but limited impact on the follicular physiology. It also showed that ibuprofen can postpone ovulation in most women for several hours.

Limitations, reasons for caution: The number of included women was limited due to the strict, within-patient inclusion criteria.

Wider implications of the findings: As the effect of ibuprofen on prostaglandin and cytokine concentrations in follicular fluid is small, the treatment with this medication around ovulation should be safe in the analysed dosage in women trying to conceive spontaneously as well as under IVF treatment.

Trial registration number: Not applicable.

P-306 Dynamic culture of human ovarian tissue in a perfusion flow-through bioreactor enhances follicle progression as compared to static culture in gas-permeable dishes

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Study question: Can in vitro continuous perfusion of human ovarian tissue strips improve follicle progression and viability of follicles over culture in gas-permeable dishes (PD)?

Summary answer: Culture in dynamic perfusion flow-through bioreactors (PB) vs static gas-permeable dishes significantly increased follicle progression to the secondary stage.

What is known already: In vitro culture of strips of human ovarian tissue in static PD enhances follicle viability and progression over conventional gas-impermeable dishes by improving oxygen availability in situ. However, progression to the secondary stage is not yet optimal for subsequent follicle isolation and culture until complete oocyte maturation. This is possibly due to suboptimal control of culture conditions and the absence of physiological biomechanical cues in static culture dishes. Dynamic continuous perfusion bioreactors may enable the physiological availaibility of solutes as well as the application of physiological fluid mechanic stresses to tissue.

Study design, size, duration: This paper reports on a comparative study of static versus dynamic bioreactors for the culture of human ovarian biopsies. Tissue strips 1×1×0.5 mm from each patient (n = 4) were cultured in static oxygen permeable dishes (PD) and in dynamic perfusion flow-through bioreactors (PB) for 6 days. Follicle quality, progression and viability were respectively assessed through histology and live/dead assay under confocal microscopy.

Participants/materials, setting, methods: Ovarian biopsies, collected from consenting patients (age <35) during laparoscopic surgery for benign gynecologic conditions, were cut with a tissue chopper and cultured in PB and PD for 6 days in alpha-MEM medium plus supplements at 37°C, 5% CO2 and 95% humidity in air. Fresh (D0) and cultured strips (D6) were fixed and stained for histological analysis or labelled with live-dead far red and Hoechst 33342 for viability assessment at the confocal microscope.

Main results and the role of chance: Overall 1349 follicles were analysed. Data showed that ovarian strips at day 0 mainly contain follicles at the primordial stage (primordial, 72.5%; primary, 24.3%; secondary, 3.2%) characterized by a good quality (grade1, 42.5%; grade2, 27.3%; grade3, 30.2%). Culture for 6 days in PB generally yielded significantly higher follicle progression (primordial, 24.4%; primary, 46.2%; secondary, 29.4%), viability (64.4%), and quality (grade1, 28.4%; grade2, 38.6%; grade3, 33%) than in PD (primordial, 34.5% ($p<0.05$); primary, 47.9% (NS); secondary, 17.6% ($p<0.01$) - viability, 64.3% (NS) - grade1, 22.5% (NS); grade2, 31.7% (NS); grade3, 45.8% ($p<0.01$)). The results suggest that dynamic culture of human ovarian tissue strips in the perfusion flow-through bioreactor for 6 days enhances follicle progression as compared to static culture in gas-permeable dishes in the patients under investigation.

Limitations, reasons for caution: Due to the high heterogeneity of ovarian tissue structure and follicle density and the limited number of patients, this preliminary study needs to be validated on a higher number of tissue strips and in a larger cohort of patients.

Wider implications of the findings: In perfusion flow-through bioreactors, oxygen perfusion, nutrients availability and biomechanical cues have a key role in the health and progression of cultured follicles. This approach may increase the yield of secondary follicles and enhance the efficiency of two-step protocols for human *in vitro* folliculogenesis.

Trial registration number: not applicable.

P-307 Thyroid autoimmunity is associated with higher risk of premature ovarian failure - A nationwide Health Insurance Research Database study

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Study question: Is thyroid autoimmunity associated with higher risk of low ovarian reserve and premature ovarian failure?

Summary answer: Thyroid autoimmunity have a significantly higher risk of premature ovarian failure.

What is known already: Premature ovarian failure is closely related with autoimmune disease and thyroid autoimmunity may account for diminished ovarian reserve in some studies. However, there is no large scale cohort study to demonstrate the association between thyroid autoimmunity and premature ovarian failure.

Study design, size, duration: A population-based retrospective cohort study on the foundation of the National Health Insurance Research Database was designed in a longitudianl-time-course fashion. National Health Insurance (NHI) program in Taiwan since March1, 1995 and included 99.9% of the 23 million population of Taiwan. Patients between January 1, 2000 to December 31, 2013 were eligible for recruitment.

Participants/materials, setting, methods: An autoimmune thyroid disease cohort was composed of patients with autoimmune thyroid disease older than 25 years of age and younger than 40 years of age. The comparison cohort consisted of patient in the National Health Insurance Research Database without autoimmune thyroid disease matched by age at a ratio of 1:3.95.

Main results and the role of chance: Overall, 2736 autoimmune thyroid disease patients and 10821 comparison patients were followed-up until a diagnosis of ovarian failure, amenorrhea or menopausal syndrome had been made. Compared with the comparison cohort, patients with autoimmune thyroid disease presented a 78% higher risk for ovarian failure (95% CI = 1.44-1.97) and a 49% higher risk of ovarian failure after adjustment (95% CI = 1.08-2.07). The cumulative incidence of amenorrhea or ovarian failure in thyroid autoimmunity group were significantly higher than in comparison group.

Limitations, reasons for caution: This is a retrospective study with ICD-9 disease code analysis for the statistical association between two diseases.

Wider implications of the findings: Given that the autoimmune thyroid disease is highly associated with early diminished ovarian reserve or even premature ovarian failure, the option for infertility treatment may be re-directed to more efficient methods in infertile patients diagnosed with autoimmune disease.

Trial registration number: non-clinical trials.

P-308 Possibilities of preimplantation genetic screening (PGS) in overcoming the impact of age on assisted reproductive technologies (ART) results

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Study question: Does PGS increase pregnancy rate in advanced age women? Is there a relationship between AMH level and the frequency of aneuploidy?

Summary answer: A strong correlation between AMH and aneuploidy was found. The frequency of clinical pregnancy is significantly higher in women of older reproductive age after CGH.

What is known already: The inefficiency of assisted reproductive technologies can be as a result of many factors. Aneuploidy is directly depends on the maternal age and rarely associated with the morphological characteristics of the embryos. PGS are used for diagnosis chromosomal rearrangements and selection balanced or healthy embryos. Consequently PGS can increase pregnancy rate and prevent transmission of unbalanced rearrangements to offspring.

Study design, size, duration: Since November 2016 to December 2017 the retrospective open comparative study of 282 embryos from 99 couples was conducted in the international clinical reproductive center "PERSONA".

Participants/materials, setting, methods: All couples were undergone IVF and FET programs. Embryos were undergone biopsy of trophoectoderm on day 5 or 6 with examination by a-CGH to diagnose chromosomal abnormalities. All 99 women were divided into two groups at different ages. The first group included 32 women under 35 years old, the average age was 29.7 years. The second group included 67 women over 36 years and older (average age 41.6 years).

Main results and the role of chance: In the first group 460 oocytes and 85 embryos were obtained. In the second group 559 oocytes and 197 embryos were obtained. Chromosomal abnormalities of embryos in both groups were identified: 45.8% and 56.3% respectivly. There was a strong correlation between the level of AMH and the probability of obtaining an euploid embryo, r = 0.99. An average AMH in group 1 was 4.79+ 2.2, the level of euploidy was 54.2%. An average AMH level in group 2 was 2.98 + 2.9, the level of embryo euploidy was 43.7%, $p<0.05$. Pregnancy occurred in 57.1% in group 1, in the 2nd group pregnancy occurred in 64.5%, $p<0.05$.

Limitations, reasons for caution: Non.

Wider implications of the findings: Applying of PGS allows to increase the pregnancy rate up to 65% for embryo transfer to reduce the risk of a chromosomal pathology with a probability of up to 95%. There is a direct relationship between AMH level and the probability of receiving an euploid embryo.

Trial registration number: 1 PK.

P-309 The most accurate approach for reproductive tract microbiome analysis

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Study question: Does primer selection have any effect on vaginal and endometrial microbiome analysis?

Summary answer: V3V5 primers sets are better suited to identify microorganisms at genus and species abundance. Thus, experimental design is essential when examining the vaginal/endometrium microbiome.

What is known already: The human microbiome is tightly related to health in disease states as well as the reproductive system. Its analysis using a marker gene and NGS became in a powerful tool. The 16S-rRNA contains nine hypervariable regions (V1V9) flanked by conserved regions, which makes an ideal target of bacterial classification. However, most NGS-platforms are not capable of covering the full-length gene. Thus, short regions have been prioritized. Currently, there is no consensus of which region best reflects the vagina/endometrium microbiome. The aim was to investigate if the reproductive-tract microbiome profile will have subtle variations by virtue of the primer set used.

Study design, size, duration: A prospective study was performed. Patients attenting to our clinic were recruited from May to December 2017. In order to detect different diversity in the microbiome, samples were taken at different stages from the same patient (n = 22): luteal phase in the previous cycle, the day of the embryo transfer, the day of the BHCG sample and the day of the ultrasound in the case of getting pregnant.

Participants/materials, setting, methods: In order to avoid confusion factors that can affect the variability and diversity in the microbiome profile we included patients that performed a frozen embryo transfer. DNA was extracted using the PureLink Microbiome DNA Purification kit. To evaluate biases introduced by primer selection V3V4 and V3V5 were used. Sequencing and bioinformatics analysis were performed according to Illumina Metagenomics protocol using the NexteraXT library on the Miseq instrument.

Main results and the role of chance: Overall, the average classification rate at genus level was 90.2% and at species level 74.32% for all samples. Regarding the sample collection, differences were shown when we compared the endometrium and the vaginal samples, for genus and species classification results (60.0 + 25.6 vs 98.7 + 0.6, $p<0.05$; 44.0+ 21.0 vs 81.1+4.1, $p<0.05$). For primer set used, no differences were reported in the classification rates for V3V4 and V4V5 at genus and species level when we consider all the samples. However, when we consider the subset of vaginal samples, higher numbers of classified microorganisms at genus level were obtained when we used V3V5 (99.0+0.1 vs 98.3+ 0.7, $p<0.05$). Regarding the diversity studies 35 different genus and 13 species at top frequencies were detected among samples, suggesting that genus studies give us more information about the microbiome diversity, revealing more than unique Lactobacillus OTU. Species identification of Lactobacillus is more difficult using a V3V4 primer set. Thus, V3V5 provides both breadth and depth of communities dominated by these genera, V3V5 amplicons will reveal either lactobacilli-dominant or lactobacilli-diminished groups. Because the reproductive tract microbiome is a Lactobacilli dominant, experimental design is essential for a broad species detection when examining the vaginal/endometrium microbiome.

Limitations, reasons for caution: The study is limited due to the sample size. A higher sample size should be used in future studies to corroborate the current findings. The lack of a standardized workflow has led to uncertainties regarding the transparency and reproducibility of microbiome studies.

Wider implications of the findings: In conclusion, the microbiome profile will have subtle variations by virtue of the primer set used. V3V5 will distinguish more accurate species in Lactobacillus predominant communities.The unification of analysis procedures and the implementation of standardized workflows may be done in order to minimize variation introduced in the results.

Trial registration number: Not applicable.

P-310 Melatonin attenuates aging of post-ovulatory mouse oocytes via a SIRT1-MnSOD dependent pathway

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Study question: The underlying mechanisms of how melatonin improves the quality of post-ovulatory aged oocytes remain largely unclear.

Summary answer: melatonin delays post-ovulatory mouse oocyte aging via a SIRT1-MnSOD-dependent pathway.

What is known already: The quality of post-ovulatory metaphase II oocytes undergoes a time-dependent deterioration as a result of the aging process. Melatonin is considered an anti-aging agent.

Study design, size, duration: *In vitro* aged MII oocytes were used in experiments at 0, 6, 12 and 24 h. For the melatonin treatment, the treated oocytes were incubated in the IVF medium with a final concentration of 10^{-7}, 10^{-5}, or 10^{-3} M melatonin.

Participants/materials, setting, methods: To collect the metaphase-II (MII) oocytes, mice were stimulated by an intraperitoneal injection of 7.5 IU of Pregnant Mares Serum Gonadotropin (PMSG), and 7.5 IU human Chorionic Gonadotropin (hCG) was injected to induce superovulation. The mice were

sacrificed 12-14 h after the hCG was used. The cumulus-oocyte complexes (COCs) were obtained by breaking the oviductal ampullae.

Main results and the role of chance: In this study, we found that there were elevated ROS levels and impaired mitochondrial function demonstrated by reduced mitochondrial membrane potential ($\Delta\Psi m$) and increased mitochondrial aggregation in oocytes aged 24 h, accompanied by an increased number of meiotic errors, unregulated autophagy-related proteins and early apoptosis, which led to decreased oocyte quality and disrupted developmental competence. However, all of these events can be largely prevented by supplementing the oocyte culture medium with 10^{-3} M melatonin. Additionally, we found that the expression of sirtuin family members (SIRT1, 2, 3) was dramatically reduced in aged oocytes. In addition, *in vitro* supplementation with melatonin significantly upregulated the expression of SIRT1 and antioxidant enzyme MnSOD, but this action was not observed for SIRT2 and SIRT3. Furthermore, the protective effect of melatonin on the delay of oocyte aging vanished when the SIRT1 inhibitor EX527 was used to simultaneously treat the oocytes with melatonin. Consistent with this finding, we found that the post-ovulatory oocyte aging process was markedly attenuated when the oocytes were treated with the SIRT1 activator SRT1720.

Limitations, reasons for caution: This study was done only in vitro.

Wider implications of the findings: Melatonin can be used in IVF for prevents oocyte aging.

Trial registration number: none.

P-311 Peripheral blood mononuclear cells in IVF practice: 2 years experience

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Study question: Do the application of intrauterine transfer of peripheral blood mononuclear cells (PMBC) improve embryo implantation rates in IVF?

Summary answer: The IVF success rates were higher in the PBMC-treated group (P <0.01) comparing with the non-treated group in "cryo" IVF cycles.

What is known already: A human endometrium is able to accept embryos only during some limited period. Endometrium should be prepared to embryo transfer to achieve embryo implantation. There is a suggestion that maternal immune cells are able to support the process of embryo implantation.

Study design, size, duration: The aim of the work was to examine the influence of intrauterine peripheral blood mononuclear cells application on embryo implantation rates for infertile patients in "fresh" and "cryo" IVF cycles. The study's protocol was approved by the Center's IRB.During January 2016 - December 2017, a total of 890 infertile couples were subjected to IVF treatment. All the infertile patients were divided into two groups: with application of PBMC and without one.

Participants/materials, setting, methods: PBMC were applied for 401 patient – 216 "fresh" IVF cycles and 165 ET after blastocysts cryopreservation. Non-PBMC group consisted of 489 patients – 240 "fresh" IVF cycles and 249 "cryo" IVF protocols. Two high quality blastocysts were transferred in each case. PBMC were applied after previous culture before the ET. Verifications of associations of PBMC application with implantation rates were accomplished by the Chi-square test ($\chi 2$ test).

Main results and the role of chance: The implantation rate was significantly higher in PBMC-treated group in "cryo" IVF protocol comparing with ETs of vitrified blastocysts without PBMC ($df = 1$, $\chi 2 = 9.852$, $\chi 2_{critical} = 6.635$, $P < 0.01$). There was no significant difference in implantation rates in both groups after "fresh" IVF attempts. We haven't considered the age of the patients in the present study. But out previous investigations showed the PBMCs affects in "fresh" IVF cycles in patients after 38 years old.

Limitations, reasons for caution: ET and vitrification of the blastocyst can be carried out for the embryos with the high quality of morphology.

Wider implications of the findings: More detailed investigations in this field could explain the role of immune system in the implantation process and early pregnancy control. PBMC therapy could improve implantation rates in patients with recurrent IVF failures with the high quality embryos.

Trial registration number: -

P-312 Use of granulocyte colony-stimulating factor during an assisted reproductive treatment does not increase the risk of birth defects

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Study question: To assess the effect of administration of granulocyte colony-stimulating factor (G-CSF) in newborns resulting from an assisted reproductive treatment.

Summary answer: Administration of G-CSF during the first steps of pregnancy does not imply a higher risk of perinatal complications or malformations in offspring.

What is known already: G-CSF illustrates the scope of reproductive immunology, as they act on reproductive function at different levels such ovulation, embryo implantation and embryo development. Despite their growing interest in the reproductive field, actions of G-CSF have still not been explained concerning its effects on the early stages of embryo development. Information available up to date indicates that administration of G-CSF is safe in terms of risk of perinatal complications on offspring, although further studies are needed to show the harmlessness of this drug in newborns.

Study design, size, duration: Retrospective study performed in 11 private clinics belonging to IVI-RMA group from January 2014 to December 2016. Our study group included 33 live-born children from a pregnancy in which G-CSF was administered compared to the control group, which contained 3798 children from couples also undergoing an assisted reproductive treatment in the same clinics and in whose pregnancy this drug was not ordered. Couples were called a month after delivery date to obtain perinatal information.

Participants/materials, setting, methods: G-CSF is essential in the uteroplacental cytokine network needed to establish and maintain pregnancy. Patients with KIR-HLA-C mismatch and recurrent miscarriage as the study group were treated as off label and after a signed informed consent, with a daily subcutaneous administration of 13 mUI of filgrastim (Neupogen, Amgen, USA) from the embryo transfer until the end of the ninth week of pregnancy. Statistical analysis was performed by ANOVA and Chi-squared where applicable.

Main results and the role of chance: There were no statistical differences in mother's age (40.9 ± 0.1 vs. 38.9 ± 1.8, $p = 0.055$), body mass index (23.2 ± 0.2 vs. 22.6 ± 1.5, $p = 0.503$), children's weight (2952 ± 200 g vs. 3145 ± 270 g, $p = 0.184$), gestational age (38 ± 1 w vs. 37 ± 1 w, $p = 0.926$) and length (50.7 ± 2.1 cm vs. 50.0 ± 1.3 cm, $p = 0.969$) between control group and women treated with G-CSF respectively. According prematurity rate, we did not also observe relevant variances in the percentage of births before week 36 (10.0% vs. 9.5%, $p = 0.783$) and week 32 (2.2% vs. 0.0%, $p = 0.585$) for the control and the study group respectively. Finally, we also analyzed the percentage of children under 2500 g (19.6% vs. 11.8%, $p = 0.570$) and under 1500 g (2.5% vs. 0.0%, $p = 0.454$) and as the previous data, we did not find significant differences for non-treated and filgrastim treated women.

According adverse perinatal outcomes, we did not find any birth defect in children included in the study group compared to a 2.1% of children affected by some congenital anomaly.

Limitations, reasons for caution: A limitation of our study is the small sample size, which makes difficult to draw conclusive results. Moreover, a consequence of a retrospective study is that not all pertinent risk factors are likely to have been identified and subsequently recorded. Therefore, only association can be inferred from the results.

Wider implications of the findings: The above analysis of the effect of G-CSF of *in vitro* fertilization perinatal outcomes in infertile women suggest the safety of G-CSF use in pregnancy, as no neonates complications have been observed. Even though, this treatment should be used carefully.

Trial registration number: Not applicable.

P-313 Control of actin nucleators by maturation regulating factors in mouse oocytes

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Study question: Does MAPK/ERK kinase (MEK) inhibition and maturation promoting factor (MPF) activation affect actin nucleators Spire-1 and Spire-2 in mouse oocytes?

Summary answer: MEK inhibition and MPF activation results in elevated levels of Spire-1 and Spire-2 at different stages of oocyte meiotic maturation.

What is known already: Meiotic resumption of the oocyte is mainly driven by MPF. During meiotic arrest, MPF is inhibited by protein kinase A (PKA), thus inactivated PKA leads to meiotic resumption. Resumption of meiosis results in two inequivalent cells: the polar body and oocyte. Chromosome migration regulated mainly by actin nucleators, takes place to achieve this asymmetric division. Actin nucleators Spire-1 and Spire-2 are known to have roles in asymmetric division of oocytes. MAPK signaling, known to regulate MPF, is also associated with asymmetric division of oocytes, though it is unclear whether it directly affects actin nucleators, or through the activation of MPF.

Study design, size, duration: The oocytes retrieved from 10 mice were pooled and divided into 4 groups for in vitro culture: MEK inhibitor PD98059 (1), PKA inhibitor H89 for MPF activation (2) and combination of these inhibitors (3), DMSO only as vehicle (4). The oocytes were analyzed at germinal vesicle breakdown (GVBD) (2 hours), spindle formation (5 hours), spindle migration (7 hours) and polar body extrusion (PBE) stages as wells as at germinal vesicle (GV) stage without culturing process.

Participants/materials, setting, methods: MEK inhibitor PD98059 and PKA inhibitor H89 for MPF activation was applied during IVM to the GV oocytes retrieved from preovulatory ovarian follicles of 3-5 weeks old female BalbC mice after follicle activation. GVBD and PBE rates of the oocytes were determined. The localizations of Spire-1 and Spire-2 were detected through immunofluorescence. Spire-1, Spire-2, pERK1/2 and pCREB protein levels were determined via Western blot for oocytes at different stages. All experiments were repeated 3 times.

Main results and the role of chance: MEK inhibitor at a concentration of 50 μM significantly reduced pERK1/2 (MEK phosphorylation target) levels of the oocytes. pCREB, the downstream target of PKA was significantly reduced at 10 μM concentration of PKA inhibitor. The inhibitors were applied at these doses during IVM of the oocytes.

GVBD rates were similar in different groups, while PBE rates were lower in MEK inhibition group. Cortical localization of Spire-1 and Spire-2 was detected in oocytes by IF at all stages of meiotic maturation. In control group, both cortical and total Spire-1 level was significantly the lowest at spindle migration stage, though Spire-2 cortical localization was the lowest in GV oocytes. MEK inhibition resulted in a decrease both in cortical Spire-1 and Spire-2 levels in PBE oocytes. PKA inhibition (MPF activation) led to increase in cortical Spire-1 levels in spindle migration stage oocytes, while it resulted in increase of cortical and total Spire-2 levels in PBE oocytes. Application of MEK inhibition and MPF activation at the same time caused an increase in Spire-1 levels at PBE oocytes, pointing out a compensation of the decreasing effect of MEK inhibition. However, Spire-2 levels remained low with no compensation of MEK inhibition in PBE oocytes.

Limitations, reasons for caution: This study only includes the inhibition of a particular member of MAPK signaling and a certain regulator of MPF. However, the relation of actin nucleators with MAPK and MPF might also be through their upstream or downstream regulators.

Wider implications of the findings: The factors affecting actin nucleation contribute to correct positioning of the meiotic spindle during oocyte maturation. Proper manipulation of actin nucleators by chemical inhibitors or activators during IVM of oocytes might enhance the development potential of the embryo leading to an increased success rate in fertility treatments.

Trial registration number: This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK).

P-314 Anti-Müllerian hormone may have a rebound effect in long-term human ovarian tissue grafts co-transplanted with Endothelial Cells

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Study question: Will co-transplantation of human ovarian tissue with Anti-Müllerian hormone (AMH) producing endothelial cells (ECs) keep a larger cohort of quiescent follicles in long-term grafts?

Summary answer: Co-transplantation of human ovarian tissue with AMH producing ECs in long-term grafts may create a rebound effect and cause follicular requirement.

What is known already: AMH has been suggested to exert a repressive input on activation and/or growth during normal folliculogenesis. We have previously shown that direct paracrine delivery of AMH via lentivirally engineered exogenous endothelial cells (exECs) reduced premature follicular mobilization and growth upon co-transplantation at 2-weeks post grafts (Man, et al., 2017). Another group, using continuous administration of AMH via osmotic pump or intraperitoneal injection of lentivirally encoded AMH in a murine model showed similar results (Kano, et al., 2017).

Study design, size, duration: Cross-sectional study, xenograft of human ovarian tissue into NOD *scid* gamma (NSG) mice with co-transplantation of control exECs (n = 7) or exECs ectopically expressing human AMH (n = 8). Grafts were harvested at 14 weeks and the ratio of follicles in each treatment was assessed in histologic sections using light and confocal microscopy.

Participants/materials, setting, methods: Human ovarian tissue, originating from 2 patients, was co-transplanted with ECs or AMH-ECs; patient-matched ovarian tissue was used in control and experimental groups. Both control and AMH expressing ECs were transduced with lentiviral vectors encoding Red fluorescent protein (RFP). Grafts were harvested at 14 weeks and a ratio of follicles in each treatment was assessed in histologic sections.

Main results and the role of chance: AMH-ECs grafts revealed a shift in the follicular pool away from quiescence with a decreased percentage of primordial follicles. In contrast, grafts co-transplanted with ECs showed a significant retention of primordial follicles at 14 weeks. In these long-term grafts, we found a two-fold increase in primordial follicles median percentage with AMH-ECs; 10.84 ± 6.98 vs. ECs 22.51 ± 7.15, P = 0.05. We found a 2-fold decrease between a median ratio of primordial to growing follicles with AMH-ECs; 8.23 ± 3.62 vs. ECs 3.44 ± 1.07, P = 0.05.

Limitations, reasons for caution: Human ovarian tissue available for research is restricted, repeating the experiment at different time points may shed more light on the phenomenon we have described here.

Wider implications of the findings: As auto-transplantation of ovarian tissue becomes more widely practiced, resolution of mechanisms mediating follicular activation and growth are increasingly relevant. Our unexpected finding, given previous results indicating a suppressive input of AMH, suggests that transient AMH overexpression may initially suppress activation, but ultimately induces a rebound effect via negative feedback.

Trial registration number: not applicable.

P-315 Effect of Ulipristal Acetate (UPA) on the gene expression profiling of endometrial cells in culture

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Study question: To assess the effect of UPA, in concentrations compatible with emergency contraception (EC), on gene profile expression of an endometrium cell line in culture.

Summary answer: The presence of UPA modifies the transcriptomic signature of endometrial cells in culture.

What is known already: Emergency contraception consists in the use of drugs or devices to prevent pregnancy after unprotected sexual intercourse. Considering the fundamental role that progesterone has throughout the process ranging from ovulation to implantation and embryo development, UPA has been developed as a selective progesterone receptor modulator (SPRM) with agonist/antagonist activity and pharmacological applications in EC.

The principal mechanism of action of UPA is the inhibition or delay of follicular rupture when administered previous to LH peak. However, given its high effectiveness in the prevention of pregnancy (up to 5 days after intercourse), post-ovulatory effects related to endometrial receptivity, cannot be excluded.

Study design, size, duration: This basic research work was conducted in 2017 and designed as a prospective study. Epithelial cells of human endometrium carcinoma cell line (HEC-1A) were exposed 24 and 48 h to UPA (100, 250, 500 ng/ml) in triplicates. Total RNA was extracted and 192 genes associated with endometrial receptivity were evaluated by quantitative PCR.

Participants/materials, setting, methods: Fluidigm Microfluidic technology combines microarrays with real time PCR, where 192 genes are analyzed. This selected panel was analyzed after 24 and 48 h in culture. From normalized Cqs with respect to the constituent genes, Fold-Change (FC ≥ 0,5 in absolute value) was calculated to determine the differential gene expression between samples treated with UPA and controls. Gene Ontology was performed to identify the biological function of those representative genes.

Main results and the role of chance: Data showed that by 24 h of incubation, there are no significant changes of transcriptomic profile in those cells exposed to UPA (100, 250, 500 ng/mL) respect to controls cultured without the drug. However, by 48 h of incubation, there is a significant ($p<0,05$) increase in total transcriptomic profile in those cells exposed to UPA (at all concentration assayed) compared to controls. To determine which genes are differentially expressed after 48 h of culture, FC of the 192 genes related to endometrial receptivity were analyzed. Absolute value of FC increases with the concentration of UPA in culture, suggesting a dose-dependent trend of down-regulation of gene expression in the panel of genes analyzed. Considering those genes with FC ≥ 0,5 in absolute value, a Gene Ontology (DAVID) was performed to identify the biological function of those representative genes as well as the possible effects of UPA on those genes. Genes related to cellular adhesion and proliferation, signal transduction, growth factors, cytokine activity and estradiol response seem to be down regulated after UPA exposure.

Limitations, reasons for caution: Our study was carried out in vitro with a human endometrium carcinoma cellular model that expresses estrogen, progesterone, molecular adhesion receptors and proteases This could limit the extrapolation of results to the UPA effects on endometrial tissue.

Wider implications of the findings: The present study suggests that there are changes in gene expression of endometrial cells exposed to UPA at concentrations compatible with EC. Our data brings new evidence for the study of the molecular mechanisms of action of UPA, used for contraceptive proposal, on endometrium receptivity associated with embryo implantation.

Trial registration number: This study was supported by SINAE, University of Rosario (BIO 486 Res. 1480/2016), São Paulo Research Foundation (FAPESP) 2015/20504-9, CONICET and ANPCyT (PICT 2016-1057).

P-316 An increase of intracellular cyclic AMP improves mitochondrial function of bovine immature oocytes

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Study question: How does a transient increase of cAMP prior to in vitro maturation (IVM) improves oocyte competence?

Summary answer: An increase of intracellular cAMP improved oocyte quality by enhancement of its mitochondrial function. On the other hand, nuclear maturation did not proceed during treatment.

What is known already: IVM of oocytes is an important technology for assisted reproduction (ART) with a wide range of research and clinical applications. However, it is generally accepted that the development of embryos produced using IVM oocytes are lower than their in vivo counterparts probably due to inappropriate status of cytoplasm. It has been also shown that an artificial increase of intracellular cAMP before IVM significantly improves oocyte developmental competence in cattle and mice. However, the precise mechanism by which cAMP improves oocyte competence during an increase of cAMP remains to be elucidated.

Study design, size, duration: The changes of gene expression in bovine oocytes and surrounding somatic cells following FSK and IBMX treatment were investigated by transcriptome analyses. A total of 458 COCs were exposed to pre-IVM treatment. As references, 491 COCs without culturing to pre-IVM were also used. Time-dependent change of nuclear maturation of oocytes and mitochondrial function in oocytes following FSK and IBMX treatment were also assessed.

Participants/materials, setting, methods: Total RNA was extracted using the RNAqueous-Micro Kit (Thermo Fisher Scientific, CA, USA) from oocytes or cumulus cells separately after isolations of oocytes and cumulus cells. RNA of Clusters were generated on a cBot (Illumina), and two lanes for the four groups were sequenced as 50-bp reads (single end) on a HiSeq 2500 (Illumina). The oxygen consumption rates (OCRs), cytochrome c oxidase (CCO) activity in mitochondria and ATP contents of oocytes were also measured.

Main results and the role of chance: Although the expression of several genes related with the progress of meiotic maturation was up-regulated, the key gene for MPF activation, Cdc25 was down-regulated ($p < 0.01$). As a result, the duration required for meiotic maturation of oocytes treated with FSK and IBMX was the same as that of control oocytes. Meiotic resumption was arrested during FSK and IBMX treatment.

The expression of genes encoding proteins which compose beta-oxidation, glycolysis, mitochondrial electron transport system, lipase maturation, and transportation of fatty acids significantly increased in oocytes following FSK and IBMX treatment ($p<0.01$). The OCRs, the proportion of mitochondria with high CCO activity and ATP content in oocytes significantly increased ($p<0.01$).

Genes involved in glycolysis and ovarian steroidogenesis were significantly upregulated in cumulus cells following FSK and IBMX treatment ($p<0.01$).

Limitations, reasons for caution: Further studies should be required to assess whether the data obtained from bovine oocytes is applicable to human ART.

Wider implications of the findings: The data of the present study revealed that FSK and IBMX treatment at prophase stage induced the gene expression of glycolysis, fatty acid degradation and mitochondrial functions in oocytes, and accordingly improved mitochondrial functions and ATP levels in oocytes.

Trial registration number: Not applicable.

P-317 Vitamin B2 and B6 deficiency and overgrowth of intestinal candida adversely affect fertilized embryos

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Study question: Could most favorable embryos be created by organic acid testing capable of realistic evaluation of nutrition deficiency and metabolism?

Summary answer: Vitamin B6 deficiency decreased the antral follicle count (AFC) and normal fertility rate. With intestinal candida overgrowth, significant decrease in good blastocyst count was observed.

What is known already: Apparently, metabolism of egg mitochondria determines the egg quality; however, most studies were based on dietary compositions with unclear nutrition details. In contrast, our study was based on organic acid testing, which can precisely assess both excess and deficiency of nutrition from metabolic products of nutrients after digestion and absorption.

Study design, size, duration: Of the patients who underwent an in vitro fertilization procedure (short or antagonist protocol) between November 2016 and December 2017, 82 patients (age: 26–45 years; mean: 38.2; 2 SD: ±8.62) who exhibited poor ovum count, fertilization rate, division rate, and/or blastocyst reach rate were subjected to organic acid testing. In this study, we retrospectively examined these patients. Of 82 patients examined, the number of patients suffering from candida overgrowth was abnormally high at 70 (80%).

Participants/materials, setting, methods: We categorized the subjects into the excessive deficiency group (<−2 SD) and normal group (>−2 SD) for vitamin B2, B6, B12, and folic acid, which are frequent and significant items among the test items, and the candida overgrowth group (>+2 SD) and normal group (<+2 SD) regarding intestinal bacteria. We assessed the AFC, ovum count, fertilization rate, and blastocyst reach rate of each group and compared by t-test.

Main results and the role of chance: Compared to the normal group, vitamin B2 deficiency group (<−2 SD) exhibited a significant decrease in the AFC ($P = 0.0056$; odds ratio, 0.73). The vitamin B6 deficiency group (<−2 SD) exhibited a significant decrease in the AFC ($P = 0.023$; odds ratio, 0.62) and normal fertility rate ($P = 0.0195$; odds ratio, 0.48) compared to the normal group. Although not statistically significant, we also observed a decline in two other items: the ovum count ($P = 0.0537$; odds ratio, 0.61) and day 3 good embryo count ($P = 0.063$; odds ratio, 0.49). Regarding intestinal bacteria, the candida overgrowth group demonstrated a significant decline in the blastocyst reach rate ($P = 0.0245$; odds ratio, 0.54) and good blastocyst reach rate ($P = 0.027$; odds ratio, 0.14) compared to the normal group. Although not statistically significant, a decrease was also observed in three other items: the day 3 good embryo count ($P = 0.087$; odds ratio, 0.70), good blastocyst reach rate ($P = 0.059$; odds ratio, 0.23), and used embryo count ($P = 0.0819$; odds ratio, 0.71).

Limitations, reasons for caution: The study cohort comprised only 82 patients; therefore, further investigation with larger sample size is warranted. In addition, the novelty of this study necessitates an additional examination by other institutes.

Wider implications of the findings: Improvement in the pregnancy rate can be anticipated by adequate supplementation of vitamin B2 and B6 and inhibition of candida overgrowth.

Trial registration number: none.

P-318 Granulosa cells provide elimination of apoptotic oocytes through unconventional autophagy-assisted phagocytosis

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Study question: Granulosa cells manifest a strong gonadotropin-dependent autophagy activity during folliculogenesis, which correlates with apoptosis. There was no data on the ability of granulosa cells to clean apoptotic oocytes.

Summary answer: Granulosa cells eliminate apoptotic oocytes and other apoptotic substrates through unconventional autophagy-assisted phagocytosis.

What is known already: The insufficiency to clean the tissues from apoptotic substrates strongly perturbs tissue homeostasis and causes autoimmunity. Premature ovarian failure (POF) is often associated with the presence of autoantibodies against ovarian proteins. The role in elimination of apoptotic oocytes during follicular atresia is commonly attributed to macrophages, which are found within the granulosa cell layer only in the most advanced stages of atresia. Living granulosa cells are known to clean their apoptotic counterparts in atretic follicles through autophagy-related mechanism. No data on the role of granulosa cells for disposal of apoptotic oocytes has been reported.

Study design, size, duration: Cultured human granulosa cells were used to study main phagocytosis (binging, ingestion, degradation) and autophagy (initiation, elongation, docking and fusion, degradation) steps after loading with apoptotic oocytes and other apoptotic substrates.

Participants/materials, setting, methods: Analysis of primary cultures of human granulosa cells and human granulosa cell line (KGN), for the presence of specific molecular markers and end-products characteristics for different steps of phagocytic and autophagic machineries by immunocytochemistry, confocal and electron microscopy and Western-blotting before and after loading with apoptotic substrates.

Main results and the role of chance: Granulosa cells manifest a strong ability to eliminate apoptotic substrates of different origin. As demonstrated kinetic studies, the rate of elimination of apoptotic substrates by granulosa cells is higher comparing with other phagocyte cells (Sertoli cells, retinal pigmented epithelium cells). The process of apoptotic cleaning by granulosa cells is strongly supported by autophagy in the process resembling LC3-associated phagocytosis (LAP). This indicates that granulosa cells should be considered as one of the most powerful non-professional phagocytes in the body, endowed with the ability to combine phagocytic and autophagy degradative pathways for ovarian homeostasis maintenance.

Limitations, reasons for caution: Thorough analysis of different steps (binding and ingestion, initiation, elongation, degradation) of both phagocytic and autophagic degradative processes depending on gonadotropin status merits to be performed.

Wider implications of the findings: Cleaning of apoptotic oocytes by surrounding granulosa cells seems likely to be a physiological mechanism involved in follicular atresia. Proper functioning of this mechanism may be a new strategy for the treatment of POF syndrome.

Trial registration number: GMR 2018-03

P-319 In vitro embryo development outcomes of DuoStim ovarian stimulation

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Study question: To investigate in vitro embryo development outcomes following DuoStim in patients with poor follicular phase (FP) ovarian response.

Summary answer: Luteal phase (LP) ovarian stimulation not only contributes to oocyte number, but also to blastocyst number, blastocyst quality, and the chance of freeze-all.

What is known already: Studies have shown that it is possible to cumulatively increase the retrievable oocyte number in poor ovarian response patients by consecutively performing FP and LP ovarian stimulation in the same menstrual cycle.

Study design, size, duration: The study is a retrospective observational study being performed at a private IVF center. Poor ovarian response patients undergoing blastocyst freeze-all IVF cycles are prospectively being included in the study, as of May 2017. All ovarian stimulations are being performed using gonadotropin releasing-hormone (GnRH) antagonist co-treatment protocols, using combinations of recombinant follicle stimulating hormone (rFSH) and

human menopausal gonadotropin (hMG), with or without clomiphene citrate (CC) priming for follicular stimulation.

Participants/materials, setting, methods: Patients with poor response (≤5 oocytes) after FP ovarian stimulation who have at least two follicles of >5 mm at oocyte retrieval are eligible for inclusion. Indomethacin is being administered from the day of trigger to the day of oocyte retrieval. Follicles <10 mm at FP oocyte retrieval are not being aspirated, with LP ovarian stimulation commencing on or after the day of FP oocyte retrieval. The primary outcome measure is oocyte number.

Main results and the role of chance: Twenty-three eligible patients have thus far been included in the study. Two patients have had their LP ovarian stimulation discontinued, as the result of poor follicular development. Two patients did not undergo LP oocyte retrieval, because of premature ovulation. From a median day2-3 antral follicle count of 4.7, 1.8 oocytes have been retrieved at FP oocyte retrieval, increasing to 3.7 after LP oocyte retrieval. Similarly, the number of blastocysts increased from 0.52 to 1.1. Eight patients had blastocysts for cryopreservation after FP oocyte retrieval, increasing to 11 after LP oocyte retrieval. Four (4/12; 33.3%) good quality blastocysts (>2CC) were obtained from the FP and eight (8/13; 61.5%) from the LP.

Limitations, reasons for caution: The use of DuoStim prevents the use of fresh embryo transfer, necessitating effective cryopreservation and frozen embryo transfer protocols. While DuoStim may improve in vitro embryo development outcomes of patient with cycle dependent poor response, this may not include Bologna poor response patients.

Wider implications of the findings: LP ovarian stimulation increases the chances of patients having blastocyst freeze-all. Developing effective DuoStim protocols may benefit patients with reduced ovarian reserve, as well as, normal ovarian reserve patients wishing to cumulatively increase their reproductive chances or expedite effective treatment outcomes.

Trial registration number: N/A.

P-320 Neuropeptide phoenixin (PNX) and its novel receptor GPR173 induce COX-1/COX-2 expression and PGE2 production through the CREB signaling pathway

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Study question: Can phoenixin in human granulosa cells has a direct effect on ovulation?

Summary answer: Phoenixin acts through its novel receptor GPR173 to induce COX-1, COX-2 expression, and PGE2 production by activating the CREB signaling pathway in human granulosa cells.

What is known already: Phoenixin is a recently discovered neuropeptide involved in regulating the reproductive system. Phoenixin activates the cAMP/PKA/CREB pathway through its receptor GPR173, a part of SREB (Super conserved Receptor Expressed in Brain) family. GPR173 also was detected at high level in the ovary. Studies have linked the CREB signaling pathway to induce COX-2 expression. Besides, cyclooxygenase (COX) enzyme is the key enzyme that controls the rate-limiting step of prostaglandin E_2 (PGE_2) synthesis, which has been recognized as a key mediator of ovulation. Whether the direct effect of phoenixin on PGE_2 biosynthesis and ovulation is still unknown.

Study design, size, duration: In vitro studies utilizing the human non luteinized granulosa cells line (HGrC1) was performed to investigate the effects of PNX on COX-1/COX-2 expression and PGE_2 production.

Participants/materials, setting, methods: HGrC1 was cultured with PNX-20 at 10 nM, 100 nM concentration with different time points, 1 hour, 3 hours, 6 hours, 12 hours and 24 hours. The levels of mRNA and protein were examined by RT-quantitative real-time PCR and western blotting. The PGE2 concentrations in the culture medium were measured by ELISA. siRNA targeting GPR173 was used to observe the influence of GPR173 receptor.

Main results and the role of chance: Our previous studies demonstrated that PNX and GPR173 present in human oocytes, granulosa cells and theca cells in various stages of development. Interestingly, GPR173 expression in granulosa cells from primary follicle and increases according to the stage of follicle development. We also confirmed the expression of PNX and GPR173 mRNA in HGrC1 cells. PNX-20 significantly induced the mRNA levels of COX-1 (2.25 ± 0.04-fold, $P < 0.001$) and COX-2 (2.76 ± 0.06-fold, $P < 0.001$) in a dose-dependent manner. The level of COX-1 and COX-2 were induced with the maximal effect observed after 3 hours treatment with PNX-20. PGE_2 production in culture medium was significantly enhanced by PNX-20 administration in a dose-dependent manner. We assessed CREB mRNA levels and found increased CREB mRNA upon PNX-20 100 nM treatment (1.44 ± 0.07, $P < 0.01$). In addition, our Western blotting results reveal that PNX-20 increased Phospho-CREB in 15 and 30 minutes of treatment (3.66-fold and 8.42-fold, respectively). Moreover, the silencing of GPR173 markedly reduced the PNX-induced increase in COX-1, COX-2 mRNA expression.

Limitations, reasons for caution: Although HGrC1 share common characteristics and are functionally similar to primary human granulosa cells, there are many differences between the primary granulosa cells and HGrC1 cells such as karyotype and the response to the stimulating factors.

Wider implications of the findings: Our results show that phoenixin act through GPR173 to induce COX-1, COX-2 expression and PGE2 production in human granulosa cells, by activating the CREB signaling pathway. Overall, these findings demonstrated that phoeinxin may participate in regulating human ovulation through the induction of cyclooxygenase-mediated PGE2 production.

Trial registration number: None.

P-321 CHK2 controls the oocyte pool in mammals

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Study question: Is the CHK2-dependent checkpoint responsible for the perinatal loss of oocytes? Does it control oocyte pool and mammalian female fertility?

Summary answer: CHK2 has a role in the pachytene checkpoint controlling the oocyte number in fetal ovaries. It also regulates the number of oocytes in adult females.

What is known already: During mammalian oogenesis, oogonia proliferate forming the so-called germinal cysts. They progress through meiotic prophase I and cysts break down due to massive perinatal oocyte death. During meiotic prophase I, double strand breaks (DSBs) are formed throughout the genome and repaired by homologous recombination. In response to errors in this process, the DNA damage response kinase CHK2 is activated promoting cell cycle arrest or even apoptosis. Contrary to what occurs in spermatocytes, oocytes present high numbers of unrepaired DSBs at pachynema, at the time of the massive oocyte death and cyst breakdown.

Study design, size, duration: We studied the number of oocytes and follicles present in control and *Chk2* mutant ovaries at different time points of animal development. We collected ovaries from $Chk2^{+/-}$ and $Chk2^{+/+}$ (control), and $Chk2^{-/-}$ (mutant) mice from 16 and 18 days *post coitum* (dpc), 0, 1, 2, 3, 40 and 400 days *post-partum* (dpp). For the fetal mice, the females were breed and timing was counted from the day when the vaginal plug was observed.

Participants/materials, setting, methods: For perinatal mice the ovaries were collected, fixed in 4% Paraformaldehyde, processed and sectioned at 7 µm. Every second section was stained by immunofluorescence against the germinal cell marker DDX4 with DAPI as counterstaining. The DDX4-positive cells were scored and classified. For adult females the ovaries were collected, fixed in Bouin solution, processed and sectioned at 7 µm. Every fifth section was stained with PAS-Hematoxylin and only the oocytes with visible nucleus were counted.

Main results and the role of chance: Control and mutant ovaries present the same number of oocytes at 16 dpc. However, at 18 dpc there is a significant two-fold increase in mutants compared to controls. This difference is still significant at 0 dpp, but is overturned during the following postnatal days. This data suggests that CHK2 regulates the number of oocytes in fetal ovaries, but there must be an alternative mechanism capable of levelling the number of oocytes

during the perinatal phase. Interestingly, while in young adult mice (40 dpp) we see no difference in the number of oocytes present in control and mutant ovaries, elder mutant mice (400 dpp) have three times more oocytes than control mice. In particular, this increase is due to an accumulation of primordial follicles, which represent the pool of oocytes available to use during the fertile period of a female. Therefore, our results show that CHK2 regulates the pool of oocytes present in adult mouse ovaries. We propose these data reflects how many oocytes may accumulate DNA damage and are naturally eliminated by a CHK2-dependent checkpoint. Thus, these findings suggest that CHK2 inhibition may extend the reproductive life of mammalian females.

Limitations, reasons for caution: The number of oocytes present in perinatal ovaries was highly variable between individuals and even between ovaries from the same mouse.

Wider implications of the findings: We found evidences that show that CHK2 controls the oocyte numbers both around birth and in adult females. Our results in elder mice suggest that inhibition of CHK2 could extend mammalian female fertility.

Trial registration number: not applicable.

P-322 Dual triggering in antagonist ICSI cycles

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Study question: Is it beneficial dual triggering with 0,1 mg/ml triptorelin asetat and 250 µcg recombinant hCG together in GnRH antagonist cycles.

Summary answer: It seems to be that dual triggering does'nt have superior effect over only rhCG triggering group in GnRH antagonist ICSI cycles.

What is known already: Final oocyte maturation is induced by human chorionic gonadotropin (hCG) after the controlled ovarian hyperstimulation (COH). According to some studies, dual triggering with a GnRH agonist and recombinant hCG does have improved oocyte maturation rates and pregnancy rates in IVF cycles compared to hCG alone groups.

Study design, size, duration: Single center, prospective, randomized clinical study.

Participants/materials, setting, methods: Two groups allocated as Group A (GnRH analog+ rhCG 56 patients) and Group B (only rhCG 66 patients) according to the triggering method. 0,1 mg/ml triptorelin asetat was used for GnRH analog and Ovitrelle for 250 µcg recombinant hCG (rhCG). Severe male infertility factor (<15 million sperm/ml) and poor ovarian reserve patients (<5 antral folicule count) were excluded from the study.When at least 3 follicules >17 mm the triggering was made randomly. P value set >0.05

Main results and the role of chance: Groups were comparable in terms of age, gonadotropin start dosage,total amount of gonadotropins, duration of stimulation (Table I)

Table I

Parameters	Group A (n = 56)	Group B (n = 66)	P value
Age	32.79 ± 5.71	34.09 ± 4.9	0,17 NS
Gonadotropin start dosage	212 ± 44	209 ± 46	0,71 NS
Total amount of gonadotropins	1948 ± 459	1991 ± 617	0,66 NS
Duration of stimulation (day)	9,39 ± 0,78	9,42 ± 0,90	0,83 NS

As main outcome of this study, collected total oocyte numbers, maturation and fertilization of oocytes and pregnancy rates were compared between the groups (Table II).

Table II

Parameters	Group A	Group B	P Value
Collected total oocyte numbers	11,13 ± 5,41	9,68 ± 4,17	0,09 NS
Mature Oocytes	8,5 ± 3,67	8,11 ± 3,46	0,54 NS
Fertilised Oocytes	6,23 ± 3,12	6,15 ± 2,52	0,87 NS
Pregnancy rates	28/56 (%50)	31/66 (%46,9)	0,73 NS

According to these results there isn't any significant difference between two groups in all parameters. We didn't see OHSS in any group.

Limitations, reasons for caution: None.

Wider implications of the findings: Dual triggering is alternative to classic only hCG trigger. Whether some studies mentioned as an significant increase in total collected oocyte number, mature oocyte number and pregnancy rates we didn't find any significant difference with these parameters. It seems that large-scale collaborative studies needed for future aspects.

Trial registration number: None.

P-323 Unravelling the molecular pathways responsible for sex determination in humans

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Study question: What are the molecular signals governing sex determination in human fetal gonads?

Summary answer: While the antagonizing sex determination mechanisms of WNT and FGF signaling appear to be conserved between humans and mice, the key determining factors are different.

What is known already: Sex determination and germ cell maturation mechanisms vary widely between species. In female mice, upregulation of WNT4 initiates a signaling cascade, leading to the development of ovaries. Conversely in males, WNT4 signaling is repressed by SOX9 and its target FGF9, shortly after activation of the male fate determining SRY gene, resulting in the formation of testes. Human (partial) sex reversal case studies suggest that the antagonistic role of WNT and FGF signaling is conserved between mice and humans. Transcriptomic analysis of human fetal gonads may provide valuable insights into the underlying mechanisms of sex determination and maturation in humans.

Study design, size, duration: Single cell sequencing data, from human fetal gonads of both female and male samples, were analyzed in R (version 3.4.2). Developmental age ranged from week (W)5 to W26 of development for female, and W4 to W25 for male cells. Data were filtered to include only cells with cell types validated through marker gene expression, retaining a final dataset of 992 female and 852 male cells of various ages from 17 female and 12 male embryos.

Participants/materials, setting, methods: Genes from WNT and FGF signaling pathways, from Gene Ontology databases, were queried in the datasets to identify age- and cell type-specific genes of interest (GOI). Heatmaps were generated of those GOI to visualize differences between various developmental clusters of germ cells and somatic cells. Differentially expressed genes (DEGs) between germ cell and somatic clusters of specific ages were identified. Volcano plots were generated to visualize significant DEGs.

Main results and the role of chance: The analysis of DEGs between W4 and W9-W10 male fetal gonadal cells (FGCs) and Sertoli cells showed that,

similar to mouse, SOX9 was differentially upregulated in human Sertoli cells. Furthermore, comparison between W5 and W7-W8 FGCs and granulosa cells in female, revealed elevated expression of multiple WNT signaling genes. Markedly, unlike mouse, the female determining factor WNT4 was not differentially expressed in somatic cells compared to FGCs at the time of sex determination. However, we observed a significant increase in WNT6 expression at W7-W8. This suggests that although similar sex determining mechanisms exist between human and mouse, other WNT genes may fulfil differing key roles in humans. Furthermore, we have identified WNT-antagonizing factors, such as SOX10 and SOX11 in both W9-W10 male and W7-W8 female somatic cells. These genes may perform undescribed regulatory functions during sex specification, in humans. Surprisingly, we also identified FGF9, a male determining factor, to be elevated in female human somatic granulosa cells, thus further highlighting interspecies differences in the underlying mechanisms of sex determination.

Limitations, reasons for caution: DEGs were identified from existing in silico databases from human fetal gonadal cells. To further validate the expression of the GOI, immunofluorescence analysis in freshly isolated human gonadal material from 7-12 W is currently ongoing.

Wider implications of the findings: Defining the key regulatory players of WNT and FGF signaling during sex determination in humans will greatly contribute to elucidating the role of these pathways during germ cell development and maturation. Adaptation of culture conditions based on this knowledge may ultimately advance efforts to derive sex-specific germ cells *in vitro*.

Trial registration number: not applicable.

P-324 Oral Vitamin D supplementation impacts luteinized granulosa-cell transcriptomics

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Study question: Can oral administration of Vitamin D (VD) to infertile women undergoing in vitro fertilization (IVF) influence luteinized granulosa-cell (GCs) gene expression?

Summary answer: Surprisingly, oral administration of VD manifests at the gene expression level in luteinized GCs, which were isolated during an oocyte retrieval procedure for IVF.

What is known already: There is evidence that circulating VD concentration in infertile women is associated with successful Assisted Reproductive Technology (ART) treatment. It is unclear whether this association is causal and no randomized clinical trial to date addressed this notion. We launched (2017) the first multi-centre controlled double-blinded randomized clinical trial to assess whether supplementation of VD will improve IVF clinical pregnancy rate. Herein we addressed one of the secondary objectives, whereby the indirect effect of VD supplementation on oocyte prognosis is explored. Specifically, we investigated the effect of the intervention on GCs transcriptomic machinery, critical regulator of oocyte maturation.

Study design, size, duration: The clinical trial aims to recruit 600 women with a baseline VD serum level <30 ng/ml, who are administered either 300,000 IU of 25-OH-vitamin D (VD group) or placebo (PL group) in a single oral shot. The duration of the study is set on three years.

Participants/materials, setting, methods: Follicular fluid (FF) samples were aspirated during the procedure of oocyte retrieval and GCs were isolated. Pilot transcriptomic analysis with RNAseq utilizing a subset of participant samples (n = 3 per group) and RT-PCR (n = 19 VD, n = 22 PL) was employed to scrutinize the differences between the two groups.

Main results and the role of chance: RNAseq analysis confirmed that GCs derived from the VD group owned a different transcriptomic signature compared to the PL group. The impact of VD on pathways involved in the GC-oocyte interaction was thoroughly explored and a number of genes were found differentially expressed between the VD and PL groups. The action of VD in GCs is plausibly mediated via the Vitamin D Receptor (VDR), the gene expression of which was 3.3 fold higher (±1.33, p = 0.046) in GCs of the VD group (n = 19) compared to PL group (n = 22). Notably, the expression of GC-specific genes traditionally associated with oocyte regulation, such as HAS2, VCAN, 3βHSD, AMHR-2, FSHR, PTGS2, GREM-1, CCND1, STAR, did not significantly change. Non-traditional GC-gene expression patterns reflecting GC-oocyte interaction following administration of Vitamin D warrant further investigation and promise to provide insight into the pathophysiology of oocyte maturation and inform future strategies for the improvement of IVF outcomes.

Limitations, reasons for caution: RNAseq validation on the protein level is pending for full interpretation of gene expression data.

Wider implications of the findings: Vitamin D supplementation may be influencing IVF outcomes via indirect control of GC-gene expression. The latter is critical for the GC-oocyte interaction and, hence, expected to impact oocyte quality.

Trial registration number: EUDRACT 2015-004233-27

P-325 Impact of the fresh cycle outcome on the subsequent frozen-thawed blastocyst transfer in medicated vs natural cycle

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Study question: Does the endometrial preparation regimen influence the outcome of frozen embryo transfer when preceding fresh transfer was unsuccessful?

Summary answer: Our study suggests that medicated frozen cycle is superior to natural cycle for patients where the preceding fresh cycle didn't result in a live birth.

What is known already: Currently, there is still no agreement whether different regimens of endometrial preparations in frozen embryo transfer cycles have equal effectiveness. It remains unclear whether hormonal preparation with estrogen and progesterone may enhance likelihood of pregnancy, when compared with a natural cycle environment.

Study design, size, duration: Between May 2015 and May 2017 the live birth rate of all naturals cycles was assessed in a case-controlled matched study with medicated frozen replacement cycles in a tertiary center.

Participants/materials, setting, methods: A total of 208 natural frozen cycles were case-matched by age, indication for ART, number of previous cycles and number of transferred thawed blastocysts with 528 medicated frozen cycles. Patients were offered natural frozen cycle if they had regular ovulatory menstrual cycle. Assuming $\alpha = 0.05$ and 80% power it was calculated that 722 frozen-thawed cycles were required to demonstrate a difference of 10% in live birth rate.

Main results and the role of chance: A total number of 981 embryos was transferred in 736 cycles were included. The mean age of patients who had natural and medicated cycles was 34.3 ± 3.8 and 33.7 ± 4.2 respectively (P = 0.07). The mean endometrial thickness was 8.7 ± 1.9 mm in natural and 9.5 ± 1.85 mm in medicated frozen cycles (P = 0.2). The mean number of transferred embryos was 1.3 ± 0.5 in the former and 1.4 ± 0.5 in the latter (P = 1.12). The live birth rate per frozen embryo transfer, was significantly lower in the natural (35/150, 23%) cycle in comparison with medicated frozen cycle (117/354 – 33%), RR 1.4, 95% CI 1.02-1.96, P = 0.036), if preceding fresh cycle did not result in a live birth. However, if fresh cycle resulted in live birth, there was no significant difference in subsequent frozen cycle whether it had natural (22/47 – 46%) or medicated (51/148–34%) preparation (RR 1.3, 95% CI 0.93 – 1.98, P = 0.11).

Limitations, reasons for caution: Although important variables such as age, indication for ART and previous attempts of transfer number of transferred embryos were comparable some bias could arise from the fact that allocation of frozen cycle was not random.

Wider implications of the findings: This study suggests that medicated cycle might be more preferential for patients in whom the fresh transfer did not result in live birth. This is a first study to report such observation and a randomised controlled study is needed to explore this hypothesis further.

Trial registration number: non-applicable.

P-326 Collagen, type I, alpha 1 (COL1A1) as potential molecular marker of ovarian aging and reproductive success in cumulus oophorus cells (CCs)

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Study question: Could the expression of some Transforming Growth Factor (TGF)-beta family members in CCs be related to reproductive aging and/or Assisted Reproductive Technology (ART) outcomes?

Summary answer: Molecular analysis combined with a multiple endpoint evaluation in ART allows the identification of transcripts and proteins in CCs that seemingly reflect reproductive success/treatment outcome.

What is known already: Success rates in ART are still unsatisfactory, partly because of insufficient oocyte quality. As this property worsens with aging, it suggests that an age-related regulatory imbalance is likely to occur in the growing ovarian follicle of reproductively aged women. Due to their prominent role in follicular growth and oocyte maturation, abnormal CCs regulation appears as an important contributor to such imbalance. Apart from producing compounds that nurture the oocyte, CCs are important interveners in a set of reciprocal interactions with the oocyte that involve different members of the varied TGF-beta family of proteins.

Study design, size, duration: CCs pools were prepared for different molecular analysis performed in the Biomedicine Department of the Faculty of Medicine-Porto University, after women undergoing ART at CETI-Centro de Estudo e Tratamento da Infertilidade (Porto, Portugal) gave their informed consent. Collection was performed intermittently over 3 years, as the work progressed, following a strict methodology based on clinical and laboratory parameters, 118 samples were grouped using multiple criteria, including cell morphology assessment and data obtained during treatment.

Participants/materials, setting, methods: CCs were mechanically dissected from CCs-oocyte complexes after ovarian puncture of women aged between 27 to 43 years old. Upon proper morphological evaluation and sampling, they were washed and preserved in liquid nitrogen until RNA extraction, followed by its amplification and quantification by quantitative PCR (qPCR), transcription levels were measured and statically analyzed. Other samples were preserved at -20°C for protein assay, they were completely lysed and analyzed by Western Blotting followed by normalized quantification.

Main results and the role of chance: The work established a methodology for grouping CCs samples, based on multiple and specific criteria, aiming to increase groups homogeneity and biomarkers reliability. Gene expression analysis used qPCR arrays to allow simultaneous quantification of 84 transcripts linked to the TGF-beta family. Major changes in expression levels were subsequently confirmed by conventional qPCR. Comparing the medians of quantifications with Mann-Whitney's test ($p < 0,05$ was established for significance level), one of the transcripts - col1a1 – corresponding to alpha-1 type I collagen, was found to be significantly expressed in younger women ($p = 0,041$) compared to the older group and in CCs associated to better prognosis ($p = 0,036$), i.e. pools of cells retrieved from apparently mature complexes, which evolved to good quality embryos, resulting in a successful treatment outcome (clinical pregnancy). Interestingly, transposing this study to the protein level and using the same statistical methodology, a refractory tendency was observed, COL1A1 appeared more abundant in CCs corresponding to poorer prognosis samples, classified with less morphological quality and linked to unsuccessful treatment outcome ($p = 0,007$), being also more expressed in CCs from older women ($p = 0,003$). These results lead us to investigate the importance of COL1A1 and its regulatory effects on CCs.

Limitations, reasons for caution: The reduced amount of CCs collected in each sampling procedure limits the use for both analytical methods of gene expression profiling and western blotting. Moreover, women biological variability limits the homogeneity of the samples. The employment of multiple endpoints of analysis was meant to minimize this obstacle.

Wider implications of the findings: Both in physiological and in-vitro aging, CCs often exhibit modifications in the compaction grade of their extracellular matrix; interestingly COL1A1 is one of its major structural constituents. As this molecule is likely to become a biomarker, its assessment should become relevant during oocyte selection in the setting of ART.

Trial registration number: Not applicable.

P-327 The motility and localization of mitochondria related with the maturation of advanced age oocytes

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Study question: What functional properties of mitochondria are induced to chromosomal error and poor quality in the maturation of advanced aged oocytes?

Summary answer: Poor motility and its movement of mitochondria could be related to chromosomal error and its poor quality in advanced aged oocytes.

What is known already: The major role of mitochondria is supported through bio-energy for oocytes' maturation and chromosomal meiosis. Advanced age oocytes have high aneuploidy ratios with poor quality, which is associated with low mitochondrial activity. However, there are still some questions about why advanced age oocytes have low functional activity in the mitochondria.

Study design, size, duration: This study is designed for motility and the localization of mitochondria with young ($n = 80$) and advanced age oocytes ($n = 60$). All experiments were repeated three times. The statistical differences were analyzed with SPSS.

Participants/materials, setting, methods: We performed confocal live imaging of young and advanced age oocytes stained with mitochondria specific staining dye from the GVBD to MII phase. Young oocytes were treated with Cytochalasin-B (CB), which is well known as a microtubule unstable agent. Next, we compared the localization and motility of mitochondria in young, old age and CB treated oocytes. In addition, we checked the speed and maturation ratio of oocytes in each group.

Main results and the role of chance: In this study, we found that young age oocytes showed dynamic mitochondrial motility gathering to the nuclear region during oocytes' maturation, whereas advanced age oocytes did not. Furthermore, the dynamic motility of mitochondria might be related with microtubule stability in oocytes. Unstable microtubules in young age oocytes showed a similar phenotype in advanced age oocytes, such as low dynamic motility of the mitochondria and slow maturation ratios from the GV to MII phase.

Limitations, reasons for caution: This is an animal study. Therefore, we needed more studies with human oocytes regarding mitochondrial activity, in order to see whether or not the motility and localization of mitochondria was related to oocyte aging in human samples.

Wider implications of the findings: Mitochondrial movement could be associated with oocyte maturation through microtubule stability, which depends on aging in mice. Therefore, these findings could contribute to rejuvenation of advanced age oocytes, such as young age oocytes. It can be a possible application to treat infertility for old women in the human reproductive medicine.

Trial registration number: not applicable.

P-328 Fertilization and embryonic development of eggs from naturally-aged mice

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Study question: According to recent paper, the major mechanism of egg activation, Ca2+ oscillations at fertilization is largely unchanged by advancing maternal ages. How about fertilization and embryonic development is?

Summary answer: Fertilization and embryo development of eggs from naturally aged mouse are not affected by the age of maternal ages at least in well care condition.

What is known already: Female fecundity decreases with age, and the decline accelerates rapidly after 35 years. The main age-dependent decline is in the number and quality of eggs. The quality of eggs mostly due to an increase of chromosomal aneuploidy with advanced maternal age. The ooplasm could be affected by ageing. According to recent paper, the major mechanism of egg activation, Ca2+ oscillations at fertilization is largely unchanged by advancing maternal ages. Here we investigate the ability of fertilization and further embryonic development in eggs from naturally aged mouse from 7 weeks to 90 weeks old.

Study design, size, duration: MII eggs were collected from B6D2F1 mice 7weeks, 62~65 weeks, 65~68 weeks, 77~80 weeks, 89~92 weeks. Naturally aged mice were acquired at 30 weeks from commercial breeders and housed for a further periods.

Participants/materials, setting, methods: At 14 hr post hCG injection, both ovaries and oviducts were collected MII-cumulus mass. In older than 65 weeks, MII eggs were obtained from antral follicles by puncture with 29 G needles. Collected MII eggs were fertilized sperm from 12 weeks male mice. Fertilization rate was calculated by two pronucleus at 8 hr post insemination. Further embryo culture were performed for 5 days for blastocyst formation. ICM/TE counts were performed by immunostaining with anti-Oct 4 and DAPI staining.

Main results and the role of chance: Number of eggs in oviducts was decline in 62 weeks female mouse ($P<0.001$). No ovulated eggs were observed in more than 65 weeks. However, preovulatory healthy MII eggs were collected from antral follicles in those mouse ovary using needle puncture. The number of eggs were decreased from 62 weeks female mouse. Chromosomal abnormality was not seen in eggs from old eggs. Fertilization rate of these preovulatory eggs from 62 weeks to 90 weeks old was no difference compared to those from 7 weeks mouse. Also, most of the fertilized eggs developed blastocysts in vitro, and the total cell count of these blastocyst was no difference compare to those from 7 weeks mouse.

Limitations, reasons for caution: This study was designed to investigate the effects of natural aging on experimental animals raised in a well-maintained environment. However, it is difficult to interpret patients with pathological causes.

Wider implications of the findings: The aging of the ovaries as a result of natural aging does not affect the quality or fertilization of the eggs, and subsequent embryonic development. Nutritional and growing environments are important for the maturation and development of eggs. This research was supported by NRF-2017R1D1A1B03028155.

Trial registration number: non-clinical trials.

P-329 Downregulation of long non-coding RNA MALAT1 inhibits granulosa cell proliferation in endometriosis by activation of p21/p53 and ERK/MAPK signaling pathways

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Study question: We want to explore the role of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), as an extensively expressed and evolutionarily conserved lncRNA, in endometriosis-associated infertility.

Summary answer: MALAT1 may play an important role in regulating the function of granulosa cells (GCs), proceeded to affect the growth and development of oocytes in endometriosis.

What is known already: Our findings first reported that MALAT1 was significantly down-regulated in endometriosis GCs and was associated with AFC. MALAT1 was primarily localized in the nuclei of GCs. Knockdown of MALAT1 in KGN cells obviously inhibited cell proliferation through restraining cell-cycle at G1/S phase, caused by p21/p53-mediated cell cycle arrest, and the activation of the ERK/MAPK pathway participates in this process.

Study design, size, duration: 80 GC samples were collected from endometriosis patients (38) and non-endometriosis women (42) undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment in the period of March 2014 to December 2015. All endometriosis patients were diagnosed by laparoscopy or laparotomy. The inclusion criteria for control women were as follows: regular menstrual cycles occurring every 25-35 days, antral follicle count (AFC) > 5, and were limited to male factor or tubal disease.

Participants/materials, setting, methods: We used human GCs and KGN cell line to investigated the potential role of MALAT1 in the endometriosis women undergoing assisted reproductive technology (ART). Quantitative reverse transcription PCR (qRT-PCR) was used to compare the expression of MALAT1 in GCs from 42 endometriosis patients and 38 controls. Then, we knocked down MALAT1 with locked nucleic acid (LNA) GapmeRs and performed CCK-8, EdU and Western blot to explore the role of MALAT1 in regulating cell proliferation.

Main results and the role of chance: MALAT1 was significantly downregulated in endometriosis GCs as compared with controls ($P<0.001$), statistical analyses indicated significant correlations between MALAT1 expression and AFC ($r = 0.343$, $P = 0.005$). The result of qRT-PCR to analyze RNA from nuclear and cytoplasmic fractions indicated that MALAT1 was predominately distributed in the nucleus. Knockdown of MALAT1, a significant increase in the protein levels of p21 and p53, and a decrease in cyclin D1 and CDK2 were observed, which are key regulators that is required for G1/S phase, however, no detectable changes were observed in apoptosis-related proteins. Moreover, MALAT1 knockdown significantly increased the levels of phosphorylated ERK1/2, while no detectable changes were observed in the total levels of ERK1/2, p38 MAPK, JNK, PI3K and AKT, as well as the levels of p-JNK, p-p38 MAPK, p-AKT and p-PI3K. With the presence of U0126 (a MAPK/ERK kinase inhibitor that inhibits MEK1/2 for downregulation of p-ERK), the upregulation of phospho-ERK1/2, p21 and p53 by the knockdown of MALAT1 was attenuated.

Limitations, reasons for caution: GCs were obtained from preovulatory follicles after Gn stimulation. The hormonal treatment used in IVF may alter the gene expression. These cells may not behave in the same way as GCs from small antral follicles. The isolation of primary cells inevitably causes contamination with other tissues, such as blood cells.

Wider implications of the findings: ERK/MAPK pathway is often aberrantly activated in human cancers and contributes to enhanced cell proliferation, however, in our study, the activation of ERK/MAPK pathway, conversely, contributes to suppress cell proliferation. Therefore, the direct link between the ERK/MAPK pathway and proliferation remains unclear and requires further study.

Trial registration number: not applicable.

P-330 Extracellular vesicles from human follicular fluid inhibit coagulation

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Study question: Do extracellular vesicles from human follicular fluid promote coagulation?

Summary answer: For the very first time, extracellular vesicles from a human body fluid are shown to inhibit coagulation.

What is known already: Extracellular vesicles (EVs) are small, cell-derived particles which are abundantly present in body fluids. EVs in human body fluids promote and/or trigger coagulation by exposing phospholipids and/ tissue factor. Although it is known that follicular fluid (FF) contains EVs, it is unknown whether these EVs promote coagulation.

Study design, size, duration: FF was collected from patients undergoing hormonal stimulation for IVF/ICSI. The ability of FF and isolated EVs from FF to trigger clotting was measured in a plasma clotting test, the fibrin generation test (FGT). This study was approved by the ethical committee and informed consent was obtained from each volunteer.

Participants/materials, setting, methods: FF was fractionated by ultracentrifugation, and by size exclusion chromatography (SEC). The latter separates EVs from soluble components such as proteins. We measured the clotting activities of FF, FF fractionated by ultracentrifugation, and of EVs isolated by SEC.

Main results and the role of chance: Surprisingly, FF or ultracentrifugation fractions of FF did *not* trigger coagulation. In fact, when EVs were isolated by SEC, these EVs even *delayed* the clotting of plasma. For example, when clotting was triggered with thromborel (extrinsic coagulation), the clotting time (CT) increased from 654-813 seconds in the absence to 1214-1609 seconds in the presence of EVs. Similarly, clotting initiated by kaolin plus phospholipid (intrinsic coagulation) increased from 656-783 seconds without EVs to 913-1280 seconds in the presence of EVs. When EVs were pretreated with heparinase, the CTs increased 120 seconds and >500 seconds for extrinsic and intrinsic coagulation, respectively. Taken together, human FF contains EVs exposing or more heparinase-sensitive factors that inhibit the common pathway of coagulation.

Limitations, reasons for caution: Additional studies are needed to elucidate the role of anticoagulant EVs in the uptake of oocytes and fertilisation.

Wider implications of the findings: Follicular EVs may play a role in the uptake of oocytes by the fallopian tube.

Trial registration number: None

P-331 Cumulus cells GPX4 expression levels are higher in patients with successful embryo implantation

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Study question: Do the redox enzyme GPX4 mRNA expression levels in cumulus cells relate to embryo implantation rates?

Summary answer: Implantation rates shows to be higher in patients with higher cumulus cells *GPX4* expression levels, regardless of patient's clinical characteristics.

What is known already: *Cumulus oophorus* cells (CCs) represent the first interface of the gamete with the ovarian environment. Therefore, they can provide valuable information concerning the viability and genetic constitution of the oocyte and its environment. CCs are responsible for protecting oocytes from oxidative stress. *GPX4* encodes GPx protein GPX4 isoform, that acts preferably reducing lipid hydroperoxides and protects cells against membrane lipid peroxidation and cell death. It is possible that oocytes protected by CCs with better defense mechanisms against oxidative stress during oocyte maturation present greater chances of becoming a good quality oocyte.

Study design, size, duration: Pooled CCs samples were collected from 23 patients submitted to ICSI procedure and subsequent embryo transfer. Patients were analyzed for B-HCG detection at day 14 after transfer, and samples were divided between Positive (n = 8) and Negative (n = 15) groups accordingly to B-HCG result. Patients clinical characteristics, as Body Mass Index (BMI), age, stimulation protocol and infertility diagnosis were considered in the analysis.

Participants/materials, setting, methods: Total RNA was extracted from CCs samples and submitted to reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Oligonucleotides were selected to be RNA specific and complementary to the human sequence of Glutathione Peroxidase 4 (*GPX4*). The relative expression (real quantitative [RQ]) of the genes analyzed was calculated for each sample by the 2-ΔΔCT method. For the multiple regression analysis, clinical and experimental data were combined.

Main results and the role of chance: A baseline model were built using B-HCG result as the dependent variable and age, diagnosis, stimulation protocol and BMI as independent variables. We compared test models composed of the four clinical variables and RT-qPCR assay data against the baseline model. With the results being submitted to the regression model considering the clinical data, *GPX4* has shown to be a potential pregnancy biomarker. A significant model ($P = 0.\ 010541996$), containing four variables indicated that *GPX4* expression levels are overexpressed in Positive group, and this significance is independent of the clinical variables of each patient.

Limitations, reasons for caution: For validation, this result need to be tested in a larger cohort, with best interest in analyzing individually collected CCs samples from patients submitted to single embryo transfer.

Wider implications of the findings: Our study revealed that *GPX4* mRNA expression levels are related to embryo implantation potential, and therefore are promising candidates for identifying oocyte quality independently of patient clinical profile.

Trial registration number: This study was approved by ethics comittee of Universidade Federal do Rio Grande do Sul under the approval number 68081017.2.0000.5347.

P-332 The effect of recent contraceptive use on the probability to conceive: A comparison between hormonal contraceptives and a fertility awareness-based method

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Study question: Are there differences in short- and long-term conception probabilities between women who recently used a fertility awareness-based contraceptive method and women who recently used hormonal contraceptives?

Summary answer: Results in this study suggest that FAB methods used for contraception increase short-term pregnancy rates with respect to hormonal contraceptives, but not ong-term pregnancy rates.

What is known already: Previous studies have indicated that the use of techniques associated with fertility awareness-based methods for contraception may enhance pregnancy rates for women trying to conceive. Such studies have so far mainly treated various verisons of the so-called Billings method and the sympto-thermal method. Several previous studies also show that the use of hormonal contraceptives may instead reduce the short-term probabilities to conceive. Most studies do not find any long-term effect of hormonal contraceptives on fecundity.

Study design, size, duration: The study is a real-life prospective observational study of women who use a mobile fertility monitoring application (Natural Cycles) in attempting to become pregnant. A total of 2934 women planning a pregnancy using the mobile application Natural Cycles between August 2014 and June 2016 were included. Of these women 1638 were previous users of hormonal contraceptives and 1296 had previously used the fertility awareness-based method provided by the Natural Cycles application to prevent pregnancies.

Participants/materials, setting, methods: Users were included if they registered as users of the mobile contraceptive app with the intent of planning a pregnancy between August 2014 and June 2016. Comparisons were performed using two complementary methods: We calculated the average Time to Pregnancy (TTP) for all women who became pregnant during the time of the

study, and performed Kaplan-Meier life-table analysis to analyze the cumulative probabilities of pregnancy including all women who entered the study.

Main results and the role of chance: We found an average Time To Pregnancy of 2.3 (95% CI: 2.1-2.4) cycles for the women who had used Natural Cycles to prevent pregnancies, compared to 3.7 (95% CI: 3.4-3.9) for women who used hormonal contraceptives prior to attempting to become pregnant. We used Kaplan-Meier life-table analysis to compare the time to reach 30% cumulated pregnancy probability for the two groups and found the time for women previously on hormonal contraceptives to be 1.6 (95% CI: 1.5-1.8) times longer than the time for women previously using Natural Cycles. When comparing 13-cycle cumulated pregnancy probabilities there were no significant differences between the two groups.

Limitations, reasons for caution: The results do not provide a comparison of neither method to other non-hormonal method of contraception. The study population only contains women who decided to use an application for fertility monitoring, which may lead to a selection bias compared to the average population.

Wider implications of the findings: The result is likely widely applicable to women who uses fertility awareness methods prior to planning a pregnancy. The results presented in this study may be of interest to women planning to become pregnant in the near future as well as for healthcare professionals counseling women on contraception and fertility.

Trial registration number: Not applicable.

P-333 Epigenetic changes in human cumulus cells during reproductive aging

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Study question: Oocyte aging is a common cause of infertility and still the mechanisms involved are unknown. Could age-related changes in DNA methylation (DNAmAge) of cumulus cells explain the reproductive decline?

Summary answer: We found suggestive evidence that chronological age correlates with DNAmAge ($r = 0.38$, $p = 0.074$). Epigenetic age acceleration exhibited moderately high correlations with variables measuring fertility.

What is known already: The study of the epigenetic profile of a tissue has been proven to reliably provide information regarding the age of the derived organ, through the analysis of 353 CpG islands across the entire human genome. The epigenetic clock algorithm identifies the discrepancies between chronological age and biological age, so called age acceleration rate (AgeAccel). Conditions such as chronic diseases, viral infection and chromosomal anomalies show an increased AgeAccel compared to healthy individuals. Our study aimed at deciphering AgeAccel in follicles of women undergoing in vitro fertilization.

Study design, size, duration: Thirty-two women undergoing IVF/ICSI were selected for the study (exclusion criteria: endometriosis, PCOS, use of PGD). Cumulus cells were removed from oocytes and pooled for each individual patient. Patient's ages ranged 24-45 y.o. (mean 36.8)

Participants/materials, setting, methods: Total DNA from cumulus cells was extracted and used for bisulfite conversion (Zymo Research). Genomewide DNA methylation profiling was carried out with 160 ng bisulfite converted DNA, using the Infinium Methylation BeadChips (Illumina) and hybridized to the Illumina Infinium 450 K Human Methylation Beadchip.

Main results and the role of chance: AgeAccel is inversely correlated to good markers of patient's prognosis, such as AMH ($r = -0.24$), number of oocytes collected ($r = -0.33$), oocyte maturity ($r = -0.31$), fertilization ($r = -0.22$) and cleavage ($r = -0.25$) and positively correlated to amount of administered gonadotropins ($r = 0.19$)

Limitations, reasons for caution: This study focused almost exclusively on assessing the epigenetic differences in CC across a population of patients undergoing IVF/ICSI. These epigenetic findings were not correlated with embryonic development and clinical outcome.

Wider implications of the findings: Our data confirms the hypothesis of a link between follicle senescence, cumulus cells and oocyte aging. The use of the age acceleration clock could, on a larger and more diverse cohort of patients, predict weather also other conditions, such as endometriosis and PCOS could be associated with follicular aging.

Trial registration number: N/A.

P-334 Effects of dietary AGE (Advanced Glycation End product) on ovarian microenvironment and oocyte quality

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Study question: Does the ovary activate an effective adaptive response to counteract the toxicity derived from dietary AGEs?

Summary answer: The ovary activates an adaptive response, which counteracts AGE accumulation but does not prevent toxic effects of dietary AGE on growing follicles and mature gamete.

What is known already: The levels of advanced glycation end products (AGEs) are increased during aging and under conditions of impaired glucose metabolism and/or oxidative stress (OS), promoting insulin resistance and other endocrine abnormalities. AGE deposition may be also ascribed to exogenous factors, such as tobacco smoking and diet. In the ovary, AGE formation has been associated to reproductive aging and polycystic ovarian syndrome (PCOS). *In vitro* exposure to methylglyoxal (MG), the main precursor of AGE, severely affects oocyte maturation, whereas *in vivo* ovarian and oocyte toxicity of MG is still unknown.

Study design, size, duration: To study the effects of dietary AGE (dAGE), 4-week-aged female CD1 mice received water ($n = 9$) or 100 mg/kg MG ($n = 9$) by gastric administration for 28 days. This treatment induces increased serum MG levels (Ghosh et al., 2006). Six mice per group were sacrificed 48-h after the last administration and ovaries were collected for further analysis. Three mice were sacrificed 15-h after induction of superovulation by PMSG-hCG protocol and MII oocytes were isolated from ampullae.

Participants/materials, setting, methods: The number of ovarian follicles was recorded in ovaries stained by ematossilin/eosin. The abundance of MG-AGEs, sirtuins (SIRT1, SIRT3), catalase (CAT), mitochondrial superoxide dismutase (SOD2), PGC1α, glyoxalase 1 (GLO1) were analysed by Western blot (WB). Spindles and chromosomes of MII oocytes were stained by immunofluorescence and digital images were analysed by ImageJ to obtain measurements of spindle dimensions (length, equator and pole width). Statistical analysis was performed by Sigma-Stat software.

Main results and the role of chance: Analysis of follicle population revealed a reduced number of primary and secondary follicles in dAGE group. Biochemical investigation demonstrated similar levels of ovarian MG-AGEs in the two groups. Nevertheless dAGE ovaries displayed an increase of SIRT1, an OS sensor, together with up-expression of CAT and SOD2. Expression of the mitochondrial sirtuin SIRT3 and PGC1α, the main regulator of mitochondrial biogenesis, was also increased. Finally, enhanced protein expression of GLO1, the main ovarian AGE detoxifying enzyme, was observed in dAGE group. Although similar ovulation rate were recorded in control and dAGE mice, spindle analysis showed that dAGE oocytes exhibited abnormal spindle size resembling those previously found in low quality oocytes (Sanfins et al, 2003).

Limitations, reasons for caution: The results on this animal model may not fully extrapolate to humans.

Wider implications of the findings: Present results suggest that AGE intake induces ovarian oxidative stress and triggers an adaptive response, which prevents ovarian AGE accumulation. Nevertheless, dAGE leads to loss of growing follicles and production of low quality oocytes. These results contribute to the knowledge of mechanisms underlying the reduced fertility in PCOS and diabetes.

Trial registration number: not required.

P-335 Bisphenol S (BPS) affects meiotic spindle formation and causes DNA damage: Implications for female infertility

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Study question: Does BPS occur in human follicular fluid? If yes, is BPS able to influence chromosome alignment and spindle formation in mouse oocyte?

Summary answer: BPS was measured in follicular fluid of women underwent ART. Accordingly, BPS in vivo treatment of mice impairs the quality of the matured oocyte.

What is known already: BPS represents the main substituent of earlier widely-used bisphenol A (BPA). In accordance with BPA detection in human follicular fluid and negative effects of both BPA and BPS is known so far, we assumed BPS presence in human follicular fluid and its negative effect on oocyte quality. Detrimental effects of BPS on oocytes were described on pig model in vitro only. On the other hand, BPS levels in human follicular fluid were not determined and influence of BPS on oocytes in vivo remains unknown.

Study design, size, duration: 35 samples of human follicular fluid were obtained from April to November 2017. Subsequently, eight-week-old ICR mice (n = 25) were treated with BPS in four different concentrations for 7 days per os. When the experiment was terminated, oocytes were isolated and matured in vitro. In total, the experiments included 127 matured oocytes.

Participants/materials, setting, methods: The samples of follicular fluid, obtained from patients of ART clinic were digested by β–glucuronidase and subjected to derivatization by 1-methylimidazol-2-sulfonyl chloride, followed by BPS measurement by LC-MS. Eight-week-old ICR mice were exposed to 0.001–100 ng BPS/g BW/day per os for 7 days. Isolated oocytes were in vitro maturated followed by TUNEL assay or spindle α-tubulin immunostaining. Oocytes were analysed by confocal microscope (Olympus, Germany) and signal intensity measurement (ImageJ, NIH, USA).

Main results and the role of chance: BPS levels were detected in samples of human follicular fluid of patients of the ART clinic, with values 3.5 ± 0.77 ng. ml-1 (median ± S.E.M.; LOD: 0.19 ng/ml, LOQ 0.65 ng/ml). The additional experiment, using mouse in vivo exposure, proved the negative impact on female reproduction. BPS treatment of mice disrupted DNA stability of matured oocytes. There was observed 4.7, 7.0 and 5.8-fold TUNEL signal intensity in metaphase chromosomes after dosing 0.001, 0.1, 100 ng BPS/g BW/day, respectively. Moreover, BPS exposure showed significantly increased the incidence of malformed spindle microtubules and chromosome misalignment. Individual BPS doses seemed to have a specific pattern of spindle abnormalities, such as extended shapes and barrel-shaped spindle in 0.001 and 10 ng BPS/g BW/day, respectively.

Limitations, reasons for caution: With respect to the observation of BPS levels in the follicular fluid, we analysed the oocyte quality after mouse in vivo exposure. Considering the results of decreasing oocyte quality arise from an animal model, it would be appropriate to evaluate the BPS effect in human oocytes.

Wider implications of the findings: Currently, the BPS exposure on mankind rise as the BPS becomes widely used, as shown with detection of BPS in follicular fluid. Moreover, concentrations of BPS closely resemble those of harmful BPA. Our findings show that these low doses of BPS significantly impact female reproduction.

Trial registration number: The sample colection and experiments with mice were performed in accordance with the Ethical Committee at Faculty Hospital in Brno and Act No 246/1992 Coll.

P-336 Circadian rhythm disruption in polycystic ovary syndrome

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Study question: Is circadian rhythm disruption involved in the pathogenic mechanism of polycystic ovary syndrome (PCOS)?

Summary answer: The chronodisruption, typically attributable to shift work, may contribute to the development of PCOS.

What is known already: Although the underlying etiology remains unclear, aberrant light–dark cycles greatly impair the ovarian function.Despite emerging evidence of a high prevalence of sleep disorders in patients with PCOS, there is limited epidemiological data concerning the association between PCOS risk and circadian disruption by sleep disturbances or night shift work.

Study design, size, duration: We performed a multicenter, questionnaire-based survey to explore the link between night shift work and PCOS risk in 436 PCOS cases and 715 controls between June 2016 and May 2017.Moreover, we conducted a series of experiments to investigate the roles of ovarian circadian disruption in PCOS pathogenesis.

Participants/materials, setting, methods: Firstly, the survey was conducted over twelve hospitals in mainland China. Only women aged 21–45 without any sleep disorders, caused by neurological, psychiatric or respiratory disorders etc., were recruited. Patients were diagnosed with PCOS according to the Rotterdam Consensus.The controls were healthy women who had participated in a health screen in these hospitals. Secondly, PCOS patients and women with tubal blockage undergoing in vitro fertilization (IVF) were recruited, GCs and follicular fluids were collected.

Main results and the role of chance: After adjusting for potential confounders, night shift workers had a significantly increased risk of PCOS compared with day workers (OR = 1.80; 95%CI = 1.18–2.75; Table 2). This association was also observed among those with rotating, but not permanent, night shifts ($OR_{(Rotating\ vs.\ day\ work)}$ = 2.00, 95%CI = 1.16–3.44; $OR_{(Permanent\ vs.\ day\ work)}$ = 1.54, 95% CI = 0.80–2.95). Compared with day workers, participants that worked night shifts for a longer period of time (more than 2 years) had a higher PCOS risk (OR = 2.08; 95%CI = 1.15–3.73). However, those that had worked night shifts for a shorter period of time (less than 2 years) did not have an increased risk of PCOS (OR = 1.56; 95%CI = 0.86–2.80). In addition, we found that in GCs of PCOS women, the overexpression of core clock gene PER1 suppressed the transcriptional activity of AR through combination, and altered androgen metabolism in GCs contributing to AR-associated hyperandrogenism.

Limitations, reasons for caution: Our survey is a retrospective investigation, further verification is required, particularly studies that evaluate individuals of different ethnicities using a prospective observational technique.The GCs used in the present study were collected from patients undergoing IVF, thus it is of significance to further confirm our findings in unstimulated and non-luteinized GCs.

Wider implications of the findings: Understanding the role of circadian rhythm disruption in PCOS could help to intervene the infertility resulted from irregular light–dark cycles common to modern-day 24-hour society.

Trial registration number: Not applicable.

POSTER VIEWING
Female infertility

P-337 Estrogen receptor 1 polymorphism (A>G, rs12199722) is associated with recurrent implantation failure

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Study question: Is there an association between any Single Nucleotide Polymorphism (SNP) in the estrogen receptor 1 (ESR1) gene and recurrent implantation failure (RIF)?

Summary answer: ESR1 polymorphism (A>G, rs12199722) is associated with RIF. Women with A/G genotype had a 60% decrease in the chance of being included in RIF group.

What is known already: Estrogen, as a crucial hormone during pregnancy, acts through its receptors alpha and beta, which are encoded by the ESR1 and ESR2 genes, respectively. These receptors are involved in reproduction and may play important roles in RIF. The uterus is a primary target of oestrogen for various functions during the reproductive cycle and pregnancy. Estrogen binds to the receptor alpha, which modulates uterine events preparing the endometrium for embryo fixation and implantation. Since genetic factors appear to be highly associated with RIF, SNPs in the ESR1 gene encoding the receptor alpha can be used to predict RIF.

Study design, size, duration: A cross-sectional study was performed between 2015 and 2017, comparing 48 women presenting RIF (two or more failed IVF/ICSI attempts and at least five or more good-morphological-quality embryos transferred) versus 2 control groups: 63 women who became pregnant in the first IVF/ICSI attempt (control group I) and 68 fertile women who had at least 2 live births and no history of miscarriage or infertility treatment (control group II).

Participants/materials, setting, methods: Genomic DNA was extracted from peripheral blood samples taken from RIF and control groups. DNA was sequenced on MiSeq(Illumina) to search for SNPs in the ESR1 gene. SNPs found by Next-Generation Sequencing were analysed to find a possible association with RIF. The A>G polymorphism (rs12199722) was identified and subsequently validated by Real Time PCR. Frequencies of genotypes and alleles were compared using Fisher's exact test. An association with RIF was calculated by logistic regression analysis.

Main results and the role of chance: Our results showed an association between A/A genotype of ESR1 gene (rs12199722) and women presenting RIF. Patients with A/A genotype had 3.0-fold increase in the chance of being included in the RIF group. On the other hand, the presence of A/G genotype was associated with a 60% decrease in the chance of being included in the RIF group. A comparison of genotype and allele frequencies of ESR1 polymorphism (A>G, rs12199722) among women with RIF and two control groups are shown in Table 1. The association between each genotype and RIF is in Table 2.

Table I Genotype and allele frequencies of ESR1 polymorphism(A>G, rs12199722).

ESR1 (rs12199722)	RIF (%) n = 48	Control group I (%) n = 63	P	RIF (%) n = 48	Control group II (%) n = 68	P
Genotype						
AA	64.6	39.7	0.02	64.6	35.3	0.008
AG	29.2	55.5		29.2	50.0	
GG	6.2	4.8		6.2	14.7	
Allele						
A	79.2	67.5	0.05	79.2	60.3	0.002
G	20.8	32.5		20.8	39.7	

Table II Logistic Regression between ESR1 polymorphism(A>G, rs12199722) and RIF.

ESR1 (rs12199722)	OR (95% CI)	P
AA	3.0 (1.53-6.08)	0.002
AG	0.4 (0.18-0.75)	0.006
GG	0.6 (0.16-2.22)	0.45

Limitations, reasons for caution: Since the basic idea of performing a test is to increase the chance of obtaining a correct diagnosis, further validation of the ESR1 polymorphism (A>G, rs12199722) is required (e.g., increasing the number of cases, expanding to different ethnic groups) to provide more information regarding its potential use to predict RIF.

Wider implications of the findings: Our results, based on two control groups, showed for the first time evidence that ESR1 polymorphism(A>G, rs12199722) can be used as a genetic marker to predict RIF allowing physicians to provide a better risk analysis for each patient, helping to make assertive decisions, improving the chances of a woman becoming pregnant.

Trial registration number: Merck Grant for Fertility Innovation (GFI-2014-16). The funders had no role in study design, data collection and analysis, or preparation of the abstract.

P-338 Analysis of 215,212 ovulation induction (OI) and intrauterine insemination (IUI) cycles reveals the high rate of multiple gestations in patients across age groups and diagnoses

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Study question: What are the ongoing pregnancy rates (OPR) and multiple gestation rates (MGR) associated with different non–*in vitro* fertilization (non-IVF) treatments?

Summary answer: Non-IVF treatment yielded OPR of 3.4%–19.0% and MGR of 3.5%–25.5%, with higher-order multiple (HOM) rates of 3.3%–6.4% in patients younger than 40 using gonadotropins.

What is known already: Non-IVF treatments with OI using oral medications (clomiphene citrate or letrozole) or injectable gonadotropins with IUI or timed intercourse (TI) are frequently used as first-line fertility treatments because of lower up-front costs compared to IVF. Since non-IVF treatments often lead to multiple birth and consequently high neonatal costs, characterization of pregnancy and multiples rates resulting from non-IVF treatments is needed to properly evaluate the cost-effectiveness of these treatment options. A study of this magnitude has been hindered to date by the lack of outcome tracking for non-IVF treatments in data registries in both the United States and Europe.

Study design, size, duration: We performed a retrospective analysis of cycles that used the OI medications clomiphene citrate or letrozole (Orals) and/or injectable FSH or hMG (Gnd) with IUI or TI with partner semen. We included 215,212 cycles from 77,303 patients treated at 13 fertility centers located within the United States. Cycles were performed between 2002 and 2017.

Participants/materials, setting, methods: We included all women who were treated with OI or IUI that were 18–45 years old, and excluded cycles utilizing donor sperm. Patients were treated according to standard clinic protocols for OI and IUI. Ongoing pregnancy outcomes were determined by the presence of a fetal heartbeat (FH) upon discharge to obstetrical care. Multiple gestations were determined by the number of FHs detected by ultrasound, and classified as either twins or HOMs (3 or more FHs).

Main results and the role of chance: The overall OPR was 11.1%, with 10.7% twin and 1.5% HOM. The OPR and MGR by age and treatment type are shown in Tables 1 and 2. Notably, the HOM rate was as high as 3.3%–6.4% in patients younger than 40 who used Gnd.

Table I Ongoing pregnancy % (N of cycles)

	<30	30–34	35–39	40–45
Orals+TI	11.2%(7831)	10.1%(16,113)	6.9%(11,922)	3.4%(5902)
Orals+IUI	11.7%(10,842)	11.1%(36,850)	9.7%(33,053)	4.9%(12,738)
Gnd+Orals+TI	14.6%(623)	12.9%(1472)	8.9%(1401)	3.9%(908)
Gnd+Orals+IUI	15.9%(2751)	14.8%(10,271)	12.6%(11,793)	6.9%(7553)
Gnd+TI	18.1%(867)	18.2%(1566)	11.4%(1257)	5.3%(1122)
Gnd+IUI	19%(3059)	17.3%(10,767)	14.2%(12,927)	6.9%(11,624)

Table II Multiple gestation % of ongoing pregnancies (N of cases)

	<30	30–34	35–39	40–45
Orals+TI: Twins	8.6%(75)	7.7%(126)	7.3%(60)	3%(6)
Orals+TI: HOM	0%(0)	0.7%(11)	0.6%(5)	0.5%(1)
Orals+IUI: Twins	7.9%(100)	8.9%(365)	8.7%(279)	6.8%(42)
Orals+IUI: HOM	0.9%(12)	0.6%(23)	0.7%(24)	0.3%(2)
Gnd+Orals+TI: Twins	17.6%(16)	13.7%(26)	11.3%(14)	14.3%(5)
Gnd+Orals+TI: HOM	2.2%(2)	1.1%(2)	1.6%(2)	2.9%(1)
Gnd+Orals+IUI: Twins	14.8%(65)	12.7%(194)	13.1%(194)	8.9%(46)
Gnd+Orals+IUI: HOM	1.4%(6)	1.7%(26)	0.9%(14)	1.2%(6)
Gnd+TI: Twins	19.1%(30)	13.7%(39)	11.9%(17)	11.7%(7)
Gnd+TI: HOM	6.4%(10)	5.3%(15)	4.2%(6)	1.7%(1)
Gnd+IUI: Twins	16.6%(96)	16.9%(315)	16.6%(306)	12.4%(100)
Gnd+IUI: HOM	3.6%(21)	4%(74)	3.3%(61)	1.6%(13)

Limitations, reasons for caution: This retrospective dataset included a variety of treatment and monitoring protocols used at multiple clinics over a large time range. In addition, the observed rates were of all treated patients, so comparison of rates between treatments, in particular TI to IUI, should be the subject of a subsequent investigation.

Wider implications of the findings: This is the first large scale study to date of the health burden associated with non-IVF fertility treatments. We observed that these treatments result in a significant rate of multiple births, including HOMs. This study sets the foundation for future cost-effectiveness studies that can improve policy-making around fertility care.

Trial registration number: N/A.

P-340 External validation of a dynamic prediction model for repeated predictions of natural conception

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Study question: How well does a previously developed dynamic prediction model perform in an external, geographical validation in terms of predicting the chances of natural conception at various points in time?

Summary answer: The dynamic prediction model performs well in an external validation on a Scottish cohort.

What is known already: We developed a dynamic prediction model for natural conception that is able to update predictions of natural conception when couples return to their clinician after a period of unsuccessful expectant management. However, it is not known how well this model performs in an external population for clinical use.

Study design, size, duration: A record-linked registry study including the long term follow up of all couples who were considered unexplained subfertile following a fertility work up at the Aberdeen Fertility Clinic in the Grampian region of Scotland between 1998 and 2011. Couples with anovulation, uni/bilateral tubal occlusion, mild/severe endometriosis or impaired semen quality according to WHO criteria were excluded.

Participants/materials, setting, methods: The endpoint was time to natural conception, leading to an ongoing pregnancy. Follow up was censored at the start of treatment, at the change of partner or at the end of study (31st of March, 2012). The performance of the van Eekelen model was evaluated in terms of calibration and discrimination at various points in time. Additionally, we assessed the clinical utility of the model in terms of the range of the calculated predictions.

Main results and the role of chance: Of a total of 1214 couples with a median follow up of 14 months after the fertility workup, 397 (33%) couples conceived naturally leading to an ongoing pregnancy. Using the dynamic prediction model, the mean probability of natural conception over the course of the first year after the workup was estimated at 25% (observed: 22%), After half a year, one year and one and a half years of expectant management after completion of the fertility workup, the average probability of conceiving naturally over the next year was estimated at 18% (observed: 15%), 14% (observed: 14%) and 11% (observed: 12%).

Calibration plots showed good agreement between predicted chances and the observed fraction of ongoing pregnancy within risk groups. Discrimination was moderate with c statistics similar to those in the internal validation, ranging from 0.60 to 0.64. The range of predicted chances was sufficiently wide to distinguish between couples having a good and poor prognosis with a minimum of zero at all times and a maximum of 55% over the first year after the workup, which decreased to maxima of 43% after half a year, 34% after one year and 29% after one year and a half after the workup.

Limitations, reasons for caution: The primary analysis showed a slight overestimation of conception by approximately 3 percentage points on group level in the first year post fertility workup and after half a year of expectant management. This is likely attributable to the difficulty in identifying the exact date of completion of the fertility workup.

Wider implications of the findings: The van Eekelen model is a valid tool in clinical practice to counsel couples with unexplained subfertility on their individualised chances of conception at various points in time, notably when couples return to the clinic after a period of unsuccessful expectant management.

Trial registration number: Not applicable.

P-341 Effects of Vitamin D supplementation on Assisted Reproduction Technology (ART) outcomes: an interim analysis of a randomized double-blind multicenter placebo-controlled trial

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Study question: Does vitamin D supplementation affect clinical pregnancy rate (CPR) per started cycle in women undergoing ART procedures?

Summary answer: No significant differences were found in CPR. A significantly higher top quality embryo rate was observed in women allocated to vitamin D.

What is known already: Several observational data from IVF cycles suggest a possible beneficial effect of vitamin D on the rate of success of the procedure. According to a recent metanalysis, the ORs of clinical pregnancy in vitamin D sufficient women was 1.33 (95%CI: 1.08-1.65) (Chu *et al.*, 2018). However, a causal relation remains to be demonstrated and interventional studies are warranted. To date, only one randomized controlled trial on the potential benefit of vitamin D for IVF has been published (Aflatoonian *et al.*, 2014). No significant benefits emerged but the sample size (n = 106) was insufficient for meaningful conclusions.

Study design, size, duration: Eligible women with a serum vitamin D level <30 ng/ml were recruited. The sample size (630 women) was calculated considering a 10% difference in CPR between the supplemented and not supplemented populations. The first visit for recruitment was 10/10/2016. Recruited women received 600,000 IU of Vitamin D or placebo (in a single oral administration) at the time of randomization. Ovarian hyperstimulation initiated within three months after randomziation.

Participants/materials, setting, methods: All participants and physicians managing the patients were blinded to trial intervention allocation. The allocation sequence was computer-generated and blinded to the investigators and participants. Inclusion criteria of women were: age 18-39 years, less than 3 previous ART attempts, body mass index between 18 m^2/Kg and 25 m^2/Kg. Exclusion criteria were: poor responders according to Bologna criteria, ART freezing-cycles. Eligible women signed an informed consent and provided a blood sample for vitamin D assessment.

Main results and the role of chance: Since the start of the study, 267 women were recruited (n = 136 women were administered vitamin D and n = 131 women placebo). No differences were observed in duration of ovarian hyperstimulation (p = 0.29) and total dosage of gonadotropins administered (p = 0.21) between the two groups. The mean (±SD) number of retrieved oocytes was 8.7 ± 6.4 in vitamin D group and 7.6 ± 4.6 in the placebo group (p = 0.09). No significant differences were found in the total number of follicles > 16 mm (p = 0.24), oocytes used (p = 0.46) and fertilization rate (p = 0.84). Cleavage rate and number of total embryos on day 3 were not different between vitamin D and placebo group (respectively p = 0.27 and p = 0.97). A statistically significant higher top quality day 3 embryos rate was found in the supplemented group (respectively 60% compared to 43%, p = 0.03). Blastulation rate was not different between the two groups (vitamin D treatment 50% and placebo 44%, p = 0.72). Due to the increased strategy to freeze embryos for clinical conditions, clinical pregnancy rate was calculated on a lower number of women (vitamin D supplemented n = 77 and placebo group n = 82) and was not different between the groups (p = 0.74).

Limitations, reasons for caution: This is a planned interim analysis performed in order to evaluate preliminary results mostly on embryological outcomes. The sample size is not powered enough to detected differences in CPR per started cycle.

Wider implications of the findings: This study can potentially influence the clinical practice of infertility treatment. Vitamin D supplementation is a simple and cheap intervention and is free of relevant side effects. It may also have beneficial effects on pregnancy outcome.

Trial registration number: EUDRACT 2015-004233-27

P-342 Only patient age - and not stimulation or relationship status - affects IUI outcome in 13-year single-centre series of 8995 consecutive cycles

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Study question: Are clinical outcomes in donor insemination cycles affected by patient age, ovarian stimulation, or relationship status?

Summary answer: Clinical outcomes are strongly associated with patient age. Ovarian stimulation, or relationship status did not significantly affect pregnancy outcomes in almost 9000 consecutive IUI cycles.

What is known already: It is well known that age affects fertility in both natural and assisted conception, with patient age to be inversely and significantly associated with positive pregnancy outcomes. Despite the improvements in semen preparation methods and controlled ovarian stimulation, the success of IUI is still lower than what is reported for other assisted reproduction procedures and we know that it is affected by many factors. Important prognostic indicators of success with IUI include the patient's age, the cause and duration of infertility, methods of ovarian stimulation, number of cycles, ovarian reserve and male factor infertility.

Study design, size, duration: Our analysis is a retrospective observation study of data collected from 8995 consecutive treatment cycles of intrauterine donor insemination performed at the London Women's Clinic between January 2004 and December 2016. The aim of the study was to examine the efficacy of IUI effectiveness in relation to patient age, ovarian stimulation, and relationship status. Statistical analysis was performed using STATA.

Participants/materials, setting, methods: Between 2004 and 2016, 3406 women attended the London Women's Clinic (LWC) for donor insemination. 1018 women were in heterosexual relationship, 4265 were in same-sex relationship, and 3712 were single. 58% of cycles were natural; the remainder stimulated (42%). The cross-section of patients - with a high number of lesbian and single women - reflects a clinical focus of the LWC and socio-legal change in the acceptance and parental rights of individuals in the UK.

Main results and the role of chance: The highest live birth rate (LBR) was found in women aged <35 years (n = 2948 cycles) with a LBR of 12.6% per cycle. Women aged 35-37 years (n = 2204 cycles) had a LBR of 10% per cycle and those aged 38-39 years (n = 1544 cycles) had a LBR of 8% per cycle. In women aged 40-42 years (n = 1621 cycles) LBR declined to 5% per cycle, while no pregnancy was observed in women aged ≥45 years.

LBRs were affected by relationship status with single women having significantly different results compared with the other groups, but this difference was fully explained after adjustment for age (p<0.000).

Single women (n = 3712) had a mean age of 39 years and a LBR per cycle of 7%, women in heterosexual relationships (n = 1018) a mean age of 34 years and a LBR of 10% per cycle, and those in same-sex relationships (4265) a mean age of 35 years and a LBR of 11% per cycle.

There was a fairly even distribution of non-stimulated (n = 5212) and stimulated cycles (n = 3783) and we found no statistically significant difference in the outcome (p = 0.70). LBR was 8.7% in non-stimulated natural cycles and 9.4% in stimulated cycles.

Limitations, reasons for caution: This study uses a large sample size of patients and is adequately powered to detect medium-size effects. The data are observational and were retrospectively analysed though so unknown confounders were not assessed. Throughout the study period stimulation protocols were modified and LBRs were calculated in varying numbers of cycles.

Wider implications of the findings: Our study has implications for clinical practice and patient counselling, with outcome following IUI dependent mainly on patient age and only marginally on ovarian stimulation and relationship status.

Trial registration number: not applicable.

P-343 Vascular endothelial growth factor polymorphism (C>T, rs3025010) can predict recurrent implantation failure

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Study question: Is it possible single nucleotide polymorphisms (SNPs) of the vascular endothelial growth factor (VEGF) gene to predict recurrent implantation failure (RIF)?

Summary answer: VEGF polymorphism (C>T, rs3025010) can predict RIF. Women with T/T genotype had a 60% decrease in the chance of being included in RIF group.

What is known already: VEGF is involved in embryonic development, decidual vascularization, fetal and placental angiogenesis and blastocyst invasion. This gene located on chromosome 6, contain eight exons and seven introns, which can form different isoforms by alternative splicing. There are several reports showing that polymorphisms in this gene can affect the expression and function of the protein, as well as serum levels of VEGF that could lead to implantation failure. The rs3025010 VEGF polymorphism site located in the intron region has been reported to be associated with another SNP in the promoter region (rs1570360), both related to implantation.

Study design, size, duration: A cross-sectional study was performed between 2015/2017 comparing 48 women presenting RIF (two or more failed IVF/ICSI attempts and at least five or more good morphological quality embryo transferred) (RIF group) with 68 fertile women who had at least 2 live births and no history of miscarriage or infertility treatment (control group).

Participants/materials, setting, methods: Genomic DNA was extracted from peripheral blood samples taken from RIF and control groups. The genomic DNA was sequenced on MiSeq system (Illumina) to search for SNPs in VEGF gene. The SNPs found by Next Generation Sequencing were analysed to find possible association with RIF. The C>T polymorphism (rs3025010) was identified and confirmed by Real Time PCR using the allelic discrimination method. Differences in genotype were compared using Fisher's exact test and logistic regression.

Main results and the role of chance: Our results showed an association between VEGF C/C genotype (rs3025010) and increased prevalence of RIF as well as T/T genotype and fertile women. Distribution of genotype and allele frequencies of RIF and control groups are represented in Table 1. These findings have been corroborated by logistic regression analysis where patients with C/C genotype had 2.8-fold increase in the chance of being included in the RIF group and patients with T/T genotype had a 60% decrease in the chance of being included in the RIF group (Table 2).

Table I Genotype and allele frequencies of VEGF polymorphism (C>T,rs3025010).

VEGF (rs3025010)	RIF group (%) n = 48	Control group (%) n = 68	*P*-value
Genotype			
CC	27.1	1.8	0.03
CT	39.6	32.3	
TT	33.3	55.9	
Allele			
C	46.9	27.9	0.003
T	53.1	72.1	

Table II Logistic Regression between VEGF polymorphism (C>T,rs3025010) and RIF.

VEGF (rs3025010)	OR (95% CI)	*P*-value
CC	2.8 (1.05-7.38)	0.04
CT	1.4 (0.63-2.96)	0.42
TT	0.4 (0.18-0.85)	0.02

Limitations, reasons for caution: Since the basic idea of performing a test is to increase the chance of attaining a correct diagnosis, further validation of the VEGF polymorphism (C>T, rs3025010) is required (i.e., increasing the number of cases; expanding to different ethnic groups) to provide more information regarding its potential use to predict RIF.

Wider implications of the findings: The ability to predict RIF using the VEGF polymorphism (C>T, rs3025010) can enable physicians to provide a better risk analysis to each patient, helping make assertive decisions, improving the chances of a woman to get pregnant, encouraging her to undergo additional cycles of ART.

Trial registration number: Merck Grant for Fertility Innovation (GFI-2014-16). The funders had no role in study design, data collection and analysis, or preparation of the abstract.

P-344 A new biochemical approach to detect oxidative stress in infertile women undergoing assisted reproductive technology procedures

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Study question: To compare blood and follicular fluid (FF) redox status in infertile women versus age-matched controls undergoing in vitro fertilization (IVF) and to establish its connection with the IVF outcome.

Summary answer: Blood oxidative stress reflects FF oxidative stress; it is significantly increased in infertile women compared to controls and correlates with IVF outcomes.

What is known already: Studies performed so far indicate that oxidative stress – characterized by an overabundance of reactive oxygen species (ROS) or/and a deficiency of antioxidants – negatively affects reproductive potential. The influence of oxidative stress (OS) on ART outcome is related to its negative impact on gamete quality and early embryo development, as reported by several studies. However, a clinically reliable, biologically accurate indicator of oxidative stress condition does not yet exist and is still difficult to identify. Antioxidant therapy can restore the redox balance and therefore potentially improve implantation, pregnancy and live birth rate and time to pregnancy.

Study design, size, duration: The study population included 45 women with female factor infertility (excluding tubal factor) and 45 age-matched controls (women from couples with pure male factor or tubal factor) undergoing ART. The biological samples used for the planned investigations were: i) blood (both plasma and leukocyte subpopulations); ii) follicular fluid (FF), including granulosa cells.

Participants/materials, setting, methods: ROS assessment (blood/granulosa cells) was performed by Cytofluorimetric analysis. OS markers (plasma/FF) were assessed by fluorometric lipid peroxidation markers (MDA) estimation. Total antioxidant capacity (plasma/FF) was performed by fluorimetric ORAC assay. Measurements of outcomes were based on the evaluation of: rate of retrieved MII oocytes, fertilization, implantation and pregnancy rates. Statistical analysis were used to correlate OS markers between blood and follicular samples and its association with embryo development and ART outcome.

Main results and the role of chance: In this study we assessed redox status in blood, follicular fluid (FF) and granulosa cells from 45 infertile women and 45 controls undergoing ART. Oxidative stress markers in blood and in granulosa cells resulted significantly ($p<0.001$) increased in infertile patients compared to controls. Moreover, we observed: i) a significant increase of OS markers in blood and in granulosa cells in infertile patients compared to controls ($p<0.001$); ii) a significant correlation between blood and FF redox markers, indicating that blood oxidative stress reflects FF oxidative stress; iii) an association between oxidative stress parameters and the considered IVF outcomes (rate of oocyte at metaphase II and fertilization rate).

Limitations, reasons for caution: Not applicable.
Wider implications of the findings: The non-invasive evaluation of oxidative stress will allow the selection of patients who could benefit from antioxidant therapy to restore redox balance before starting the IVF treatment. The improved redox environment would increase the ART efficiency, allowing the quicker achievement of a successful pregnancy.
Trial registration number: Not applicable.

P-345 Reproductive outcomes in patients with a history of retained products of conception after delivery versus abortion

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Study question: In the assessment of infertile patients - does a history of retained products of conception (RPOC) entail a similar prognosis following delivery and abortion?
Summary answer: Following hysteroscopy for RPOC after delivery and abortion similar reproductive outcomes were observed. However, following delivery, subsequent pregnancies entailed a higher rate of abnormal placentation.
What is known already: RPOC are associated with the formation of intrauterine adhesions and subsequent infertility, while hysteroscopic treatment is associated with less adhesions as compared to curettage. A single study to date compared post-partum and post abortion RPOC, and noted no difference in fertility and pregnancy outcomes between cases following delivery and first trimester abortion. However, study patients were not uniformly treated with hysteroscopy, now the gold standard for RPOC.
Study design, size, duration: This is a cohort of operative hysteroscopies performed between 2011-2015 for suspected RPOC. Variables were compared between cases following delivery (n = 85), and following abortion (n = 96).
Participants/materials, setting, methods: Patients included underwent an operative hysteroscopy at our university hospital due to RPOC, during which confirmed trophoblastic tissue was removed. Computerized files were reviewed. For patients who did not deliver at our institution following the hysteroscopy – a telephone survey was performed, during which we inquired regarding infertility or subsequent pregnancies.
Main results and the role of chance: A history of infertility following hysteroscopy, defined as at least one year of failure to conceive, was noted in 11 patients (11.8%) in the post abortion group, four of whom were receiving assisted reproductive treatment, as compared to two patients (2.3%) in the post-delivery group, one of whom was receiving assisted reproductive treatments (p = 0.01). None of the cases were attributed to mechanical female factor (intrauterine adhesions). A similar rate of patients in both groups had experienced an abortion since their hysteroscopy. In the post-delivery group – 34 patients delivered following hysteroscopy (40.0%) for a total of 36 deliveries, while in the post-abortion group 37 delivered (39.7%) a total of 42 deliveries. Deliveries in the post-delivery group were notable for a higher rate of abnormal placentation – 30.5% - including low lying placenta and placenta accreta. A significant rate of vaginal deliveries in both groups entailed manual removal of the placenta or manual exploration of the uterine cavity (23.5% and 10.5%, p = 0.20).
Limitations, reasons for caution: The study is retrospective and data obtained was that available in patient files and through telephonic survey. In addition, the post abortion group included missed abortion and termination of pregnancy, and medically and surgically treated cases. These entities may entail different outcomes, but were not powered for seperate analysis.
Wider implications of the findings: In the assessment of infertility patients, a history of past RPOC following delivery or abortion entails a similar prognosis, and warrant a similar suspicion for intrauterine adhesions. During pregnancy follow up – cases of RPOC following delivery necessitate careful attention to placentation.
Trial registration number: Not applicable.

P-346 Predicting the cumulative chance of live birth over multiple complete cycles of in vitro fertilization (IVF): an external validation study

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Study question: Are the published pre-treatment and post-treatment McLernon models, predicting cumulative live birth rates (LBR) over multiple complete IVF cycles, valid in a different context?
Summary answer: With minor recalibration of the pre-treatment model, both McLernon models accurately predict cumulative LBR in a different geographical context and a more recent time period.
What is known already: Previous IVF prediction models have estimated the chance of live birth after a single fresh embryo transfer, thereby excluding the important contribution of embryo cryopreservation and subsequent IVF cycles to cumulative LBR. In contrast, the recently developed McLernon models predict cumulative LBR over multiple complete IVF cycles at two certain time points: a) before initiating treatment using baseline characteristics (pre-treatment model) and b) after the first IVF cycle adding treatment related information to update predictions (post-treatment model). Before these models are used in clinical practice, their predictive performance needs to be validated in an independent cohort.
Study design, size, duration: External validation study in an independent prospective cohort of 1515 Dutch women who participated in the OPTIMIST study (NTR2657) and underwent their first IVF treatment between 2011 and 2014. Participants underwent a total of 2882 complete treatment cycles, defined as all fresh and frozen thawed embryo transfers resulting from one episode of ovarian stimulation. The follow up duration was 18 months after inclusion, and the primary outcome was ongoing pregnancy leading to live birth.
Participants/materials, setting, methods: Model performance was evaluated up to three complete treatment cycles, using the linear predictor as described by McLernon to calculate the probability of live birth. Discrimination was expressed by the c-statistic and calibration was depicted graphically in a calibration plot. In contrast to the original model development cohort, antimullerian hormone (AMH), antral follicle count (AFC) and body weight were available in the OPTIMIST cohort and evaluated as potential additional predictors for further model improvement.
Main results and the role of chance: Applying the McLernon models to the OPTIMIST cohort, the c-statistic of the *pre-treatment model* was 0.62 (95% CI 0.59-0.64) and of the *post-treatment model* 0.71 (95% CI 0.69-0.74). The calibration plot of the *pre-treatment model* indicated slight overestimation of the cumulative LBR. To improve calibration, the *pre-treatment model* was adjusted by subtracting 0.40 from the intercept. The *post-treatment model* calibration plot revealed accurate cumulative LBR predictions. After addition of AMH, AFC and body weight to the McLernon models, the c-statistic of the *updated pre-treatment model* improved slightly to 0.66 (95% CI 0.64-0.68), and of the *updated post-treatment model* remained at the previous level of 0.71 (95% CI 0.69-0.73).

As example for clinical application, using the recalibrated pre-treatment model, a woman aged 30 years with 2 years of primary infertility who starts ICSI treatment for male factor infertility has a probability of success of 0.39 in the first ICSI cycle, increasing to 0.71 over 3 complete cycles. Then using the post-treatment model, if this woman had 10 oocytes retrieved in the first cycle, embryos cryopreserved and a single fresh cleavage stage embryo transfer, the cumulative probability of success rises to 0.83 over 3 cycles.

Limitations, reasons for caution: The OPTIMIST study contained two randomized controlled trials (RCT) evaluating the effectiveness of gonadotropin dose individualization on basis of the AFC. The strict dosing regimens, the RCT in- and exclusion criteria and limited follow up time of 18 months might have influenced model performance in this independent cohort.

Wider implications of the findings: The McLernon prediction models can be introduced in clinical practice, after local recalibration, to inform patients and to complement clinical reasoning. These new counselling tools are the first to offer an objective and personalized estimate of the cumulative probability of live birth over multiple complete IVF cycles.

Trial registration number: Not applicable.

P-347 Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: a randomized controlled prospective study

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Study question: Is there any effect of anti-oxidant treatment with coenzyme Q10 (CoQ10) on ovarian response and embryo quality in young low-prognosis patients with POR?

Summary answer: Women in CoQ10 group had more retrieved oocytes, higher fertilization rate and more high-quality embryos, less cancelled embryo transfer, more available cryopreserved embryos.

What is known already: It has been suggested that poor responders could assist in evaluation of optimal management strategies for these patients. CoQ10 acts as an antioxidant by inhibiting lipid peroxidation and DNA oxidation, Several animal studies have demonstrated that CoQ10 protects ovarian reserve, counteracts physiological ovarian ageing by restoring mitochondrial function In the clinical setting, led to better response to ovulation induction and decreased odds of fetal aneuploidy in 35–43-year-old women. To date, however, no study has investigated whether CoQ10 pretreatment could improve the ART treatment outcomes in young subpopulation of poor responders in a randomized setting.

Study design, size, duration: A prospective, randomized controlled study included 186 consecutive patients with POR stratified according to the POSEIDON classification group 3 (age < 35, poor ovarian reserve parameters). The participants were randomized to the CoQ10 pre-treatment for 60 days preceding IVF-ICSI cycle or no pre-treatment. The number of high quality embryos was a primary outcome measure.

Participants/materials, setting, methods: This was a prospective randomized controlled study conducted at the Reproductive Medical Center of the Peking University Third Hospital, a tertiary university hospital and a center of excellence in Reproductive Medicine in China. The study is reported according to the CONSORT guidelines.

Main results and the role of chance: A total of 169 participants were evaluated (76 CoQ10 and 93 controls) and 17 women were excluded due to low compliance with CoQ10 administration. The baseline demographic and clinical characteristics were comparable between the groups. CoQ10 pretreatment resulted in significantly lower gonadotrophin requirements and higher peak E2 levels. Women in CoQ10 group had increased number of retrieved oocytes (4, IQR 2-5), higher fertilization rate (%) and more high-quality embryos (1, IQR 0-2); $p<0.05$. Significantly less women treated with CoQ10 had cancelled embryo transfer because of poor embryo development than controls (8.33% vs. 22.89%, $p = 0.04$) and more women from treatment group had available cryopreserved embryos (18.42% vs. 4.3%, $p = 0.012$). The clinical pregnancy and live birth rates per embryo transfer and per one complete stimulation cycle tended to be higher in CoQ10 group, but did not achieve statistical significance.

Limitations, reasons for caution: The important limitation of our study was its small sample size and we were unable to detect significant differences in clinical outcomes.

Wider implications of the findings: Pretreatment with CoQ10 improves ovarian response to stimulation and embryological parameters in young women with poor ovarian reserve in IVF-ICSI cycles. Further work is required to determine whether there is an effect on clinical treatment endpoints.

Trial registration number: Chinese Clinical Trial Registry (trial registration number: ChiCTR-IPR-17010945).

P-348 Investigating the effect of lifestyle risk factors upon the number of aspirated and mature oocytes in in vitro fertilization cycles: interaction with antral follicle count

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Study question: Is there a cumulative effect of smoking and Body mass Index on the reproductive outcomes after the first completed IVF cycle. Is there any possible interaction with antral follicle count.

Summary answer: There is an independent and a cumulative effect of smoking and BMI on the number of aspirated oocytes and mature oocytes in fresh IVF treatment-cycles.

What is known already: There is evidence demonstrating that lifestyle factors have a detrimental effect on fertility, however the data examining the effects of lifestyle changes on fertility treatment outcomes are limited. Furthermore, little is known about the cumulative impact of these unhealthy factors. Despite the lack of clear preconception guidelines intended for people seeking fertility treatment, most patients undergoing IVF treatment are willing to modify their lifestyle behavior. It is therefore crucial that they are aware of the precise lifestyle changes they can implement in order to enhance the likelihood of IVF success.

Study design, size, duration: Cohort study based on 2 different prospective cohorts. In total, 242 participants (hypothesis generating cohort) from the 'Lifestyle study cohort" in which data collection started in May 2013 and ended in September 2015. In addition, 432 participants (validation cohort) from the 'UppSTART cohort" starting their first fresh IVF cycle between September 2011 and December 2013 were included. Both public and private fertility clinics in mid-Sweden region were included in both studies.

Participants/materials, setting, methods: Number of aspirated oocytes, number of mature oocytes, number of embryos created, number of utilizable embryos and proportion of mature oocytes were the main outcome measures. The lifestyle factors considered were age, body mass index (BMI), smoking, alcohol consumption, daily caffeine consumption, physical activity score and history of depression. A regression model were performed to identify potential risk factors in the 'Lifestyle study cohort". The results were then validated in the UppStART cohort.

Main results and the role of chance: The median age of the population at cycle start was lower in the 'Lifestyle study" cohort 31.1 (IQR = 6.8) years compared to the 'UppStART study" validation cohort 34.0 (6.0) years. However, there were more women with university education in the 'UppStART study" validation cohort compared to the 'Lifestyle study" cohort.

Significant risk factors were identified in the 'Lifestyle study cohort": smoking and BMI. Women with both risk factors had (on average) 25% less aspirated oocytes than women without risk factors (IRR of 0.75,95% CI 0.61–0.94). Women with these risk factors had also an IRR of 0.78 (95% CI: 0.62–0.98) for the proportion of mature oocytes (in relation to the total number of aspirated oocytes), meaning that they would (on average) have 22% fewer mature oocytes than women with no risk factors. For the outcome of proportion of mature oocytes (in relation to the total number of aspirated oocytes), the number of risk factors was found to have a borderline significant interaction with

AFC (p = 0.099): the larger the value of AFC, the less harmful the effect of the risk factors.

Limitations, reasons for caution: The relatively small sample size, especially in the 'Lifestyle study", might introduce some problems with statistical power. This might be reflected in the borderline statistical significance of some of the results, where, on the other hand, notable and stable trends are demonstrated.

Wider implications of the findings: These negative lifestyle factors are easy to detect at an early stage of the assessment process and might allow for optimization of the treatment outcome. The results provide evidence on the significance of preconception guidance for the optimization of the treatment.

Trial registration number: Not relevant.

P-349 Outcome of Progestin-primed ovarian stimulation protocol vs. ultra-long protocol in patients with a first IVF/ICSI cycle: a randomized clinical trail

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Study question: Could Progestin-primed ovarian stimulation protocol or ultralong protocol be a preferred alternative for infertile women during IVF/ICSI.

Summary answer: Both regimens can get similar top-quality embryos with good developmental potential. They all have their benefits and are irreplaceable.

What is known already: Ultralong protocol with fresh embryo transfer is a typical ovarian stimulation regimen using a depot of long acting GnRH agonist. It is well recognized and widely used. Progestin-primed ovarian stimulation (PPOS) based on a freeze-all strategy is a new ovarian stimulation regimen that uses progestin as an alternative to a GnRH-a for preventing a premature LH surge. Ultralong protocol has some weakness of being increased procedure complexity, higher fees, and greater risk of ovarian hyperstimulation syndrome (OHSS) in addition to its benefits. There is an unmet need for new ovarian stimulation protocols with improved efficacy and user convenience.

Study design, size, duration: A prospective RCT including 260 patients was performed between 1 July 2017 to 31 December 2017. Computerized randomization was conducted to assign participants at a 1:1 ratio into two treatment groups: PPOS (130 patients) or ultralong protocol (130 patients) followed by their first IVF/ICSI with fresh/frozen embryo transfer. The primary outcome was the number of top-quality embryos. The sample size was chosen to detect a difference of two oocytes with a power of 85%.

Participants/materials, setting, methods: Patients with normal ovarian reserve undergoing their first IVF/ICSI procedure were included. MPA and HMG was simultaneously administered at menstrual cycle 3 in PPOS group. Oocyte maturation was co-triggered by a GnRHa and hCG(1000IU). In the ultralong protocol group, pituitary down-regulation was obtained in early follicle phase, Ovarian stimulation started after 35 days and hCG(5000IU) was used to trigger. Only the first embryo transfer cycle was followed-up. The embryological and clinical outcomes were measured.

Main results and the role of chance: Basic characteristics, such as age, BMI and infertility duration, in both groups were comparable. There was no significant difference in the number (mean ± SD) of top-quality embryos [3.9 ± 3.0 for PPOS vs 3.4 ± 2.5 for ultralong protocol] or the number of oocytes retrieved [11.8 ± 6.5 for PPOS vs 11.1 ± 5.7 for ultralong protocol] between the groups. During the whole process of ovarian stimulation, the LH level in the PPOS group was always higher than that in the ultralong protocol group but lower HMG dosage was administrated in the PPOS group [2027.9 ± 333.9 for PPOS vs 2689.0 ± 624.9 for ultralong protocol, P = 0.00]; however, no patient from either group experienced a premature LH surge. Besides, there was no moderate or severe ovarian hyperstimulation syndrome during the ovarian stimulation in PPOS group while three patients suffered it in the ultralong protocol group. No significant difference was found in the clinical pregnancy rate of the first embryos transfer cycle between the two groups (P = 0.89): 56.5% for the PPOS group (65/115) versus 57.4% for the ultralong protocol group (70/122).

Limitations, reasons for caution: Patients and physician were not blinded to the study. Moreover, some patients had not finish their first embryo transfer cycle at the end of the trial. It would be better if the live birth rates were observed in the follow up period and the result could be demonstrated.

Wider implications of the findings: PPOS effectively reduces the HMG dosage and the incidence of moderate and severe OHSS during COH while having a weakness to use freeze-all strategy only. It can be an alternative of the treatments for infertile woman undergoing IVF as well as traditional protocols.

Trial registration number: ChiCTR-INR-17012089.

P-350 Arterial stiffness during controlled ovarian hyperstimulation and early pregnancy in women exposed to assisted reproduction

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Study question: To study the effects of controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF), and early pregnancy, on arterial stiffness determined by digital pulse wave analysis (DPA).

Summary answer: We found an increased systemic arterial stiffness, assessed by DPA, in luteal phase of IVF but no change during ovarian stimulation or in early pregnancy.

What is known already: Among female sex hormones, estrogen has a vasodilatory effect whereas the vascular effect of progesterone is not yet fully determined. A person's vascular condition can be expressed in terms of arterial stiffness, as measured by pulse wave analysis. Photoplethysmographic DPA is an operator-independent method to estimate arterial stiffness and endothelial function in large and small arteries, and changes in DPA parameters towards increased stiffness have shown good correlations with age and cardiovascular pathology and morbidity. To our knowledge, there are no previous studies investigating the effects of IVF and COH on arterial stiffness.

Study design, size, duration: Prospective observational longitudinal study including 68 women, starting before conception and performed during COH and IVF treatment until the seventh gestational week.

Participants/materials, setting, methods: 68 women planned for IVF treatment were asked to participate. The women were examined with DPA at first visit to the clinic (baseline), during IVF stimulation, after embryo transfer, and in early pregnancy. Heart rate, mean arterial pressure (MAP) and DPA variables cardiac ejection elasticity index (EEI), *b/a,* dicrotic index (DI), *d/a*, and the global variable aging index (AI), were measured. The ovarian response to gonadotropin stimulation was estimated with ovarian stimulation index (OSI).

Main results and the role of chance: Heart rate was significantly increased at all measuring points ($p \leq 0.003$) but MAP only at embryo transfer ($p = 0.007$). DPA variables representing large arteries (EEI, *b/a*) and peripheral arteries (DI, but not *d/a*), and AI, all indicated increased arterial stiffness at embryo transfer compared with baseline ($p \leq 0.035$). No DPA variable was significantly changed during stimulation or at pregnancy measurements. Independent of the influence of age, basal measurements of *b/a* and AI indicated higher arterial stiffness in poor ovarian responders.

Limitations, reasons for caution: The stimulation measurements, which showed no significant DPA variable changes, may have been performed too early during the stimulation (stimulation day 7), before the full effect of stimulation had been reached. Baseline measurements were obtained without considering the menstrual cycle.

Wider implications of the findings: We expected a vasodilatory effect of COH since estrogen is a vasodilator, but found the opposite. The unexpected findings could be a progesterone effect, or activation of the renin-angiotensin-aldosterone-system (RAAS), eclipsing the estrogen effects. The association between arterial stiffness and poor ovarian response, independent of age, deserves further exploration.

Trial registration number: The study was approved by Regional Research Ethics Committee in Lund, Sweden, Dnr 2014/648.

P-351 Identification of empty follicles or oocyte-containing follicles by ultrasound images using K-means method and principal component analysis assessing several parameters with artificial intelligence

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Study question: To investigate whether we can identify the existence of oocytes from ovarian follicles by ultrasound images using artificial intelligence (AI).

Summary answer: We can retrospectively classify ovarian follicles with or without oocytes by ultrasound images using K-means method and principal component analysis assessing several parameters with AI.

What is known already: We cannot distinguish follicles with or without oocytes using ultrasound, because a diameter of oocyte is below minimum detection levels of usual ultrasound devices. When we choose 8 MHz as a frequency of ultrasound, minimum detection levels are estimated to be 0.2 mm and 0.48 mm with a simple calculation and in consideration with distance-based resolution, respectively. Therefore, physicians and patients cannot speculate how many oocytes could be retrieved before oocyte retrieval, or whether a follicle would be empty in a cycle with timed intercourse or intrauterine insemination. New idea to detect oocytes by ultrasounds has been desired.

Study design, size, duration: We hypothesized the follicles with oocyte might be more rigid (i.e. less elastic) than those without oocytes, because the former has thicker follicle membrane as cumulus-oocyte-complex. If they were, deformity patterns with or without oocytes might be different when a needle was going to insert into the follicles. With a similar concept, elastography has been utilized for prediction of breast cancer. We performed a pilot study in a single center on October in 2015.

Participants/materials, setting, methods: With informed consent, 3 patients participated in the study. Ultrasound (B-mode) moving images were recorded throughout the oocyte retrieval under anesthesia, each follicle was aspirated separately using different syringe followed by confirmation with microscopy. Aspirated follicles and moving images were numbered in numerical order. The image data of 16 follicles (11 contained oocytes, 5 did not) were analyzed with AI in order to distinguish the differences with or without oocytes using several parameters, retrospectively.

Main results and the role of chance: We chose six parameters from the moving images when a needle was going to insert into the follicles: #1: Circularity of outline of the follicles ($C = 4\pi S/L^2$, where S is the area surrounded by the outline of the follicles and L is the perimeter of the outline), #2: Angle formed between the two lines defined by the two points just before piercing the follicles ($\theta_{1/\sqrt{2}} = D_{1/\sqrt{2}}$, where D is the distance between the convex hull and the outline for each angle, and $D_{1/\sqrt{2}}=D_{max} \times 1/\sqrt{2}$), #3: $\theta_{0.01} = D_{0.01}$ where D = 0.01 mm, #4: D_{max} difference between just before piercing the follicles and 1 mm in front of piercing the follicles ($D_{max}{}^{diff}$), #5: $\theta_{1/\sqrt{2}}$ difference between just before piercing the follicles and 1 mm in front of piercing the follicles ($\theta_{1/\sqrt{2}}{}^{diff}$), #6: $\theta_{0.01}$ difference between just before piercing the follicles and 1 mm in front of piercing the follicles ($\theta_{0.01}{}^{diff}$). By K-means method and principal component analysis with AI, oocytes-containing follicles can be predicted in 8/10 and empty follicles can be predicted in 5/6.

Limitations, reasons for caution: This was a retrospective pilot study with a sample size, and was a single-center study. Moreover, this method requires needle aspiration. Further prospective studies in large samples size without needles are needed.

Wider implications of the findings: We can retrospectively classify ovarian follicles with or without oocytes by ultrasound images with AI. If we could prospectively recognize ovarian follicles with or without oocytes by ultrasound image only (without needles), we could predict retrieval oocyte number and locations of empty follicles at daily office gynecology.

Trial registration number: N/A.

P-352 Is the follicular flushing usefull for poor ovarian response, a prospective controlled randomised trial

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Study question: Does follicular flushing with a double-lumen needle improve the number of oocytes collected in a poor responder population?

Summary answer: Follicular flushing does not improve, number of oocytes collected, numbers of top embryos, oocyte quality and pregnancy rate and could decrease the number of oocytes collected.

What is known already: Follicular flushing in poor ovarian response is a usual practice but benefit is unproved. No strong statistical power prospective randomised study has been produced at this time.

Study design, size, duration: A monocentric controlled randomised prospective study leaded in Strasbourg fertility unit from March 2011 to June 2016. We compared two technics of oocyte retrieval: direct aspiration with a single lumen aspiration needle vs flushing method with a double lumen aspiration needle (EchoTip® Cook® Double Lumen Aspiration Needle K-OPSD-1735-B-L).

Participants/materials, setting, methods: Patients with 4 or less follicles bigger than 14 mm on ovulation induction day in IVF cycle were included. Patients were randomized between a FLUSH group with a double-lumen needle with flushing and a NO FLUSH group with a classic single-lumen needle without flushing. The primary outcome was the number of oocyte collected. The secondary criteria was the pregnancy rate and the number of embryos.

Main results and the role of chance: 250 patients were included: 125 patients were included in the FLUSH group and 125 in the NO FLUSH group. The two groups were comparable about Age, BMI, FSH and AMH before stimulation and number and follicle and oestradiol on ovulation induction day. The number of oocytes retrieval was highest in the NO FLUSH group (2.35 in the flush group versus 3.43 in the non flush group, p<0,001), as the number of meta II oocytes (p = 0,003).

Limitations, reasons for caution: Several operators participated to this study, each one had some differences in their own practice of flushing even if the amont of flushing medium was standardised. Those differences of practice could be a bias to interpret our results.

The volume of flushing fluid was limited that's why for some patients included in FLUSH GROUP, few oocytes were not collected by flushing procedure.

Wider implications of the findings: Follicular flushing with double-lumen needle for poor responder patients doesn't increase the number of oocyte collected.

Trial registration number: PRI 2010 HUS n°4781 **2010-A00589-30**

P-353 Circulating microRNAs as biomarkers for controlled ovarian stimulation

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Study question: Are circulating microRNAs (miRNAs) able to predict the response to controlled ovarian stimulation (COS) in patients undergoing assisted reproduction cycles?

Summary answer: The presence of specific miRNAs in serum may be a tool to predict the ovarian response to COS.

What is known already: Most assisted reproduction cycles use COS before oocyte pick up, to increase the number of embryos. However, the optimal

stimulation protocol has not been established. The need for COS individualization to attain an optimal oocyte yield while minimizing the risk of an excessive response, has been recognized. Therefore, the development of tolls to predict the response to COS is extremely important. MiRNAs are small non-coding RNAs that can be detected in the extracellular environment. Circulating miRNAs emerged as diagnostic and prognostic markers of several diseases; however, its utility as a biomarker of response to COS is still unknown.

Study design, size, duration: This study included 15 serum samples split into three groups, and polled together, according with the response to COS: poor response: < 4 retrieved MII oocytes (Poor, n = 5), normoresponse: ≥ 4 and < 25 retrieved MII oocytes (NR, n = 5), and hyper response: ≥ 25 oocytes (Hyper, n = 5).

Participants/materials, setting, methods: Serum samples were collected from patients < 36 years-old, body mass index (BMI)>18,5 and <25 Kg/m^2, prior to the COS for intracytoplasmic sperm injection (ICSI), in a private university-affiliated IVF center, between January/2017 and June/2017. Patients presenting endometriosis grades III and IV were excluded. Circulating RNA was extracted, cDNA was synthesized and large-scale analysis of miRNA was performed (miScript miRNome qPCR Array, QIAGEN). The cel-miR-39 was spiked-in to calibrate the gene expression analysis.

Main results and the role of chance: A total of nine miRNAs were differentially detected in serum from the pool of patients who presented hyper response, in comparison with the pool of patients who responded normally (NR). Four miRNAs were exclusively detected in patients on the Hyper group, with undetectable levels in the NR and Poor groups. On the other hand, five miRNAs were differentially detected in patients with poor response, four of them exclusively detected in patients from Poor group. Moreover, we identified the absence of one miRNA in Poor group, with normal detection levels in NR and Hyper groups. These findings suggest these miRNAs could be explored as specific serum predictive markers for poor or hyper response to COS.

Limitations, reasons for caution: These analyses were performed in pooled samples. The next step includes the validation of these candidate miRNAs in a larger cohort of samples and the correlation with clinical data from the patients.

Wider implications of the findings: Individual heterogeneity in ovarian response limits the implementation of individualized COS protocols. Currently, there is no consensus regarding the factors to be considered when determining the gonadotropin dose in individualized protocols. Detection of specific circulating miRNAs prior to the treatment could guide the COS and improve the outcomes of IVF.

Trial registration number: None.

P-354 Vitamin D deficiency leads to increased insulin resistance and advanced glycation end-product (AGE) accumulation and decreased DHEA-S levels and oocyte maturation rates

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Study question: Is vitamin D deficiency associated with hormone levels, oxidative stress, insulin resistance, advanced glycation end-products (AGEs), and the outcome of IVF-ET in infertile patients?

Summary answer: Vitamin D deficiency has been associated with increased insulin resistance and AGE accumulation and decreased DHEA-S levels and oocyte maturation rates.

What is known already: Vitamin D is necessary for maintaining calcium homeostasis and promoting bone metabolism. However, vitamin D deficiency is still common in the general population, particularly in women of reproductive age. Vitamin D receptors are expressed in a number of tissues including the skeleton and parathyroid glands as well as reproductive tissues. Vitamin D has been shown to exert effects on female reproduction.

Study design, size, duration: The present study enrolled 510 infertility women who received IVF treatment at the HORAC Grand Front Osaka Clinic between July 2016 and August 2017. Patients were divided into two groups based on their serum 25-hydroxyvitamin-D3 [25(OH)D] levels: Group A < 20 ng/ml and Group B ≥ 20 ng/ml, and follicular fluid 25(OH)D levels: Group C < 20 ng/ml and Group D ≥ 20 ng/ml. This study was approved by the local Ethics Committee.

Participants/materials, setting, methods: Vitamin D insufficiency and deficiency corresponded to 25(OH)D levels < 30 and < 20 ng/ml, respectively. Serum 25(OH)D, AMH, basal FSH, testosterone, and DHEA-S were measured at a commercially available laboratory. Reactive oxygen metabolite (d-ROM) and biological antioxidant potential (BAP) levels were measured as indicators of the degree of oxidative stress. AGEs were measured using the AGE Reader. A follicular fluid sample was obtained from preovulatory follicles ≥ 18 mm in diameter.

Main results and the role of chance: The numbers (%) of women with a vitamin D deficiency and insufficiency were 337 (66.1%) and 141 (27.6%), respectively. No significant differences were observed in age (38.4 ± 4.7 vs. 38.1 ± 5.0 years) or BMI (21.1 ± 4.1 vs. 21.0 ± 3.8 in kg/m^2) between groups A (n = 337) and B (n = 173). DHEA-S was significantly lower in group A than in group B (165.9 ± 90.4 vs. 188.1 ± 115.4 μg/dL, $p < 0.05$). HOMA-R was significantly higher in group A than in group B (1.81 ± 1.5 vs. 1.28 ± 0.6, $p < 0.01$). AGEs were significantly higher in group A than in group B (213.6 ± 60.5 vs. 192.9 ± 29.4 AU, $p < 0.01$). However, no significant differences were observed in AMH, testosterone, basal-FSH, PRL, d-ROM, or BAP. Oral vitamin D supplementation increased follicular fluid 25(OH)D levels in deficiency and insufficiency cases (from 16.0 ng/mL to 29.7 ng/mL, $p < 0.01$). Oocyte maturation rates were significantly lower in group C than in group D (73.7 vs. 83.1%, $p < 0.05$). However, no significant differences were observed in the rate of fertilization, good quality embryos, blastocysts, or good quality blastocysts.

Limitations, reasons for caution: The vitamin D status fluctuates and may be influenced by several external factors such as sun exposure, seasonality, and diet. Therefore, the limitation of the present study is that vitamin D levels may have varied.

Wider implications of the findings: The present results demonstrated that serum vitamin D levels are increased by oral vitamin D supplementation and this may have a significant impact on insulin resistance, DHEA-S levels, AGE accumulation, and oocyte maturation. A vitamin D deficiency may be involved in the pathogenesis of infertility.

Trial registration number: Not applicable.

P-355 Factors associated with livebirth in couples undergoing their first ICSI cycle: an internally validated prediction model

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Study question: What factors are associated with livebirth in couples without children undergoing ICSI cycles for the first time?

Summary answer: Our seven-parameter model shows good precision and calibration for the prediction of live birth and can be reliably used for estimation and counseling.

What is known already: Predictive models are useful for counseling patients and guiding treatment decisions. Most prediction models rely on demographic and ovarian reserve parameters without incorporating cycle specific information which can be vital. The precision of the existing models is most often suboptimal for clinical use. Also, there is a paucity of information about the variables which are most useful predicting success in the first ICSI cycles.

Study design, size, duration: This was a cohort study including ICSI cycles managed at Ankara University, Department of Reproductive Health and Infertility between years 2010 and 2017. A total of 488 women were included.

Participants/materials, setting, methods: Couples with no prior children undergoing their first ICSI cycles using fresh embryos were included in the study. Exclusion criteria consisted patients with secondary infertility, younger than 45 years, and cycles in which more than two embryos were transferred. The cohort was partitioned for model building (n:305) and internal validation via bootstrapping. The prediction model was built using a variable selection approach, and parameter estimates were obtained using Bayesian logistic regression with a vague prior.

Main results and the role of chance: A total of 488 women with a live birth rate of 27.9% were included. The model variables consisted female age ≥38

years (OR:0.05,95% CrI:0.00-0.44), baseline luteinizing hormone (LH) levels above 6mIU/mL (OR:2.46,95% CrI:1.33-4.52) and antral follicle count below 5 (OR:0.13,95% CrI:0.02-0.60), duration of induction cycle longer than 10 days (OR:2.31,95% CrI:1.18-4.61), endometrial thickness at the day of transfer greater than 9 mm (OR:2.16,95% CrI:1.02-4.75), transferred embryo grade and count. The model showed good precision (AUC: 0.76) and good calibration (Hosmer-Lemeshow test, P = 0.624) in the internal validation cohort. The baseline model without using cycle specific information also showed modest precision (AUC:0.68).

Limitations, reasons for caution: Our cohort size is smaller than optimal, and some parameter estimates may be unreliable despite using a Bayesian framework with Markov Chain Monte Carlo simulations. An external validation study is required for the generalization of these results to broader populations.

Wider implications of the findings: Our model uses variables which are easily obtained and widely recorded during ICSI cycles worldwide. If proven to be stable in external validation studies, our model offers the highest precision reported up to date.

Trial registration number: Not applicable.

P-356 Cheaper, simpler, better: oral desogestrel versus antagonist injections for LH suppression in corifollitropin-stimulated cycles in the same oocyte donor. A crossover study

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Study question: Is the ovarian response to Corifollitropin (CFT) among oocyte donors (OD) different when treated with oral Desogestrel (DSG)or subcutaneous injections of antagonist (ANT) for LH suppression?

Summary answer: Donors response to CFT-DSG compared to CFT-ANT was similar in number of oocytes and pregnancy rates in recipients, with less injections and lower medication costs.

What is known already: A single injection of CFT replaces the first seven daily injections of any rFSH preparation. Initiating CFT seven days after discontinuation of the oral combined contraceptive pill (OCP) instead of 5 days, results in a significant reduction of the additional consumption of rFSH in oocyte donors(OD) on an antagonist protocol. Patients showed high degree of satisfaction and preference for this preparation. A new form of controlling the endogenous LH peak has been put forward: the so-called "progesterone protocols". DSG is a progestogen-only pill (POP) that at a dose of 75 mcg inhibits ovulation by suppressing endogenous LH peak.

Study design, size, duration: An observational cross-over study, including 35 donors treated at a private, university-based IVF centre between Feb'15 and May'17. Recruited donors had undergone two stimulation cycles within <20 months under CFT. Exclusion criteria: AFC >20, hypersensitivity to CFT, presence of ovarian cysts or enlarged ovaries, risk or history of ovarian hyperstimulation syndrome (OHSS). Fifty-four corresponding recipients of freshly donated oocytes were analysed (29 in the CFT-ANT group and 25 in the CFT-DSG group).

Participants/materials, setting, methods: Healthy donors, pre-treated with OCP and CFT. On stimulation day 8, daily FSH doses were added if needed. In CFT-ANT cycles, daily antagonist was administered since follicle >14 mm until trigger. Subsequently, in CFT-DSG cycles, donors received daily DSG starting right after OCP until trigger. All donors were triggered with a GnRH agonist bolus. Outcomes of both cycles and clinical pregnancies among corresponding recipients were compared. Paired statistical test was used to compare variables.

Main results and the role of chance: Mean donors age was 25.94 ± 5.37 years, mean basal AMH was 3.57 ± 2.07 ng/mL, and mean AFC was 19.35 ± 5.07. Interval between both treatment cycles was 0.91 ± 0.70 months.

Compared to CFT-ANT cycles, cycles under CFT-DSG received less number of injections (10.34 ± 2.83 vs 5.03 ± 2.12, p<0.05), a lower total supplementary rFSH dose (497.4 ± 338.9 IU vs 442.9 ± 332.8 IU, p<0.05); all this translated in a lower total cost of medication (1018.6 ± 191.0€ vs. 813.8 ± 145.9€, p<0.05).

Compared to CFT-ANT, donors treated with CFT-DSG had significantly higher mean serum estradiol at trigger (1505.4 vs 2406.0 pg/ml, p<0.05). There were no differences in the total number (17.4 ± 7.5 vs 18.6 ± 89.0) and mature oocytes retrieved (14.1 ± 6.9 vs. 15.9 ± 8.1). There were no cycles cancelled due to low ovarian response or premature LH rise prior to GnRH trigger. There were no cases of OHSS.

The average number of embryos transferred was 1.3 ± 0.7 in the CFT-ANT group vs. 1.1 ± 0.6 in the CFP-DSG group(p> 0.05). Clinical pregnancy rate/embryo transfer was similar between groups: 52.0% vs. 58.6% respectively (p>0.05).

Limitations, reasons for caution: No cycles were cancelled due to low ovarian response and it should be interpreted with caution. Given that included patients were OD that had undergone both treatment cycles, with good ovarian reserve markers, and none previous cancelled cycles.

Wider implications of the findings: In OD programs, treatment cost savings are important, but so are patient safety, comfort and compliance, and clinical results among recipients. The main advantage of this strategy is its simplicity, an aspect of utmost importance in the management of ODs. This information could be useful for PGS and fertility preservation.

Trial registration number: The study was in clinicaltrials.gov (Trial number: NCT03354494).

P-357 A multivariate logistic regression model for embryo selection improves pregnancy outcomes in frozen-thawed embryo transfer

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Study question: Can a validated model, based on biological variables, be developed to predict successful pregnancy outcomes in frozen-thawed embryo transfer (FET)?

Summary answer: Our logistic model with six variables shows good performance on validation of the values for the prediction of pregnancy in single-blastocyst transfers.

What is known already: Although three morphological parameters have routinely been used to select the best blastocyst for transfer (degree of blastocoele expansion and appearance of the trophectoderm (TE) and the inner cell mass (ICM)), their independent ability to predict pregnancy outcomes remains unclear. A simple scoring system derived from a mathematical model that can be easily implemented in clinical practice has not been previously described for the prediction of ongoing viability.

Study design, size, duration: We performed a retrospective cohort study. Retrospective evaluation of data extracted from implantation data originating from 4717 blastocyst transfers conducted at a single clinic between January 2012 and December 2015 in phase I. Data from 490 FETs were collected between January 2016 and February 2017 in phase II.

Participants/materials, setting, methods: We obtained the regression functions for the establishment of pregnancy for each factor of the ASEBIR embryo assessment system (Hum Reprod, 2011) and of conventional clinical information, which were statistically related to the establishment of pregnancy confirmed by univariate analysis. A multivariate logistic regression was conducted, which was not affected by multicollinearity, which provided the probability of the establishment of pregnancy with calculated values from actual data from the regression functions.

Main results and the role of chance: In phase I of the study, 4717 single-blastocyst transfers were performed that resulted in 1826 pregnancies (38.7% of the women). The prediction model, built after multivariable logistic regression analysis, demonstrated that blastocyst diameter (odds ratio (OR) 271.03, 95% confidence interval (CI): 62.93-1167.34), ICM (OR 4.36, 95% CI: 1.22-15.59), and TE (OR 3.03, 95% CI: 1.12-8.22) were predictive factors for successful pregnancy outcomes. Cryopreservation day (OR 14.40, 95% CI: 2.03-102.18), ET attempt (OR 11.18, 95% CI: 2.06-60.63), and female age (OR 11.16, 95% CI: 4.89-25.51) were predictive for unsuccessful pregnancy

outcomes. The area under the receiver operating characteristic curve for the model for successful pregnancy outcomes was 0.692 (95% CI: 0.669-0.716). Sensitivity and specificity were 0.611 and 0.667, respectively; positive and negative predictive values were 0.540 and 0.729, respectively. In phase II, a validation study (n = 490) showed that the application of this logistic model was associated with a significantly higher ongoing clinical pregnancy rate (54.2% versus 43.4%) with an odds ratio of 1.62 (95% CI: 1.12-2.36, P = 0.011).

Limitations, reasons for caution: In this study, the morphokinetic variables provided by time-lapse incubators could not be assessed, and embryo evaluation was limited to distinct time-point observations. The interpretation of our findings is limited by the retrospective nature of the analysis and the potential for unmeasured confounding.

Wider implications of the findings: This prediction model incorporates readily available data that are routinely collected in clinical practices and have been shown to significantly improve pregnancy outcomes. Further study is required to add morphokinetic information and thereby improve implantation rates.

Trial registration number: Not applicable.

P-358 Patients with Inflammatory Bowel Disease (IBD) have similar reproductive outcome compared to the general infertile population

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Study question: Is reproductive outcome compromised in patients with IBD who undergo IVF with transfer of single euploid blastocyst?

Summary answer: IBD Patients who pursue a single euploid embryo transfer have similar odds of implantation, clinical pregnancy and pregnancy loss compared to the general infertile population.

What is known already: Often, women with IBD experience challenges to family building throughout the course of their reproductive years. Patients may encounter decreased fertility and fecundity associated with pelvic adhesive disease from surgery and/or by the disease itself. IBD has been postulated to cause infertility due to an adverse autoimmune influence on embryo quality and/or endometrial receptivity. Currently, there is limited data assessing the implantation rate in IBD patients following IVF. This study aims to evaluate embryo transfer outcome in IBD patients who undergo transfer of screened embryos.

Study design, size, duration: This was a retrospective cohort study of patients who underwent IVF with preimplantation genetic testing and subsequent euploid single embryo transfer (SET) of a vitrified-warmed blastocyst in a synthetically prepared endometrial cavity from Jan 2012 to January 2018. Trophectoderm biopsies (Day 5-6) were analyzed by Next-Generation Sequencing or quantitative Polymerase Chain Reaction (qPCR). A sample size of 36 patients per group was needed to detect a 25% difference in implantation rates with 80% power (alpha = 0.05).

Participants/materials, setting, methods: Natural language processing was used to identify from the electronic medical records a cohort of women with the diagnosis of IBD. A matched 3:1 ratio cohort of control subjects were identified using a propensity-score matching algorithm based on oocyte age, BMI, and ovarian reserve markers. Cases involving the transfer of fresh and/or multiple embryos were excluded. Also, uterine factor infertility, ovum donation, recurrent pregnancy loss, recurrent implantation failure and severe male factor infertility were excluded.

Main results and the role of chance: Of 3274 euploid, single, vitrified-thawed blastocyst transfers included, 38 patients with an IBD diagnosis were compared to 114 control patients. IBD and control patients had a similar implantation rate (71.0% vs. 78.0% (p = 0.68)), clinical pregnancy rate (50.0% vs 60.5% (p = 0.68)) and pregnancy loss rate (37% vs 25.8% (p = 0.25)) respectively. An IBD diagnosis was not found to significantly modify the odds of implantation (adjusted OR = 0.6 [CI 95% -1.2–0.8]) after the data was evaluated using a GEE model that accounted for patients who underwent multiple cycles and controlled for oocyte age, body mass index, anti-müllerian hormone, basal antral follicle count and endometrial thickness at embryo transfer. Additionally, the odds of implantation were not altered by IBD patients having an ulcerative colitis or Crohn's disease diagnosis. (OR = 0.4 CI95% 0.1 – 1.9).

Limitations, reasons for caution: We acknowledge the potential weakness of this study. The retrospective nature of the study might create a possible selection bias. The study's current results must therefore be considered with some caution. Larger, well-controlled studies are needed to verify the study's findings.

Wider implications of the findings: Patients who suffer from Inflammatory bowel disease have comparable embryo transfer outcomes to the general infertile population when pursuing a euploid, single, cryo-thawed embryo transfer. Patients and physicians can be reassured that an IBD diagnosis does not appear to adversely impact embryo quality and endometrial receptivity.

Trial registration number: This study was approved by the Western Institutional Review Board (Study Number: 1167398).

P-359 Association of follicular fluid levels of adrenomedullin 2, vascular endothelial growth factor and its soluble receptors with ICSI outcome

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Study question: Association between follicular fluid (FF) levels of adrenomedullin 2 (ADM2), vascular endothelial growth factor (VEGF) and its soluble receptors with ICSI outcome.

Summary answer: The FF ADM2 could be a potential predictive marker for ovarian non-responders with cutoff value of 348.55 (pg/ml).

What is known already: ADM2 and VEGF are involved in ovarian function especially angiogenesis, follicular development, and ovulation. VEGF action could be antagonized by its soluble receptors, soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble VEGF receptor 2 (sVEGFR-2), via decreasing its free form, thereby decreasing angiogenesis. It has been reported that sFlt-1 and sVEGFR-2 have low expression in post-selected follicles (POF) compared with preselected follicles (PRF). However, after dominant follicle selection, sVEGFR-2 expression increases while sFlt-1 expression reduces along with the days of cycle.

Study design, size, duration: Ninety non-smoker women aged from 20-40 years with fallopian tube obstruction, idiopathic infertility and male factor infertility (varicocele and oligospermia) were enrolled in this study. The long GnRH agonist–recombinant FSH protocol was used for all patients and a single FF aspiration without blood was done. All patients were treated with ICSI and were categorized as non-, poor-, normo- and high-responders according to the retrieved oocytes number.

Participants/materials, setting, methods: The fertilization rates were determined by dividing the number of fertilized oocytes with the number of mature oocytes and the clinical pregnancy was evaluated by existence of the intrauterine gestational sac via transvaginal ultrasound. Implantation rate was defined as the quantity of visible sacs per number of transferred embryos. FF ADM2, VEGF, sFlt-1 and sVEGFR-2 levels were determined by ELISA kits and the VEGF/sFlt-1 and VEGF/sVEGFR-2 ratios were also calculated.

Main results and the role of chance: In the present study, for first time existence of ADM2 in FF was reported; its mean level was 62.2 ± 66.86 pg/ml. Also, it was found that age of the patients was significantly correlated with ADM2 (r = 0.268, p = 0.049). ADM2 level was positively correlated with VEGF and sVEGFR-2 levels (r = 0.586, p = 0.001 and r = 0.482, p = 0.001, respectively). Our results showed that the levels of ADM2 in FF were significantly higher in ovarian non-responders in comparison to patients with poor-,

normo- and high- ovarian responses (p<0.05). Also, the FF levels of VEGF, and sVEGFR-2 were significantly higher in the non-responders than in poor-responder patients (p<0.05). We found no significant association between FF levels of ADM2, VEGF and sVEGFR-2 with clinically pregnant. Based on receiver operating characteristic (ROC) analyses, cutoff value for ADM2 as a non-responder predictor was 348.55 (pg/ml) with sensitivity and specificity of 67.7 % (CI, 67.21–68.25%) and 94.6% (CI, 94.11–95.08%), respectively.

Limitations, reasons for caution: It is a preliminary report covering existence of ADM2 in FF and further studies are required to clarify mode of action of ADM2 in ovarian angiogenesis. The number of subjects was almost low and there may have been other confounding factors that we were unable to account for.

Wider implications of the findings: We found higher FF levels of ADM2 in non-responder women and proposed ADM2 as a potential predictive marker for non-responders. Besides, positive correlations of ADM2 with VEGF and sVEGFR-2 were obtained, what could be considered as an important clue regarding the roles of ADM2 in ovarian angiogenesis.

Trial registration number: Not applicable.

P-360 Bacterial infection other than Chlamydia associated to tubal factor of infertility suggests new approach

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Study question: Is the presence of endocervical bacteria associated to hysterosalpingography outcomes in patients screened for infertility?

Summary answer: Endocervical bacterial is associated to tubal factor infertility and most prevalent etiologic agents were Ureaplasma and Mycoplasma, suggesting that not only Chlamydia must be screened.

What is known already: It is known that microorganisms infecting vaginal flora as Chlamydia trachomatis and Neisseria gonorrhoeae cause pelvic inflammatory disease (PID) in at least 50% of cases. A strong association exists between chlamydia infection and tubal factor infertility, leading PID-related morbidity (i.e., infertility and ectopic pregnancy) and is a substantial public health problem worldwide. Considering the uterine and tubal factors are responsible for 20% of infertility causes, the hysterosalpingography is part of infertility investigation. Besides that, in developing countries, the female infertility by infection as Chlamydia trachomatis is highly prevalent and correlated to tubal alterations.

Study design, size, duration: Cross sectional study reviewed 156 clinical charts of women who underwent infertility investigation during 2016 and 2017 in a private reproductive medicine center in Brazil. The inclusion criteria were women who had hysterosalpingography realized and collected endocervical secretion for bacteria screening.

Participants/materials, setting, methods: Women had complete infertility investigation including anamneses, serum hormone measurements, transvaginal ultrasound, hysteroscopy and biopsy if necessary, hysterosalpingography and endocervical bacteria screening. The hysterosalpingography results were classified as normal tubal or abnormal tubal and endocervical bacteria's screened were Chlamydia Trachomatis, Neisseria Gonorrhea, Ureaplasma Urealíticum, Mycoplasma Hominies and Gardnerella by using PCR. Patients with bacteria positive received antibiotic treatment before IVF cycle.

Main results and the role of chance: Patients were 22 to 48 years of age (36.3 ± 4.6), had a mean of 4.3 years of infertility time, basal FSH of 9.4 ± 14.5 and anti-mullerian hormone of 2.2 ± 2.0. The hysterosalpingography was abnormal in 53.2% of patients, which determined tubal factor of infertility. The prevalence of endocervical bacteria's was 20.5%, as Chlamydia Trachomatis 3.2%, Neisseria Gonorrhea 0.0%, Ureaplasma Urealíticum 7.7%, Mycoplasma Hominies 5.1%, Gardnerella 2.6%, other bacteria 9.6% (Staphylococcus Epidermidis, Streptococcus Agalactiae, Enterococcus Faecalis, and Escherichia Coli). Patients presenting abnormal hysterosalpingography had a higher prevalence of endocervical bacteria (27.7%) compared to normal hysterosalpingography (12.3%; p = 0.014), independently of the type of bacteria. From women with endocervical bacteria positive (n = 32), 34.4% presented more than one type of bacteria positive, and from those, 81.8% (9/11) had tubal factor of infertility.

Limitations, reasons for caution: The number of cycles included in this study is relatively small and larger prospective studies should be carried out to confirm the results. The prevalence of each bacteria individually analyzed in this study was low and it was not possible to correlate each one with hysterosalpingography outcomes.

Wider implications of the findings: The prevalence of endocervical bacterial infection was higher among patients with tubal factor infertility, supporting the association between bacterial infection (other than Chlamydia) and tubal factor. The prevention of complications associated with bacterial infection is an indication for screening at the time of IVF and even at gynecological routine.

Trial registration number: not applicable.

P-361 Progesterone dose adjustment and transfer postponement in patients with low progesterone levels following hormonal replacement therapy for frozen thawed embryo transfer

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Study question: Is progesterone (P) dose adjustment and postponement of frozen thawed embryo transfer (FET) effective to improve outcome in patients with low P levels following hormonal replacement therapy?

Summary answer: Dose adjustment to obtain serum P levels >10 ng/ml and transfer postponement accordingly gave similar outcome to those of normal P and 'in phase" transfers.

What is known already: Several studies have demonstrated that, in HRT cycles, low serum P levels on the day of FET were associated with decreased pregnancy and live birth rates. However, in our hands, P dose adjustment after ET to obtain P levels >10 ng/ml was ineffective to correct impaired outcome. Therefore, we decided to measure serum P levels and adjust P dose to obtain a serum P level >10 ng/ml prior to ET and to postpone ET by one or two days accordingly.

Study design, size, duration: This retrospective study included data from March to October 2017 obtained in a cohort of 139 patients, undergoing FET following HRT. Endometrial preparation was achieved by sequential administration of vaginal estradiol until endometrial thickness >7 mm, followed by transdermal estradiol combined with 600 mg/day vaginal micronized P (200 mg three times a day).

Participants/materials, setting, methods: This study was conducted in a university hospital. Serum P was measured on D2 following exogenous P introduction in the evening (referred as D0). When P levels were >10 ng/ml, ET was performed on D2, D3 or D5 depending on embryo stage at cryopreservation. When P levels were <10 ng/ml, P dose was increased to 1200 mg/day. Serum P level was checked one day later and the next day if necessary. ET was postponed accordingly.

Main results and the role of chance: Mean serum P level on D2 was 12.1 + 3.52 ng/ml and serum P <10 ng/ml were observed in 28% of cycles. Cycles were cancelled in 11 patients because of inability to obtain P >10 ng/ml despite increased progesterone supplementation (n = 5) or failure in performing P measurement timely (n = 6). ET was postponed in 26 patients after P dose adjustment until reaching serum P values beyond 10 ng/ml. This strategy led to similar positive pregnancy test (42.3 % vs 43.1 %, NS), heartbeat activity at 6 weeks (30.7 % vs 27.5 %, NS) and ongoing pregnancy rates at 12 weeks (26.9 % vs 25.7 %, NS) when compared with 102 'in phase" transfers performed in patients with P >10 ng/ml at first evaluation.

Limitations, reasons for caution: The number of treated patients has to be extended to confirm these preliminary data.

Wider implications of the findings: These results suggest that serum P measurement prior to ET and further adjustment of exogenous P dose and postponement of transfer might optimise the outcome of FET cycles performed using HRT.

Trial registration number: not applicable.

P-362 Mitochondrial function in granulosa cells of preovulatory follicles may be diminished in infertile women with polycystic ovary syndrome

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Study question: Could polycystic ovary syndrome (PCOS) affect the mitochondrial function in granulosa cells (GCs) of preovulatory follicles?

Summary answer: Mitochondrial function in GCs of preovulatory follicles may be diminished in infertile women with PCOS.

What is known already: Several problems in patients with PCOS undergoing IVF have been reported including decreased fertilization rates, abnormal embryo development, high incidence of miscarriage and increased likelihood of ovarian hyperstimulation syndrome (OHSS). In addition, Infertile women with PCOS frequently have higher concentrations of inflammatory cytokines such as tumor necrosis factor (TNF)-alpha and androgens in follicular fluid (FF). These unfavorable intrafollicular environment may have a negative effect on the mitochondrial function in GCs. However, the study on the mitochondrial activity in GCs of infertile patients with PCOS undergoing COS for IVF has not been reported.

Study design, size, duration: This retrospective study included 51 infertile patients with PCOS (PCOS group) and 95 infertile patients with normal ovulatory cycle (control group) who underwent IVF/ICSI in controlled ovarian stimulation (COS) cycles between June 2015 and December 2016.

Participants/materials, setting, methods: IVF cycles in which GnRH antagonist protocol was used for controlled ovarian stimulation (COS) were included for this study. FF from at least 2 follicles (range 17-21 mm) at oocyte retrieval was collected. IVF results and relative amounts of succinate dehydrogenase complex subunit A (SDHA) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha) mRNA in GCs were compared between the two groups. The expression of *SDHA* and *PGC-1alpha* mRNAs was analyzed by realtime RT-PCR.

Main results and the role of chance: Patients' age and infertility duration were similar in the two groups. In the PCOS group, total dose of recombinant human FSH (rhFSH) used for COS was significantly lower and the number of oocytes retrieved was significantly higher than in the control group. However, number of embryos fertilized was comparable between the two groups. FF TNF-alpha and IL-6 concentrations at oocyte retrieval were significantly higher in the PCOS group ($P<.001$, $P<.001$). Relative amount of *SDHA* mRNA in GCs was significantly lower in the PCOS group of 4.58 ± 2.87 compared with 9.62 ± 3.90 in the control group ($P<.001$). Relative amount of *PGC-1alpha* mRNA in GCs was also significantly lower in the PCOS group ($P<.001$).

Limitations, reasons for caution: This study have limitations to evaluate the mitochondrial function due to a small numbers of sample available and marker genes investigated and its retrospective nature. Therefore these results should be taken with caution until well-designed controlled studies will be presented.

Wider implications of the findings: Mitochondrial function in GCs may be impaired in infertile women with PCOS and these results may be associated with the unfavorable intrafollicular environment including higher levels of FF pro-inflammatory cytokines.

Trial registration number: None.

P-363 Expression of various genes in granulosa cells derived from women undergo IVF procedures

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Study question: Do telomerase and topoisomerase expression and activity in luteinized granulosa cells derived from woman undergoing IVF treatments correlate with woman infertility?

Summary answer: The results show variations and correlations between enzymes activities and expressions with women's age, cause of infertility and estrogen levels with/without telomerase activating compounds.

What is known already: Telomerase (Telo) and topoisomerase (Topo) are nuclear enzymes with essential activities needed for maintaining the normal functions and survival of cells and tissues in organisms.

Granulosa cells (GCs) are somatic cells, surrounding each developing oocyte, required for the development and functionality of ovarian follicle in vivo. These enzymes exist in all living organisms, in which they play a crucial role in genomic stability. Little is known about in their functions and activities in developing GCs. Thus, deep understanding of the enzymes participating in the maintenance of oocytes and GCs quality and functionality is important for improving human fertility.

Study design, size, duration: GCs were collected from 80 IVF patients following oocyte retrieval and formal consent. The GCs were immediately subjected to protein or RNA extract procedures or incubated for various intervals with/without AGS (telomerase activating compounds).

The extend of the activity and expression of the enzymes and other genes (markers of GCs) in the GCs of each woman were correlates to various parameters including woman age, cause of infertility, number of oocytes and estrogen levels.

Participants/materials, setting, methods: GCs were collected and isolated from follicular fluids of patients undergoing IVF treatments. In some experiments GCs were treated with telomerase activating compounds (AGS) Telomerase activity was determined in whole cells (WC) and DNA bound (DB) extracts using TRAP assay. Topoisomerase I activity was assayed by the relaxation of supercoiled DNA plasmid. The expressions of the enzymes and other relevant genes were examined by RT-PCR using specific primers.

Main results and the role of chance: Variations in telo and topo I activities in the GCs derived from the examined women were detected. A negative correlation between topo I or telo activities and blood estrogen levels was observed. The patients were divided into two groups according to the cause of infertility. Low but not significant, telo activity was observed within the group diagnosed as "female infertility" and not in "male infertility" group. An increase in Telo expression with AGS, was shown in older women–in female infertility group, while in male infertility group, the increase in Telo expression was determined in all ages.

Specific GCs genes expression (known as markers for GCs: CAMTA1, UNC-5, 3bhsd, B4galt 2), showed different expression pattern due to the presence/absence of AGSs. We found that the expression pattern of CAMTA and B4galt 2 in correlation with woman age, was the opposite to that observed for telo, but 3bhsd expression was very similar to Telo expression. No dependency with woman age was observed for the expression of Unc5 and STAR genes in GCs. Generally, Telo expression decreases with woman's age, but in the presense of AGS's, Telo expression was increased, especially in older women.

Limitations, reasons for caution: The Gcs in our experiments were derived from woman exposed to gonadotropins and maturating oocyte agents therefore they represent the state of luteinized GSc.

Wider implications of the findings: Telomerase and topoisomerase are nuclear enzymes.

Deep understanding of the enzymes participating in maintenance of oocytes and GCs quality and functionality is important for the design of strategies to treat human infertility. Identification of GCs response to AGS compounds, could provide a new and promising insights into IVF procedures.

Trial registration number: All the human samples used in this study were approved by Helsinki committee no. 0003-15-SOR

P-364 Anti-Mullerian hormone can predict pregnancy, live birth and miscarriage?

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Study question: Is the serum Anti-Mullerian hormone (AMH) level in infertile women associated with the outcome of fertility treatment?

Summary answer: AMH cannot be a predictive value for pregnancy, live birth or miscarriage 1 to 1.5 years after starting fertility treatment.

What is known already: AMH is an established marker of ovarian reserve and a good predictor of ovarian response for controlled stimulation, and previous research has shown that AMH is associated with implantation and clinical pregnancy rate effectuated from assisted reproductive medicine treatments. However, it is still uncertain whether AMH can predict the final outcome of every type of fertility treatment.

Study design, size, duration: This retrospective cohort study was designed to identify the correlation between serum AMH levels and fecundity by reviewing infertile couples' medical records. Four hundred and eighty-one women had the AMH test at their first consultation visit from January 2015 until June 2016. The history of 477 women who received any kind of fertility care was examined only if the couples reached clinical pregnancy, live birth, or miscarriage by June 2017.

Participants/materials, setting, methods: This study was performed at a private fertility clinic located in Japan's second city, Osaka. AMH was measured by an enzyme-linked immunosorbent assay (gen II). The couples suffered from various causes of infertility and underwent different fertility treatments including, timed intercourse direction, intrauterine insemination, assisted reproductive technology, and/or alternative medicine. The data was analyzed with the Games-Howell multiple comparisons procedure.

Main results and the role of chance: The average age of the women was 38.9 (SD = 4.6, range 25-49), and the average AMH was 2.4 (SD = 2.4, 0-33.8) ng/mL. Two hundred and forty-one were younger than 40 years old (group I), and two hundred and thirty-six women were in their forties (group II). The data was analyzed according to their AMH levels, a: <0.1, b: 0.1-0.49, c: 0.5-0.99, d: 1.0-1.49, e: 1.50-1.99, f: 2.0-2.49, g: 2.5-2.99, h: 3.0-3.99, i: 4.0-4.99, j: ≥5.0 ng/mL. The pregnancy rate, the live birth rate including the ongoing pregnancy rate after the 12th week of gestation, and the miscarriage rate were compared among different AMH sub-groups (a-j) at the point of 1 to 1.5 years after their first visit. The pregnancy rate in group I was a: 0.31, b: 0.53, c: 0.46, d: 0.52, e: 0.57, f: 0.62, g: 0.60, h: 0.77, i: 0.59, j: 0.66. The live birth rate was a: 0.23, b: 0.40, c: 0.38, d: 0.41, e: 0.54, f: 0.58, g: 0.47, h: 0.65, i: 0.53, j: 0.58. No significant difference was found among the AMH dependent subgroups. This trend was the same in the elderly group (II). The analysis on miscarriage rates showed no relation between AMH and miscarriage development.

Limitations, reasons for caution: Small sample size. There is possible dispersion of the cause of infertility, the patients' back ground and treatment policy.

Wider implications of the findings: AMH is not associated with the outcome of fertility treatment. Low AMH doesn't necessarily mean a poor prognosis of fertility treatment. With adequate management for poor ovarian responders we anticipate just under 40% of infertile couples can achieve conception.

Trial registration number: not applicable.

P-365 A personalized maturation trigger administered 36 hours before IUI improves the pregnancy rates

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Study question: If a maturation trigger of human chorionic gonadotropin (hCG) is administered 36 hours before intrauterine insemination (IUI), will this improve the pregnancy rate?

Summary answer: A maturation trigger of hCG administered 36 hours before intrauterine insemination (IUI) improves the pregnancy rate.

What is known already: In ART treatment, 36 hours is accepted as the optimal duration between a maturation trigger and oocyte pick-up (OPU) in order to obtain mature oocytes. With a shorter duration, the chances for immature oocyte obtainment rate are higher; when the duration is longer, the ovulation rate becomes higher. Therefore, approximately 36 hours following the maturation trigger could be a suitable duration for both ovulation and fertilization naturally without ART. In our country, self-injection kits with a prefilled syringe containing either recombinant-human chorionic gonadotropin (r-hCG) syringe or Gonadotropin-releasing hormone agonist (GnRH-a) are available as maturation triggers.

Study design, size, duration: This was a retrospective cohort study of total 425 patients who received IUI form the husband's semen in our clinic between January and April 2017. The patients were classified into one of three groups: the r-hCG group included 73 patients who received r-hCG 36 hours before IUI; the u-hCG group included 233 patients who received urinary-hCG 24 hours before IUI; and, the GnRH-a group included 119 patents, who received GnRH-a 36 hours after IUI.

Participants/materials, setting, methods: Some patients were received Clomiphen citrate or recombinant follicle stimulating hormone (r-FSH) as ovarian stimulation, but others received no drugs for stimulation. Luteal support was not performed for all participants. Factors that included male infertility and advanced maternal age of the female partner were excluded in this study. A clinical pregnancy was defined as the confirmation of a gestational sac via ultrasound. The clinical pregnancy and miscarriage rates were evaluated for the three groups.

Main results and the role of chance: The mean age of the patients in the r-hCG, u-hCG and GnRH-a groups were 36.5, 36.1 and 35.7, respectively, and there were no significant differences. The mean number of previous IUI attempts (times) and the percentage of stimulated cycles in the r-hCG, u-HCG and GnRH-a groups were 1.2 and 19.2%, 1.4 and 15.5%, and 1.6 and 20.2%, respectively, with no significant differences among the three groups. The clinical pregnancy rate in the r-hCG group was 16.4%, which was significantly higher than that for the u-hCG group (8.2%, $p<0.05$). But there were no significant differences between the r-hCG and GnRH-a groups (20.2%). The miscarriage rates for the r-hCG, u-hCG, and GnRH-a groups were 0, 26.7, and 36.8%, respectively, with no other significant differences among the three groups.

Limitations, reasons for caution: This study was not a randomized controlled trial. The r-hCG has only been available since March 2017. Prior to that, u-hCG wss the only agent available for hCG preparation.

Wider implications of the findings: The miscarriage rate in the GnRH-a group tended to be higher than that in the r-hCG group. One of the reasons could involve the half-life of each agent, because r-hCG shows a longer half-life than GnRH-a. Nobody received luteal support, which suggested that r-hCG might support the luteal phase.

Trial registration number: This study had no RCT status, and was not assigned a trial registration number.

P-366 Impact of yoga- and meditation-based lifestyle intervention on depression and quality of life in infertile couples: a randomized controlled trial

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Study question: What is the impact of yoga- and meditation-based lifestyle intervention (YMLI) on depression severity, quality of life, and accelerated cellular aging in infertile couples with major depressive disorder (MDD)?

Summary answer: A brief 12-week YMLI significantly decreases depression severity, increases the quality of life, and reverses the biological aging in infertile couples with major depressive disorder.

What is known already: Depression and Infertility are comorbid and significantly affect pregnancy outcomes. Modern lifestyle factors, including the psychological stress of infertility and treatment failures, contribute to depression. The depression-related accelerated cellular aging that includes neurodeficits,

regulatory feedback dysfunctions, affect reproductive functions and quality of life in both infertile men and women. In addition, it can lead to poor quality gametes and severe complications in the offspring like neurodevelopmental disorders and cancers. Targeted interventions are needed to increase the success of pregnancy and healthy offspring.

Study design, size, duration: A randomized clinical trial of 12−week YMLI for infertile couples (n = 80 couples) with MDD was designed to evaluate the impact on depression severity, quality of life, and systemic biomarkers of cellular aging.

Participants/materials, setting, methods: Infertile couples with MDD were randomly assigned to a 12−week yoga intervention group (n = 40) or to a control group (n = 40). Clinical parameters and blood samples were collected before and after the intervention.Assessment of depression severity was by Beck Depression Inventory–II scale (BDI-II)], and quality of life was by abbreviated 26 item version of World Health Organization Quality-of-Life Scale (WHOQOL−BREF). Systemic biomarkers of cellular aging were assessed as per manufacturer protocols.

Main results and the role of chance: YMLI led to a significant decrease [difference between means, (95% CI)] in BDI-II score [-5.83 (-7.27, -4.39), p < 0. 001]. There was significant improvement in all domains of the quality of life: physical [11.3 (4.2 to 21.3), p < 0. 001]; psychological [13.2 (3.9 to 22.4), p < 0. 001]; social [4.6 (1.6 to 12.3), p < 0. 033]; and environment [3.4 (1.1 to 7.8), p < 0. 039]. There were significant improvements in the 12-week levels of the systemic biomarkers of accelerated aging in yoga group compared to control group [decrease in ROS, 8OH2dG, cortisol, and IL-6, and, increase in TAC, COX2 activity, telomerase activity, BDNF, serotonin, melatonin, and sirtuin 1) (all P<0.05). Although YMLI did not differentially improve telomere length (P = 0. 084), telomere length was maintained. These findings were in parallel with the significant reduction in BDI-II scores and increase in WHOQOL-BREF scores.

Limitations, reasons for caution: Interpretation of the study findings will be more precise only after further studies of the impact of YMLI on pregnancy outcomes in infertile couples with MDD and their future offspring become available. It should also be noted that chronological age is an independent risk for infertility.

Wider implications of the findings: Reversal of depression associated accelerated aging by YMLI in infertile couples with MDD improve all domains of the quality of life. YMLI induced improvement in neural networks and integrative mechanisms contribute to improvement in both male and female reproductive systems, gametes, pregnancy outcomes, and future offspring.

Trial registration number: Clinical Trial Registry of India (CTRI) REF/2014/09/007532

P-367 Live birth rate in Turner syndrome patients after oocyte donation: a matched-cohort study over a 10-years period

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Study question: Is the live birth rate in Turner syndrome women after oocyte donation (OD) comparable to that of oocyte recipients without Turner syndrome?

Summary answer: The live birth rate in Turner syndrome patients after IVF with donated oocytes (OD) is 35.4%, comparable to other women accessing this treatment.

What is known already: Turner syndrome is one of the most common female chromosomal abnormalities with gonadal dysgenesis, due to a total or partial (mosaicism) absence of one X chromosome. Turner syndrome usually causes infertility and gestational complications, thus spontaneous pregnancies in these women are rare, and pregnancy outcomes involve an increased risk of miscarriage and stillbirths. Assisted reproduction techniques, especially OD, can give women with Turner syndrome the possibility to become pregnant and delivery healthy children.

Study design, size, duration: Retrospective matched (1:2) cohort study including 252 OD patients. The exposed group ("Turner", n = 84) were women affected by Turner syndrome performing their first fresh embryo transfer (ET) between 2010 and 2017. The non-exposed group ("no-Turner"; n = 168) were women without chromosomal alterations also performing their first ET in the same time period. Endometrial preparation was performed with estrogens, administered either orally or transdermally, while oocyte donors were stimulated using GnRH antagonist protocol triggered with GnRH-agonist.

Participants/materials, setting, methods: Turner women in this study had different karyotypes: 45,X (4 women, 4.8%) and several mosaics such as 45,X/46,XX and 45,X/46,XY. No-Turner women required OD mainly due to age. Cases and controls were matched by date of ET, number of embryos transferred, and developmental stage of the embryo at the time of ET. Differences in reproductive outcomes between groups were assessed using Chi-square tests. Additionally, we analyzed the subset of 147 cycles with single ET (SET).

Main results and the role of chance: Women in the Turner group were on average 32.2 years old (SD 4.8, range 22-43), while women in the no-Turner group were 41.8 (SD 4.3, range 25-50). All other parameters were similar in both groups: mean BMI was 23.9 (SD 4.2), mean number of mature oocytes (MII) assigned to each woman was 7.3 (SD 1.5, range 4-14), and they were fertilized with partner's sperm in 86.8% of cases. ET was performed on day 2-4 in 165 (66.7%) cases, with 1 embryo transferred in 147 (58.3%) of cycles.

Reproductive outcomes were slightly higher in the Turner group, although not statistically significant. Biochemical pregnancy rate was 54.9% vs. 47.2% (p = 0.28). Clinical pregnancy rate was 39.5% vs. 37.7% (p = 0.82). Ongoing pregnancy rate was 36.3% vs. 30.6% (p = 0.38), and live birth rate was 35.4% vs. 29.6% (p = 0.36). Comparable results were obtained when analyzing the subset of SET: biochemical pregnancy rate was 57.5% vs. 50.5% (p = 0.44); clinical pregnancy rate 43.5% vs. 38.3% (p = 0.56); ongoing pregnancy rate 40% vs. 30.4% (p = 0.27); live birth rate 38.6% vs. 29.7% (p = 0.30).

Limitations, reasons for caution: Women in the Turner group were on average 10 years younger; however reproductive outcomes are not affected by the patient's age when donated oocytes are used, and the mean donor age was 26 in both groups.

Wider implications of the findings: OD is an assisted reproduction technique that offers excellent results in women with Turner syndrome, especially in single ET, which is the recommended treatment option for these patients.

Trial registration number: Not applicable.

P-368 National U.S. utilization patterns and live birth rates of various ovarian stimulation protocols for IVF

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Study question: How does the U.S. utilize various ovarian stimulation protocols for in vitro fertilization (IVF) and what are resulting live birth rates?

Summary answer: Paradoxically, minimal stimulation, natural cycles and *in vitro* maturation (IVM) are utilized primarily in poor prognosis patients, where their utility is the lowest.

What is known already: Alternative ovarian stimulation protocols for IVF have grown in popularity. Yet, patient populations best suited for these protocols have not been defined.

Study design, size, duration: This is a retrospective study of aggregate data published by the Society for Assisted Reproductive Technologies (SART) for autologous IVF cycles performed in the U.S. during 2014 and 2015.

Participants/materials, setting, methods: IVF cycles were stratified based on ovarian stimulation protocols and included 205,705 conventional stimulations, 4,397 minimal stimulations, 2,785 natural cycles and 514 in vitro maturation (IVM) cycles. The main outcome measures were utilization patterns and age-specific live birth rates for the different ovarian stimulation protocols.

Main results and the role of chance: With advancing female age, utilization of conventional stimulation protocols decreased, while minimal stimulation and natural cycle IVF increased. LFOR diagnoses were in all age groups less prevalent in patients undergoing conventional stimulation than with all other protocols. Live birth rates were uniformly the highest with conventional stimulation at 42.4%, 33.1%, 22.1%, 11.7% and 3.9% for ages <35, 35-37, 38-40, 41-42 and

>42, respectively. Moreover, pars in live birth rates between conventional stimulation and other protocols widened with advancing age from 1.6-3.9-fold among women <35 years to 4.4-6.6-fold among women > 42 years.

Limitations, reasons for caution: Current U.S. national IVF outcome reporting allows for only limited interpretation of data: One such limitation is absence of a standardized definition of what represents minimal stimulation. Another limitation is that SART reports only present aggregate data. Our ability to adjust for confounding patient characteristics was, therefore, limited.

Wider implications of the findings: Conventional stimulation protocols still represent the gold standard of care. Alternative protocols should only be used in younger patients with normal FOR if specific indications exist which negate lower pregnancy and live birth chances but should be avoided in older women and in younger patients with LFOR.

Trial registration number: not applicable.

P-369 Is there a difference between blastocyst formation and cumulative pregnancy rates between cycles of recipients who received fresh or vitrified eggs from the same donor?

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Study question: Do frozen-thawed oocytes affect the blastocyst formation and pregnancy rate compared to fresh oocytes from the same donor in *in vitro* fertilization (IVF) cycles?

Summary answer: Frozen-thawed oocytes do not affect blastocyst formation and pregnancy rates in donation cycles when compared to fresh oocytes from the same donor.

What is known already: An efficient oocyte cryopreservation method is mandatory to establish a successful egg-banking program. There are increasing reports showing good clinical outcomes after oocyte cryopreservation, but there is still a lack of large controlled studies evaluating the effectiveness of oocyte cryo-banking. Although previous studies suggest that vitrified oocytes result in similar pregnancy rates when compared to fresh oocytes, no data is available regarding blastocyst formation and pregnancy rates from the same oocyte donor.

Study design, size, duration: Cross-section study from an oocyte donation program, including 20 oocyte donors, that obtained ≥14 MII oocytes/cycle and divided into 49 different recipient's, 20 receiving fresh and 29 frozen eggs, from 2013 to 2017.

Participants/materials, setting, methods: All oocyte donors (23.5 ± 2.91 years old) were stimulated using the same protocol. Recipient's patients (42.04 ± 4.14 years old) received at least 07 mature oocytes from fresh (n = 20) or frozen-thawed (n = 29) cycles, according to uterine synchronization. For all recipients, fertilization were carried out with ICSI and the embryos produced within the cycle were transferred on blastocyst stage (2.02 ± 0.59).

Main results and the role of chance: There was no difference between fresh and frozen-thawed oocyte cycles rates for fertilization (83.21% vs 81.57%, p = 0.6765), D3 embryo formation (93.44% vs 94.42%, p = 0.6038), top quality D3 embryo (total TQ D3/total D3 embryos: 46.24% vs 42.14%, p = 0.3719), blastocyst formation (55.78% vs 54.59%, p = 0.8324), top quality blastocyst (total TQ Blastocyst/total Blastocyst: 67.69% vs 62.99%, p = 0.5945), pregnancy (75.86% vs 65.52%, p = 0.5648) and implantation (48.15% vs 44.44%, p = 0.7074). Paired T-test or Fisher´s test was applied as appropriated.

Limitations, reasons for caution: The egg donation is usually performed by healthy and young woman (≤29 years) and could be speculated that oocytes from women below 30 years old may be more resistant to vitrification than those from women of advanced age. Moreover, male factor was not considered.

Wider implications of the findings: Using eggs from the same donor to fresh or frozen-thawed cycles produce an excellent clinical outcome. Moreover, a consistent egg vitrification program could be extended to accumulate oocytes from successive stimulations from the same donor, decreasing risk of ovarian hyperstimulation and improving fertility preservation and/or oocyte donation program outcomes.

Trial registration number: Not applicable.

P-370 Dual triggering with GnRH agonist plus hCG versus triggering with hCG alone for IVF/ICSI outcome in GnRH antagonist cycles

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Study question: Is dual triggering with GnRH agonist plus hCG as efficacious as hCG triggering in terms of oocyte and pregnancy outcomes?

Summary answer: Results indicate comparable or significantly improved outcomes with the use of GnRH agonist plus hCG as compared with hCG alone for triggering of oocyte maturation.

What is known already: Final oocyte maturation triggering in *in vitro* fertilization cycles using GnRH antagonist protocols is conventionally achieved with hCG, which is associated with an increased risk of ovarian hyperstimulation syndrome (OHSS). Triggering with GnRHa has been used as an alternative to hCG; however, previous findings indicate poorer pregnancy outcomes with this method.

Study design, size, duration: A comprehensive literature search was performed to identify randomized controlled trials comparing IVF outcomes between women receiving combined administration of hCG with GnRHa and those receiving hCG alone for triggering of final oocyte maturation.

Participants/materials, setting, methods: Meta-analysis.

Main results and the role of chance: Four studies including 527 patients eligible for inclusion in meta-analysis were identified. No significant difference in the number of mature oocytes or fertilized oocytes retrieved was found between groups. Clinical pregnancy rate with dual triggering was significantly higher as compared with hCG-alone triggering (pooled OR = 0.48, 95% CI: 0.31 to 0.77, P = 0.002), but there was no significant difference in the ongoing pregnancy rate between groups (pooled OR = 0.88, 95% CI: 0.29 to 2.66, P = 0.826).

Limitations, reasons for caution: Only 4 studies were eligible for inclusion and the quality of the studies was only moderate overall.

Wider implications of the findings: None.

Trial registration number: None.

P-371 Relationship between pregnancy rate and post sperm wash motile sperm count in IUI cycles: perspective from a developing country

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Study question: What is the least total Motile sperm count in a post wash IUI semen sample for a reasonable pregnancy rate?

Summary answer: No pregnancies occured when the total motile sperm count of the post wash semen sample was < 10million.

What is known already: IUI is an efficacious, cost effective treatment of infertility. In developing country like India where IVF is not covered by health insurance, the affordability of common man for IVF is low. Also there are various societal taboos associated with IVF. Hence, we are expected to offer IUI

for those with higher degrees of male factor infertility. The quality of sperms is an important determinant of outcome. KoyunOk et al, suggested that an average post-wash total motile sperm count of 10×10^6 may be a useful threshold value for IUI success, but more studies are needed to determine a cut-off value.

Study design, size, duration: It was a prospective observational study carried out between November 2015 and November 2017. The sample included 422 consecutive IUI cycles. All IUI cycles using husband sperm were included and those using donor sample were excluded. Post sperm wash motile sperm count(PMSC) was calculated as Post sperm wash (Total sperm count x Percentage motility)

Participants/materials, setting, methods: The patients underwent standard protocol of ovulation induction using clomifene and gonadotropins as per institutional protocol. Follicular monitoring was done using Mindray Resona 6 using 7.5 MHz transvaginal probe.Ovulation trigger with purified urinary HCG 10,000 units was given and IUI done 36-40 hours later. The ovulation was checked prior to IUI and done only if follicles had ruptured. Semen sample was collected by masturbation and processed by WHO prescribed density gradient method and IUI done.

Main results and the role of chance: In the 422 patients included in the study, the range of female partner age and male partner age (mean) was 18-38 yrs(29.8) and 23-42 yrs(31.3) respectively.312 and 110 patients had primary and secondary infertility respectively. The number of patients with PMSC <10,10-20,20-30,30-40,40-50,>50 million was 43,51,77,103,80,68 respectively and number of pregnancies(pregnancy rate) was 0(0%),17(33.3%),15 (19.4%),21(20.3%),17(21.3%),24(35.2%) respectively. Out of the 422 patients who underwent IUI, 94 conceived, making pregnancy rate 22.2%.

There were no pregnancies when the total motile sperm count of the inseminated sample was < 10million. However, the motile sperm count >10 million gives good pregnancy rate. This can be used to explain prognosis to patients undergoing IUI and counsel them for other options such as IVF/ICSI in the subsequent cycle.

Limitations, reasons for caution: The study has a low sample size and is an observational study. It is a single centre study.

Wider implications of the findings: Further multicentre studies with larger number including the combined impact of post wash motility/morphology must be carried out. This can be used to define criteria for explaining the limitations of IUI treatment and next step to offer after IUI fails: whether to go ahead with IUI or offer IVF/ICSI.

Trial registration number: RM003

P-372 Pain scores during oocyte retrieval: a retrospective cohort study comparing three different protocols

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Study question: Pain scores during oocyte retrieval in three Dutch clinics: a comparison of oral analgesics with intravenous or intramuscular opioids.

Summary answer: The lowest pain scores were achieved by intramuscular administration of alfentanil, followed by intravenous fentanyl and, finally, non-sedative oral analgesics.

What is known already: No particular method is superior in providing effective conscious sedation and analgesia for pain relief during oocyte retrieval, as described in a 2013 Cochrane review (Kwan, et al.). Conscious sedation is most commonly used for pain relief in the USA and UK, whereas pain protocols in the Netherlands vary from oral analgesics to various administration methods of opioids or a paracervical block. Comparison of these methods has never been done before. We aimed to compare these methods and thereby identify the most effective and convenient pain relief protocol, ultimately enabling the creation of a uniform protocol across the Netherlands.

Study design, size, duration: A retrospective cohort study was conducted in three hospitals in the Netherlands. A total of 2.134 oocyte retrievals between May 2012 and May 2017 were included in the analysis. Each clinic has a different protocol for pain relief during oocyte retrieval: clinic 1. oral analgesics (1000 mg paracetamol and 500 mg naproxen), clinic 2. intramuscular opioids (0.01 mg/kg alfentanil), clinic 3. intravenous opioids (50ug fentanyl). We excluded patients where pain relief was not given according to local protocol.

Participants/materials, setting, methods: In all three clinics, pain scores were registered during every oocyte retrieval on a scale from 0 to 10. Clinic one and three used the numerical rating scale, clinic two used the visual analogue scale. Pain scores and additional variables, such as female demographics, treatment type and cycle (in vitro fertilisation or intracytoplasmic sperm injection) and oocyte number were recovered from the national electronic fertility database or from the local electronic file.

Main results and the role of chance: A mixed linear model was performed, since some patients had had multiple follicle punctures. Mean pain scores and their 95% confidence intervals were: 5.6 (5.3-5.8) in the oral analgesics group, 5.1 (5.0-5.3) in the intravenous opioids group and 4.0 (3.9-4.2) in the intramuscular opioids group. Furthermore, age, number of oocytes and number of previous retrievals had a significant effect on pain scores. A lower age, higher number of follicles and having undergone a retrieval in the past were associated with higher pain scores. The largest effect was found on having undergone a previous oocyte retrieval, the mean pain score increases with 0.28 (95% CI 0.17-0.38) per previous puncture. In view of the large size of the participant population it is unlikely that outcomes are the result of chance.

Limitations, reasons for caution: Potential limitations of this study include; the retrospective design, the use of only one type of analgesia per centre for comparison and the use of verbal and visual pain scores (although both using a scale from 0 to 10 with whole numbers).

Wider implications of the findings: Acceptable pain scores were demonstrated following intramuscular administration of opioids during oocyte retrieval. The use of intramuscular alfentanil is unaccustomed for this procedure worldwide, but could be a cost effective and less invasive alternative for conscious sedation. Further research is needed to compare post-procedure pain, side effects and patient satisfaction.

Trial registration number: *N17.059*, trial registration number from the local Medical Ethical Committee.

P-373 Embryo implantation failure as a prognostic factor of ART outcomes: An evaluation of 3315 ART cycles

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Study question: Can the number of previous implantation failures (NIF) alone be a useful tool for predicting the outcomes of ART cycles?

Summary answer: An increase in NIF is associated with a worsening of ART outcomes but is closely related to patient age.

What is known already: As one of the main reasons for the high rate of abandoning ART programmes, implantation failure (IF) is a challenging condition. Adjuvant treatments and diagnostic investigations to improve the chances of pregnancy have been proposed. However, the use of IF alone as a prognostic factor for ART outcomes has not been evaluated.

Study design, size, duration: A single-centre prospective-cohort study on data from 3315 couples submitted to IVF/ICSI cycles (one per couple) was conducted. Only fresh embryo transfers were considered. The couples were categorized into three groups according to female age: ≤35 years, 36–39 years and ≥40 years. The NIF of each couple was recorded at the first appointment. Exclusion criteria included abnormal karyotype, uterine defects, evidence of hydrosalpinx, infections, endocrine problems, coagulation defects or thrombophilia and autoimmune defects.

Participants/materials, setting, methods: Potential confounding factors, including age, aetiologies, duration/type of infertility, type of ovarian stimulation, endometrial thickness, and number/quality and development stage of the transferred embryo, were included as variables. NIF was correlated with the presence or absence of clinical pregnancy (CP) and live birth (LB). Simple logistic regression (considering only the independent variable NIF), multiple logistic

regression (considering other cycles variables together with NIF) and ROC curve analysis were used to evaluate the results.

Main results and the role of chance: Simple logistic regression revealed that the likelihoods of CP and LB were significantly (P<0.0001) associated with NIF. Multiple logistic regression confirmed this results, although to a smaller degree. However, this influence of NIF seemed to be important only in younger women (≤35 years). In older age groups, an influence of NIF on clinical pregnancy and live birth was not observed (Table 1). In the younger group (≤35 years), for the probability of CP and LB occurrence, the ROC curve set the threshold at ≤1 for the best prognostic value.

Table 1 Regression analysis. OR:odds ratio CI: confidence interval.

	Simple Regression			Multiple Regression		
Total (3315)	**OR**	**95%CI**	***P***	**OR**	**95%CI**	***P***
CP (n: 1091)	0.88	0.85-0.92	<0.0001	0.94	0.90-0.98	0.007
LB (n: 840)	0.86	0.82-0.90	<0.0001	0.94	0.89-0.98	0.01
≤35 years (n: 1572)						
CP (n:651)	0.88	0.82-0.94	0.0004	0.90	0.84-0.97	0.005
LB (n:547)	0.87	0.81-0.93	0.0004	0.90	0.84-0.97	0.008
36-39 years (n:1049)						
CP (n: 321)	0.97	0.91-1.0	0.40	0.97	0.91-1.04	0.43
LB (n: 227)	0.96	0.88-1.0	0.30	0.96	0.89-1.0	0.34
≥40 years (n: 694)						
CP (n: 119)	0.96	0.88-1.04	0.31	0.94	0.86-1.03	0.19
LB (n: 66)	0.99	0.90-1.10	0.94	0.98	0.88-1.10	0.78

Limitations, reasons for caution: Including the number of transferred embryos can change the results.

Wider implications of the findings: This study highlights the importance of NIF alone in the prognosis of clinical outcomes after IVF/ICSI cycles. Changes in therapy with inclusion of procedures that may enhance the results (e.g., increasing the number of embryos transferred, PGS, time lapse) would be advisable in young patients with ≥2 IF.

Trial registration number: The local ethics committee authorised this study.

P-374 In vitro fertilization (IVF) outcomes after dramatic weight loss linked to bariatric surgery: a case-control study

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Study question: Do women having undergone bariatric surgery achieve similar IVF outcomes as compared to women matched at the first IVF for age and body mass index?

Summary answer: Cumulative live birth rates are comparable, even after adjustment for confounding variables. Smaller weight for gestational age was observed in newborns of bariatric surgery group.

What is known already: Bariatric surgery leads to long term significant weight loss, improves spontaneous fertility, obstetrical and neonatal prognosis but increases incidence of newborn's small weight for gestational age. So far, articles concerning IVF outcomes after bariatric surgery are sparse; they included small cohorts, ranging from 5 to 40 cases, comparing IVF results both before and after surgery, or cases to obese and normal-weight patients. This is the first and the largest study matching bariatric surgery patients undergoing IVF to patients of similar Body Mass Index (BMI) and age and without a history of significant weight loss.

Study design, size, duration: This case-control study was performed from the retrospective analysis of three IVF center databases. Data from 10,287 IVF/ICSI cycles between January 1st, 2012 and December 31st, 2016 were extracted and analyzed, and all patients were followed until the pregnancy issue including newborn's state of health. Inclusion criteria for all cases were women aged 18 to 43 years old with a previous history of bariatric surgery and undergoing their first IVF cycle during this time frame.

Participants/materials, setting, methods: Each case was matched with two following controls of the same IVF centre, of similar BMI (±2 kg/m^2) and age (±2 years) and without a history of significant weight loss. A total of 249 women were included: 83 cases and 166 controls.The main outcome measure was the cumulative live birth rate after the first IVF cycle; secondary outcomes were number of mature oocytes and embryos obtained in the first IVF cycle and neonatal outcomes.

Main results and the role of chance: No significant difference in cumulative live birth rates was found between cases and controls (22.9% vs. 29.5%, respectively, p = 0.339), nor was any significant difference observed in cumulative pregnancy rates (36.1% vs. 38.6% respectively p = 0.817). Average number of mature oocytes (6.9 ± 4.9 vs 7.4 ± 4.9, p = 0.189) and embryos (4.4 ± 4.2 vs. 4.9 ± 3.8, p = 0.239) were similar between both groups. A lower BMI and higher number of good-quality embryos appeared to have a significant positive impact on the occurrence of live birth in both groups. However, multivariate conditional logistic regression analysis showed no significant difference in cumulative live birth rates after adjustment for potential confounding factors.

Additionally, miscarriage rates were found to be 41% in cases and 33% in controls (p = 0.613), and a significantly smaller weight for gestational age was observed in newborns of cases versus controls (2849 g±687 vs 3170 g±863; p = 0.002).

Limitations, reasons for caution: This is the largest study concerning live birth results in IVF bariatric surgery patients and the first to match IVF patients having undergone bariatric surgery to patients of similar BMI and age. Nonetheless, the sample size remains limited and further studies, with prospective inclusion, are needed to confirm these conclusions.

Wider implications of the findings: Bariatric surgery allowed IVF prognosis of patients with previously morbid obesity to be similar to that of comparable age and BMI women. Higher BMI negatively impacts live birth occurrence. Smaller weight for gestational age necessitates investigations concerning impact of surgery types and fresh versus frozen embryo transfers on neonatal outcomes.

Trial registration number: This study was approved by our Institutional Review Board (IRB) and registered in clinicaltrial.gouv under the number NCT02884258

P-375 Pregestational thyrotropin level and reproductive outcome of in vitro fertilisation in healthy euthyroid women

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Study question: Does pregestational thyrotropin (TSH) level above 2.5 mIU/L predict delivery rates in euthyroid healthy women undergoing first in vitro fertilization (IVF)?

Summary answer: In euthyroid women TSH above as compared to below 2.5 mIU/L was associated with lower odds for clinical pregnancy and delivery.

What is known already: Thyroid hormones (TH) are vital for achieving and maintaining pregnancy. Pregnancy inflicts a vast increase in demands for TH. This impact is even greater in the setting of fertility treatment due to ovarian hyperstimulation. Elevated TSH in early pregnancy is associated with increased risk of miscarriage. Current guidelines recommend levothyroxine supplementation in women with elevated TSH undergoing IVF procedures, aiming at TSH 2.5 mIU/L. However, data on impact of pregestational TSH above 2.5 mIU/L in euthyroid women on success of IVF is sparse.

Study design, size, duration: This is a retrospective cohort study of 623 women comparing success of first IVF according to pregestational level of TSH above or below 2.5 mIU/L.

Participants/materials, setting, methods: Women with first referral for fertility treatment at Aarhus University Hospital, Denmark, January 1st 2012 until March 31th 2014 were included. Exclusion criteria were chromosomal abnormalities, comorbidity, prior levothyroxine (LT4) treatment, and lack of TSH measurement. TSH and anti-thyroperoxidase-antibodies (TPOab) were measured as part of infertility work-up. Impact on success of IVF was assessed using logistic regression adjusted for BMI, smoking, age, fertility status, and initiation of LT4. P-values of <0.05 were considered significant.

Main results and the role of chance: Overall live birth rate was 27.0 % (n = 168). 14.5 % (73) miscarried, 24.5 % (59) before week 8 of gestation. 18.3 % (114) had TSH above 2.5 mIU/L. Baseline demographics according to grouping of TSH showed no differences in age, BMI, history of smoking, fertility status or infertility diagnosis. However, women with TSH above 2.5 mIU/L had lower change of clinical pregnancy (21.1 % vs 31.0 %, p = 0.035) and delivery (18.4 % vs 28.9%, p = 0.023), despite comparable chance of conception (22.2% vs 39.9%, p = 0.19). These associations were confirmed in the adjusted analysis with odds ratio for clinical pregnancy 0.49 (95% CI: 0.27-0.90, p = 0.022) and odds ratio for delivery 0.54 (95% CI: 0.29-1.00, p = 0.049), comparing TSH above vs below 2.5 mIU/L. In crude analysis odds for any pregnancy loss was significantly higher among women with TSH above2.5 mIU/L, but not in the adjusted analysis.

TPOab was only available in a subcohort. In subanalysis adding TPOab to the adjusted regression analysis, the adverse impact of TSH on success of IVF remained regarding clinical pregnancy. In line with this finding, odds for early pregnancy loss was higher in women with TSH above 2.5 mIU/L (OR 3.43 (95% CI:1.03-11.40, p = 0.044).

Limitations, reasons for caution: TPOab are associated with increased risk of miscarriage. TPOab were available only in a subcohort. However women with or without available sample were comparable, and a subanalysis adjusted for TPOab displayed similar adverse impact of TSH above 2.5 mIU/L as in our main analysis indicating validity of our findings.

Wider implications of the findings: RCTs show improved IVF outcome when treating pregestational TSH above 4.0 or 4.5 mIU/L with LT4. Our data, though retrospective, suggests adverse IVF outcomes with TSH above 2.5 mIU/L. Future intervention studies should clarify whether additional improvement of reproductive success in IVF is obtainable treating pregestational TSH above 2.5 mIU/L.

Trial registration number: None.

P-376 Double embryo obtaining using the 1st polar body transfer technique: is it a fact or a myth?

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Study question: Could transfer of the patient's 1st polar body (PB) to donor oocyte cytoplasm be used to obtain viable embryos?

Summary answer: The 1st PB transfer technique can be used to double the number of embryos suitable for embryos transfer.

What is known already: Mainly the increase of oocytes number in patients with poor response are conducted in stem cells studies. But the 1st PB transfer technique, which is mainly applied in case of oocyte cytoplasm donation, could be used to double oocyte number for further fertilization.

Study design, size, duration: The study was performed in the Medical Centre IGR from March 2017 to January 2018. It involved 60 oocytes in each group (groups A and B) obtained from 16 patients and 11 donors, respectively. The mean age of oocyte donors and patients was 28.2 ± 2.4 and 40.4 ± 3.9 years, respectively. We evaluated embryos' euploidy and the number of zygotes and viable embryos at 48, 72, 96 and 120 hours after fertilization.

Participants/materials, setting, methods: Oocytes obtained from 16 patients with poor response. Donor oocytes had been pre-enucleated and modified by the transfer of patient's 1stPB with further fertilization. The procedure was carried out using Nikon Ti Eclipse(Japan) inverted microscope, Saturn 3 laser console(UK). Blastocyst biopsy was performed for the preimplantation genetic screening (PGS), samples were diagnosed using Ion S5 by Thermo Fisher Scientific(USA). Statistical analysis was carried out using Shapiro-Wilk test for normality, Chi-square test.

Main results and the role of chance: The number of normal fertilized oocytes in both groups was comparable and reached 46 (76.7%) zygotes in group A and 45 (75.0%) zygotes in group B. Nine oocytes (15.0%) in group A had abnormal fertilization pattern and 5 oocytes (8.3%) degraded after ICSI, whilst 14 oocytes (23.3%) in group B had abnormal fertilization and 1 oocyte (1.7%) degraded after sperm injection. Then embryos developed as follows: in 48 hours after fertilization there were 41 (68,3%) and 49 (81.7%) embryos, in 72 hours - 39 (65.0%) and 47 (78.3%), in 96 hours - 33 (55.0%) and 40 (66.7%), in 120 hours – 27 (45.0%) and 30 (50.0%) embryos, where 16 (59.3%) and 20 (66.7%) blastocysts were of high quality (5AA) in group A and group B, respectively, and there was no statistically significant difference between the groups (p>0.05). Embryos distribution by euploidiy/aneuploidy was as follows: group A –1:1 (12 euploid and 12 aneuploid embryos), group B – 1:2 (8 euploid and 16 aneuploid embryos). Next combinations of embryos' chromosome sets (obtained from patients oocytes and modified donor oocytes) were detected: euploidy/euplody (5 pairs), euploidy/aneuploidy (4 pairs), euploidy/mosaic (2 pairs), euploidy/polyploidy (1 pair), aneuploidy/euploidy (3 pairs), aneuploidy/polyploidy (2 pairs), aneuploidy/aneuploidy (7 pairs).

Limitations, reasons for caution: The 1st PB chromosome set corresponds to the oocyte nucleus and and unequal meiosis-I will result in two aneuploid embryos But the availability of two euploid oocytes obtained by transfer doesn't guarantee the presence of a normal embryo's chromosomal set, since two different spermatozoa are used for fertilization.

Wider implications of the findings: The 1st PB transfer is useful to increase the patient's chances of having a biologically native child and avoiding the oocytes donation. Also the use of this technique makes it possible to detect quantitative chromosomal abnormalities of embryos introduced by spermatozoa, in particular polyploidy and mosaicism, without any additional analyses.

Trial registration number: Not applicable.

P-377 Predictive factors for pregnancy after controlled ovarian stimulation and intrauterine insemination: A retrospective analysis of 4146 cycles

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Study question: What are the predictive factors for clinical pregnancy (CP) and live birth (LB) in intrauterine insemination (IUI) cycles following controlled ovarian stimulation (COS)?

Summary answer: Age <38, anovulatory infertility, ≥2 preovulatory follicles, total motile sperm count ≥5 million, and IUI cycle rank ≤3 are associated with a good outcome.

What is known already: IUI is a widely used infertility treatment in various indications, and is frequently offered first-line because it is simple, non-invasive, has a low cost and minimal risk of complications. However, its overall efficiency remains questioned, whether in natural cycles or following COS. Despite being

recommended by many international societies, it is sometimes overlooked in favor of *in vitro* fertilization (IVF), which offers higher success rates, but at a considerably higher cost. Studies have shown that many factors can affect outcomes, such as age and cause of infertility, but the impact of many other variables remains unknown.

Study design, size, duration: A retrospective unicentric study at the department of reproductive medicine of Institut Mutualiste Montsouris, a university affiliated private IVF center in Paris, France, between January 2009 (date when patient data became computerized) and December 2016.

Participants/materials, setting, methods: Patients aged 18 to 43 years who had an IUI following COS with gonadotropins were included. Donor cycles were excluded. Cycles were monitored by transvaginal ultrasounds and hormone assays. Ovulation was triggered with hCG when 1-3 follicles reached >16 mm with an endometrial thickness >7 mm, and insemination performed 36 h later. When serum LH was >10 IU/L, IUI was performed the day after hCG. Statistical analysis was performed using Chi square and logistic regression.

Main results and the role of chance: 4146 cycles (1312 couples) were included. Mean age was 34.7 ± 4 years. CP rate (CPR) and LB rate (LBR) were 11.5% and 8.1%, respectively. CPR/cycle was significantly higher in women <38 years compared to ≥38 years (12.6% versus 8.3%, p = 0.0001), in cycles with ≥2 preovulatory follicles (>16 mm) compared to 1 follicle (14.4% versus 11%, p = 0.01), and when TMSC was ≥5 million compared to <5 million (12.4% versus 7.7%, p = 0.0002). LBR/couple was 39% for anovulatory infertility (WHO group II), significantly higher (p<0.05) than unexplained infertility (28.6%), mixed (23.4%), male factor (20.1%), unilateral tubal (14.2%), low ovarian reserve (13.2%), and endometriosis (stage I and II) (11.1%). However, it was comparable to cervical factor infertility (29.3%, p = 0.11). Multivariate analysis showed the following factors were associated with CP: Cycle rank ≤3 (Odds ratio (OR) = 1.5, 95% CI: 1.2-1.9, p<0.001), age <38 years (OR = 1.5, 95% CI: 1.2-2, p<0.001), ≥2 preovulatory follicles (OR = 1.4, 95% CI: 1.1-1.8, p = 0.004), TMSC ≥5 million (OR = 1.8, 95% CI: 1.3-2.4, p<0.001). Compared to anovulatory infertility, endometriosis, low ovarian reserve, unilateral tubal, and male factor were associated with a lower CPR (OR = 0.3, 95% CI: 0.1-0.5, p<0.001; OR = 0.4, 95% CI: 0.3-0.7, p<0.001; OR = 0.5 95% CI: 0.3-0.9, p = 0.01; OR = 0.6, 95% CI: 0.4-0.8, p = 0.002 respectively).

Limitations, reasons for caution: The main limitation of our study is the unicentric retrospective design, although the large number of cycles and the fact that only COS with gonadotropins was included make the findings interesting. Moreover, a cost analysis would have been relevant in order to compare the efficacy of IUI and IVF.

Wider implications of the findings: Our study confirms that IUI can be an efficient treatment in selected indications. Young patients with anovulatory infertility seem to be the ideal candidates, with a 39% LBR per couple. These data need to be validated in prospective trials, but can be used to council patients on the treatment choice.

Trial registration number: Not applicable.

P-378 In vivo conceived embryos recovered by nonsurgical uterine lavage: Preliminary experience

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Study question: After controlled ovarian hyperstimulation (COH) and intrauterine insemination (IUI), can embryos be recovered by uterine lavage using an automated programmable lavage catheter?

Summary answer: In a series of 69 cycles of superovulation followed by IUI, uterine lavage recovered 48 embryos in 25 (36.2%) of cycles.

What is known already: Early work performed by Buster and colleagues in 1983 recovered embryos from the uterus by lavage in natural cycles. Prior attempts performing lavage after COH and IUI failed to recover embryos consistently and resulted in unintended pregnancies in the ovum donors. The current uterine lavage catheter prior to its use in this study underwent extensive bench and in vivo testing for fluid dynamics and the ability to recover known numbers of implanted artificial and human tissue (discarded oocytes and in vitro conceived embryos). Subjects gave their consent for any embryo recovered to be donated to research.

Study design, size, duration: We performed a single site, prospective, feasibility study in 54 women to evaluate the safety and efficacy of a new uterine lavage system (Previvo Genetics, Inc., San Carlos, CA). Subjects gave their written informed consent for the research protocol approved by Western Institutional Review Board. All lavages were performed in Punta Mita, Mexico during August 2017 to January 2018 by a single investigator. Subjects were followed for 30-days post-lavage to monitor for complications.

Participants/materials, setting, methods: 54 women were pretreated with oral contraceptives and stimulated with gonadotropins for 69 COH cycles. Ovulation was triggered with either 5,000 IU of human chorionic gonadotropin (hCG) or leuprolide acetate 4 mg and 2,500 IU of hCG, followed by IUI of donor sperm 36 hours after trigger. Uterine lavage occurred 4-6 days after the IUI. After lavage, uterine curettage performed and a GnRH antagonist injection (0.25 mg/day) was given for 3 days.

Main results and the role of chance: A total of 69 lavages were performed in 54 women with a mean age of 25.7 years (range 19-38) and 44% were nulliparous. Mean subject BMI was 25.4 kg/m^2 (range 17.8-36.7). In the COH cycles, subjects were mildly stimulated an average of 9.2 days of gonadotropins (mean rFSH 733 IU total dose, mean hMG 722 IU total dose). Ovulation trigger was given when there was at least 2 dominant follicles ≥18 mm. After IUI, uterine lavage recovered embryos in 25 (36.2%) cycles (range 0-4/cycle). A total of 48 embryos were recovered: 18 (37%) multi-cell embryos, 7 (15%) morulas and 23 blastocysts (48%). Six (12%) morulas progressed to blastocysts, for a total of 29 blastocysts (60%). Blastocysts were of high quality, 79% (23/29) were 3BB or higher. All embryos regardless of stage were biopsied, vitrified and cryopreserved. All subjects menstruated after the GnRH antagonist injections. Eight cycles (11.6%) had detectable hCG levels (>2 mIU/mL) after their menses, that regressed spontaneously in two, declined after curettage in one and persisted after curettage in five. Persistent hCG levels (highest level 2,652 mIU/mL) were treated with one dose of methotrexate (50 mg/m^2) in 3 cycles and two doses in 2 cycles.

Limitations, reasons for caution: Although sample size is small, there were progressive increases in embryo recovery due to improvements in lavage technique. It is difficult, however, to determine the efficiency of each lavage, because we are unable to verify the number of oocytes ovulated and number of embryos available for recovery.

Wider implications of the findings: Uterine lavage offers a nonsurgical minimally invasive approach to recovering embryos in patients at risk for a multiple gestation after IUI or desire preimplantation genetic screening or diagnosis for genetic conditions without IVF.

Trial registration number: Trial registration number is pending.

P-379 Frozen-thawed embryos generated from a successful fresh IVF cycles have higher potential for favorable outcome than frozen-thawed embryos obtained from unsuccessful fresh cycles

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Study question: Whether a successful fresh in-vitro fertilization (IVF) cycle correlates with better outcomes of frozen-thawed embryo transfers (FT-ET) from same ovum pick-up.

Summary answer: Frozen-thawed embryos generated from successful fresh embryo transfer cycles have a higher potential to implant when compared to frozen-thawed embryos obtained from unsuccessful fresh cycles.

What is known already: Many variables, including woman's age, embryo quality and endometrial receptivity, influence the success rate of either fresh embryo transfer (ET) or FT-ET cycles. Patients frequently ask whether previous successful fresh IVF attempt is a positive prognostic factor for their FT-ET.

Study design, size, duration: A retrospective cohort study comprised of 2218 patients that underwent IVF treatment in a single IVF unit between 1997 and 2017.

Participants/materials, setting, methods: Patients who underwent fresh ET and at least one consecutive FT-ET cycle at Hadassah Medical center IVF unit were included in the study. Comparison of FT-ET cycles details and outcomes were assessed in relation to the outcome of the preceding fresh ET cycles. Statistical analyses were performed to evaluate the correlation between the outcome of fresh ET attempt and its consecutive FT-ET cycle.

Main results and the role of chance: Six hundred and seven out of 2218 patients achieved clinical pregnancies in their fresh ET (27.4%). No difference was found in the number of retrieved oocytes, maturity rates, fertilization rates and embryo quality distribution between fresh cycles resulting in clinical pregnancy and failed fresh cycles. When the outcome of the consecutive frozen cycles were compared between patients with successful and unsuccessful fresh ET cycles, a significant higher pregnancy rates were observed, for either per patient or per treatment cycle (37.2% vs. 32.1% and 26.6% vs. 21.8%; $p = 0.01$ and $p = 0.026$, respectively). This was substantiated by the McNemar test which showed a significant association between a successful fresh cycle and positive outcome in the consecutive FT-ET cycle ($P<0.001$).

Limitations, reasons for caution: The retrospective design of our study makes it more prone to bias. Assessment is performed for a long period of time where culture condition and laboratory work might be differing between patients and for each particular patient.

Wider implications of the findings: A successful fresh IVF-ET attempt that resulting in a clinical pregnancy can serve as a positive prognostic factor for the subsequent FT-ET cycle. Among other parameters, this finding provides an important information for the clinician when counseling patients before carrying out their frozen thawed ET cycle.

Trial registration number: Not applicable.

P-380 Results from a novel transnational fresh egg donation program based on frozen sperm and embryos shipping

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Study question: To investigate the clinical efficacy of a novel fresh oocyte donation program based on transportation of semen and embryos between two countries.

Summary answer: This novel fresh-egg donation program based on international transportation of frozen embryos is highly effective. When blastocysts are available, the success rate is very high.

What is known already: Despite several advantages of oocyte vitrification in donation cycles, recent large cohort studies suggest that oocyte vitrification may lead to lower live birth rates compared with fresh gametes. On the contrary, increasing evidence suggest comparable outcomes with frozen compared to fresh embryo transfer. Hence, a new strategy could be to treat recipient in countries with limited availability of oocyte donors: the shipping of frozen sperm of the partner to the center where the donor will undergo pick up, insemination of fresh oocytes, the vitrification and shipment of the resulting embryos and to the country of origin.

Study design, size, duration: A retrospective cohort study of donor oocyte IVF cycles with elective vitrified-thawed embryo transfer and shipping from December 2015 to July 2017 between two IVF clinics in different European countries. All donors were stimulated with a GnRH antagonist protocol with GnRH agonist trigger; oocytes were inseminated by ICSI with frozen/thawed semen. Recipients' endometrium was prepared with a standard dose of 6 mg estradiol valerate and 800 mg vaginal progesterone in the last days before the transfer.

Participants/materials, setting, methods: A total of 630 patients were available for statistical analysis. In 11% of cases the semen used was from a donor, in the remaining 89% it was from the male partner and was frozen and transported to the oocyte donation clinic. Resulting embryos were vitrified between with an open system on day +2 or on +5, and shipped back to the original clinic for transfer.

Main results and the role of chance: The main reasons for treatment were female age (40%), reduced ovarian reserve or menopause (25%) and previous IVF failures (21%). The mean (±SD) age of the patients was 42.3 ± 4.2 years. The oocytes were obtained from healthy donors aged 26.2 ± 4.4 years. The number of mature oocytes assigned to each recipient was 6.8 ± 1. The mean number of 2PN and viable frozen embryos per patient was 4.9 ± 1.56 and 4.17 ± 1.5, respectively. For the 630 patients, a total of 1066 embryos were warmed; the survival rate was 98.5% (n = 1051). After the first embryo transfer, the clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR) were 43 and 31.9% respectively. The embryos were transferred at the cleavage stage (day 2 or 3) in 476 patients (75.5%) and the OPR was 30.6%. The single and twin pregnancy rates were 73 and 27%, respectively. Vitrified blastocysts were available for 154 patients and the CPR and OPR were 44 and 37%, respectively. The median number of days between donor oocyte pick up and the frozen embryo transfer was 63.

Limitations, reasons for caution: Limitations include the retrospective design, sample size and data from a single institution.

Wider implications of the findings: This study reports an oocyte donation program based on transfer of frozen embryos transported between countries. This system eliminates the need to align the timing of donor oocyte retrieval with the embryo transfer to recipients, while using fresh oocytes for treatment.

Trial registration number: Not Applicable.

P-381 The effect of the oocyte donor's and recipient's ages the on cumulative live birth rate: a population-based cohort study

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Study question: What is the impact of the donor's and recipient's ages on the cumulative live birth rate (CLBR) in oocyte donation cycles?

Summary answer: Recipients using oocytes from donors aged 35 years and older had a significantly lower CLBR compared to recipients using oocytes from donors aged <35 years.

What is known already: Cycle-based statistics show that the donor's age is a major determinant of success in oocyte donation/recipient programmes and that the age of the oocyte recipient has less impact on pregnancy and live birth rates. There is no existing evidence about the impact of the donor's and the recipient's ages on CLBR in oocyte donation cycles.

Study design, size, duration: A population-based retrospective cohort study used data from the Victorian Assisted Reproductive Treatment Authority in Victoria, Australia. This study included 1,490 women commencing assisted reproductive technology using donated oocytes between 2009 and 2016. Demographic characteristics, treatment type and resulting pregnancy and birth outcomes were recorded for the stimulated cycle and associated thaw cycles until 30 June 2016, or until a live birth was achieved, or until all embryos from the stimulated cycle had been used.

Participants/materials, setting, methods: Women using donated oocytes in Victoria, Australia from 2009-2016 were included. The association between the donor's/recipient's ages and CLBR was measured by adjusted hazard ratio (AHR) in multivariate Cox regression. The following covariates were included: cause of infertility, parity and number of embryos transferred (1 or 2). Donor

age was grouped as <30, 30-34, 35–37, 38-40, and 41+ years and recipient age as <35, 35–37, 38–40, 41-42, 43-44 and 45+ years.

Main results and the role of chance: The mean age of the oocyte donors was 33.7 years (range 21 to 45 years) with 64.9% under 35 years. The mean age of the oocyte recipients was 41.4 years (range 19 to 45 years) with 60% of the oocyte recipients aged under 40 years and 25.4% aged 45+ years.

There was a significant relationship between donor's age and CLBR. The CLBR for recipients with donors aged <30 years and 30-34 years was 44.7% and 43.3% respectively. This decreased to 33.6% in donors aged 35-37 years, 22.6% in donors aged 38–40 years and 5.1% in donors aged 41+ years. Compared with recipients with donors aged <30 years, recipients with donors aged 38-40 years had 22.1% less chance to achieve a live birth (AHR 0.60, 95% CI 0.43–0.86) and recipients with donors aged 41+ years had 39.6% less chance to achieve a live birth (AHR 0.14, 0.04–0.44). The multivariate analysis showed no significant differences in the success by recipient's age. Advanced male age was not associated with a decrease in the likelihood of live birth.

Limitations, reasons for caution: The use of data from one state in Australia limits the generalizability of the results. Number of cycles was used as the time variable in Cox regression, which does not account for the time difference between cycles among recipients.

Wider implications of the findings: These estimates can be used when counselling women about their likelihood of having a baby using donated oocytes, and to inform public policy as currently there are no national Australian recommendations on the age of women who donate their oocytes.

Trial registration number: Not applicable.

P-382 Fully automated assay assessment of intra- and inter-cycle variation for Anti-Müllerian hormone during the natural cycle

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Study question: Does Anti-Müllerian Hormone (AMH) have important intra- and inter-cyclic variations during the natural cycle when a fully automated assay is used for the sample analysis?

Summary answer: With the use of a fully automated assay, AMH shows significant intra- and intercycle variations during the natural cycle, not caused by analytical variability.

What is known already: AMH has emerged as one important clinical marker for ovarian reserve. Fully automated assays reduce analytical variability and enables more accurate evaluation of biological variations. The hormone shows strong correlation to the number of antral follicles and provides an important baseline assessment for individualizing of the therapeutic strategy in assisted reproductive techniques (ART). AMH has been considered a reliable test to be measured any day of the cycle without substantial variation. However, recent data have pointed towards important inter- and intra-cycle fluctuations and questioned whether a single AMH measurement is sufficient for decision-making in ART.

Study design, size, duration: Prospective study including 22 volunteers from April to December 2017. Blood samples for AMH, FSH, LH, estradiol, and progesterone were obtained by venous puncture during the natural cycle on day 2/3, day 10, day of LH surge (LH surge was defined as LH concentration rise by 180% above the latest serum value), luteal phase (confirmed by elevated progesterone levels 8 days after LH surge), and day 2/3 of the subsequent bleeding.

Participants/materials, setting, methods: Healthy volunteers between 18–37 years of age were included, with regular menstrual cycles and a BMI between 18-28 kg/m2. Exclusion criteria: Intake of hormonal contraceptives for two previous months, pregnancy, breastfeeding, previous conditions with possible impact on the ovarian reserve (ovarian surgery, chemotherapy, radiation of the pelvis, …). Blood for AMH-evaluation was stored at -20°C. For batch analysis, two kits of Elecsys® AMH automated (Roche for Cobas 601 platform®) were used.

Main results and the role of chance: A total of 99 samples from 22 women with a median age of 30,7 ± 4,11 years and a BMI of 23,2 ± 3,63 were analysed. A substantial longitudinal fluctuation in AMH levels was found, expressed as coefficient of variation (CV) intra-cycle of 0,207, calculated for all individual readings per cycle day. Clinically, AMH value varied 20,7% throughout the cycle from the first serum analysis obtained at the beginning of the cycle with no clear pattern of fluctuation. Serum AMH levels were significantly altered by BMI and age ($p<0,001$), both negative correlated with AMH values. A positive correlation between LH and AMH concentrations was found ($p = 0,01$), but not to other hormones. Absolute intra-individual inter-cyclic variability of AMH (cycle-day 2-3 in two consecutive cycles) was 0,75 ng/mL (range: 0,03 ng/mL – 2,81 ng/mL) and inter-cycle CV was 0,28 (Confidence interval: 0,16-0,39; $p<0,0001$). No differences were found comparing variability of AMH between the assays used. The observed AMH fluctuations across the cycle are noticeably higher than expected from the assay imprecision and therefore seem more likely to be caused by biologic variability.

Limitations, reasons for caution: This study reports limited number of study subjects. However, an average of 4.5 blood-samples study subject were assayed in one run, eliminating inter/intra-assay bias in the normal cyclic women included. Further studies will have to investigate, whether AMH-fluctuations are also present in other subgroups of population.

Wider implications of the findings: The large intercycle and intracycle AMH fluctuations in the same woman during her natural cycle using an automated assay keeps the question open on the best time to measure AMH for decision-making in daily practice. Further RCTs evaluating AMH dynamics and its correlation with treatment outcomes should be conducted.

Trial registration number: This trial has been registered for clinical.trials.gov: NCT03106272.

P-383 Can "patient-friendly" salivary hormone measurements replace serum monitoring of IVF patients? An European multi-center assessment

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Study question: Can a saliva based Estradiol (E2) assay replace the use of blood monitoring during a controlled ovarian stimulation (COS) cycle for IVF/ICSI?

Summary answer: Salivary E2 based hormone testing provides an equivalent alternative to serum based assessment and may become the preferred method of COS monitoring in the future.

What is known already: Salivary diagnostic testing is emerging as a less invasive, inexpensive alternative to serum analyte measurements with proven diagnostic accuracy in clinical settings. This study aimed to determine the performance of a salivary hormone competitive immunoassay for monitoring patient E2 levels during monitored cycles for infertility treatment. Such treatments encompass: Undergoing monitored infertility treatment employing controlled ovarian stimulation (COS) with oral agents or gonadotropins for Ovulation induction/ IUI and COS for IVF/ICSI.

Study design, size, duration: The prospective study was performed at 4 different European clinics (Italy, Spain, Belgium, France), between 9/2016-11/2017, whereby at each venipuncture appointment, subjects also collected a saliva sample via passive drool according to the assay specifications. In total 152,

214, 184 and 104 samples were collected from 59, 122, 40 and 40 patients respectively. Between 1 and 6 samples were collected from each individual patient. 122 patients collected ≥3 samples in an individual cycle.

Participants/materials, setting, methods: Saliva samples were coded, de-identified and stored frozen. They were then run blinded in a separate facility. Saliva and serum correlation were calculated using the matched mean saliva and serum assay results for all E2 data points collected. Pearson product moment correlations of the serum and saliva were conducted using the natural log transformed data. Data was assessed per clinic and overall taking into account the different serum E2 testing platforms used per clinic.

Main results and the role of chance: One to six salivary E2 samples were analyzed for each patient. In the four clinics the correlation coefficients of Saliva E2 to serum values were Italy: r = 0.69, p < 0.001; France: r = 0.53, p <0.001; Belgium: r = 0.75, p < 0.001; Spain: r = 0.79, p <0.001. Mean (±SD) serum E2 values (pg/ml) differed between clinics reflecting different demographics and approaches to patient stimulation [Italy: 803 ± 760, France: 862 ± 892; Belgium: 1174 ± 933; Spain: 1276 ± 1026. The respective mean (±SD) saliva values (pg/ml) were 17 ± 9, 26 ± 15, 24 ± 14 and 24 ± 14. When examining individual patients who collected ≥3 samples in one cycle, more than 75% of patients showed an individual within cycle correlation of >0.7 and 62% a correlation of >0.9. Patients with discolored saliva samples generally showed poor correlations, indicating that they failed to collect according to protocol. Patient surveys have also shown that saliva based hormone testing is associated with improved patient satisfaction and decreased stress.

Limitations, reasons for caution: The results could be limited as the study was conducted in an unselected population of all patients performing IVF. A further limitation was that although patients were informed on collection protocols it was evident that some patient saliva samples may have been sub optimally collected which influences the assay outcome.

Wider implications of the findings: Rapid, salivary E2 based hormone testing provides an equivalent alternative to serum based assessment. The ease of saliva sampling allows a reduction in treatment burden, improved patient satisfaction and decreased stress. Saliva based hormone tests may become the preferred method of hormone monitoring for fertility treatments in the future.

Trial registration number: NCT03162809.

P-384 Premature progesterone elevation is associated with decreased uterine artery blood flow and clinical pregnancy rate

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Study question: Could premature progesterone elevation (PE) affect the uterine artery (UA) perfusion and IVF outcome?

Summary answer: PE on the day of hCG is associated with decreased UA blood flow and clinical pregnancy rate (CPR) in infertile patients undergoing IVF.

What is known already: Controlled ovarian stimulation (COS) is correlated with premature PE and it is generally believed to have an adverse effect on pregnancy outcome in infertile patients undergoing IVF. Although many possible mechanisms of decreased pregnancy rate by premature PE have been discussed, the causes has not been completely elucidated. Moreover, the study on the effect of premature PE on UA blood perfusion has not been reported.

Study design, size, duration: This retrospective study included 108 infertile patients who had their blood taken for measurement of serum progesterone on the day of hCG in stimulated IVF cycles between February 2015 and January 2017.

Participants/materials, setting, methods: The association of serum progesterone levels (group 1: ≤ 1.5 ng/ml, group 2:1.5-2.0 ng/ml, group 3: > 2.0 ng/ml) measured on the day of hCG with IVF outcome and resistance index (RI) and pulsatility index (PI) of UA on the day of embryo transfer (ET) was evaluated in 108 cycles corresponding to 108 patients who underwent IVF in COS cycles using GnRH antagonist protocol.

Main results and the role of chance: Patients' characteristics were comparable among three groups. Total days of gonadotropins used for COS was significantly longer in group 1 than in group 2 or 3 (*P*=.012 vs group2, *P*=.040 vs group 3, respectively). The number of oocytes retrieved was significantly higher in group 3 than in group 1 (*P*=.029). However, there were no differences among three groups with respect to the numbers of mature oocytes, fertilized oocytes and good quality embryos. RI of UA was significantly higher in group 3 than in group 1 or 2 (*P*<.001, *P*<.001). PI of UA was also significantly higher in group 3 than in group 1 or 2 (*P*<.001, *P*<.001). CPR seemed to be lower in group 3, but this difference did not attain the statistical significance (*P*=.076 between group 1 and 3, *P*=.087 between group 2 and 3). When CPR was compared between the two groups with each progesterone levels ≤ 2.0 ng/ml and > 2.0 ng/ml, CPR was significantly lower in the group with PE (*P*=.034).

Limitations, reasons for caution: This study has a limitation to evaluate the impact of premature PE on UA blood flow and IVF outcome due to a retrospective nature and small number of sample available. The effect of premature PE should be investigated according to the different responders to COS in a bigger patient pool.

Wider implications of the findings: Premature PE (especially > 2.0 ng/ml) can result in a decrease of UA blood flow and it can be one of contributing factors of decreased CPR by premature PE in IVF cycles.

Trial registration number: None.

P-385 Does the effect of body mass index on in vitro fertilization with GnRH-antagonist protocol in polycycstic ovary syndrome vary with the level of androgen?

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Study question: Does the effect of body mass index on in vitro fertilization with GnRH-antagonist protocol in polycycstic ovary syndrome vary with the level of androgen?

Summary answer: Economic benefits were achieved in hyperandrogenism-PCOS patients with less cost and more available embryos. Overweight and hyperandrogenism both have significantly negative effects on pregnancy outcomes.

What is known already: Endocrine disturbances are complicated in polycycstic ovary syndrome (PCOS) patients. hyperandrogenism (HA), and obesity interact with each other and promote PCOS progression. However, few studies to date have investigated body mass index (BMI) and androgen level together. Moreover, there are limited literatures that investigate the clinical phenotype of PCOS patients that can benefit most from the GnRH-antagonist protocol during in vitro fertilization (IVF). Therefore, the present study was designed to evaluate whether the effect of BMI in PCOS varies with the level of androgen on IVF with GnRH-antagonist protocol.

Study design, size, duration: A total of 583 infertile women between 20 and 35 years of age who underwent IVF using the conventional GnRH antagonist protocol at a single center between January 1, 2013, and December 31, 2015, were included in this retrospective cohort study.

Participants/materials, setting, methods: Patients were divided into 4 groups: BMI<25 kg/m^2 with normal androgen level (group 1, n = 218), BMI<25 kg/m^2 with hyperandrogenism (group 2, n = 152), BMI≥25 kg/m^2 with normal androgen level (group 3, n = 117), BMI≥25 kg/m^2 with hyperandrogenism (group 4, n = 96).

Main results and the role of chance: Number of retrieved oocytes was significantly higher in lean PCOS patients compared with overweight PCOS patients. Available embryos were significantly higher whereas Gn stimulation days and total Gn dosage were significantly lower in the HA PCOS group, compared with non-HA PCOS patients. Clinical pregnancy rate was of no significant difference among four groups. Live birth rates in overweight PCOS groups were significantly lower when compared with group 1 (23.9%, 28.4% vs. 42.5%, P<*0.05*). Abortion rate in group 4 was significantly higher than group 1 (45.2% vs. 14.5%, P<*0.05*). Logistic regression analysis revealed that BMI and androgen level both acted as significant influence factors for abortion rate.

Limitations, reasons for caution: One major limitation of our study is the retrospective design. hyperandrogenism, obesity, and hyperinsulinemia are the mainly endocrine disturbances in PCOS patients. Prospective studies should

investigate the effects of hyperinsulinemia and BMIs on IVF outcomes in patients undergoing the GnRH-antagonist protocol to determine their impact on HA.

Wider implications of the findings: Economic benefits were achieved in HA-PCOS patients with less cost and more available embryos. Due to the high abortion rate and low live birth rate, a freeze-all approach might be a preferable option for HA-PCOS patients so as to create a buffer for reducing androgen levels before transferring freeze-thawed embryos.

Trial registration number: None.

P-386 Excessive intrauterine interventions negatively affect in vitro fertilization (IVF) outcomes in women with repeated IVF failure

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Study question: Does the number of performed intrauterine interventions have an impact on IVF outcomes in women with repeated IVF failure?

Summary answer: The previous history of an excessive number of intrauterine interventions appears to have an adverse effect on IVF outcomes in women with repeated IVF failure.

What is known already: The necessity for comprehensive assessment of endometrial morphology and function in women with repeated IVF failure along with the lack of the non-invasive assessment methods oblige us to perform an invasive intrauterine procedure to get endometrial samples. However, even the minimally invasive intrauterine procedures carry a slight risk of infection and can cause destruction of the endometrium right through to its basal layer. The damaged endometrial basal layer can be responsible for residual fertility impairing changes. However, there is still a lack of systematic analysis to assess the number of intrauterine interventions as a risk factor for IVF outcomes.

Study design, size, duration: A retrospective observational cohort study includes 131 women with previously repeated IVF failure undergoing fresh IVF or cryo cycle in a single University-affiliated infertility clinic. The study was conducted from September 2011 to February 2014.

Participants/materials, setting, methods: The female patients with repeated implantation failure (after at least two previous attempts of embryo transfer) under the age of 40 years with two or more high-quality embryos were enrolled for the study. The history of previously performed intrauterine interventions (both diagnostic and curative, not only a uterine curettage but also minimally invasive procedures) was taken into account.

Main results and the role of chance: On average each woman included in the study had 5,32 ± 0,23 intrauterine interventions (1,85 ± 0,10 uterine curettage procedures and 4,29 ± 0,21 minimally invasive procedures). The number of performed uterine curettage procedures didn't correlate to the IVF outcome (R = 0,031, p = 0,76), while there was a moderate, but significant negative correlation between the number of minimally invasive intrauterine procedures and the implantation rate (R=−0,21, p = 0,013). A logistic regression analysis demonstrated that more than three previously performed intrauterine procedures in women with repeated IVF failure were associated with subsequent implantation failure (OR 2,20; 95%CI 1,06-4,56).

Limitations, reasons for caution: In the retrospective study not all pertinent risk factors could be identified and subsequently recorded. So only association, and not causation, can be inferred from the results. These data for the risk factor analysis were received just from one center. It should be validated using the dataset from other centers.

Wider implications of the findings: These data may provide information for the development of an effective strategy of preconceiving care in women with repeated implantation failure with reasonable rejection of excessive uninformative ineffective intrauterine procedures. The data also could be considered for explanatory and prognostic purposes.

Trial registration number: Not applicable.

P-387 The correlation between oocyte diameter and blastocyst quality

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Study question: Is there an association between the diameter of the oocyte and the quality of its corresponding blastocyst?

Summary answer: When comparing the ratios of oocyte diameters, the lower the ratio the higher the chance of developing to better quality blastocyst.

What is known already: The reproductive capacity of females decreases significantly in the fourth decade which is directly correlated to an age-related decrease in oocyte quality and quantity. The fact that the live birth rates from oocyte donation in older women is consistent suggests that oocyte quality is the major factor responsible for infertility. Decreased substrate for ATP production and increased mitochondrial deoxynucleic acid (mtDNA) mutations might be involved in increased loss and decreased quality of ovarian follicles in older women. An association between low mitochondrial DNA copy and the ability of the oocyte to become fertilized has been described.

Study design, size, duration: It is a retrospective study conducted at Trio Fertility Center in Toronto, Ontario, Canada affiliated with University of Toronto. We collected all the data from the IVF cycles conducted in 2015 in which an embryoscope was used to visualize the progression of the embryos.

Participants/materials, setting, methods: We retrospectively analyzed the cycles of 470 women where the embryoscope was used which gave us a total of 1914 embryos. The embryoscope's diameter function was used to measure the mature oocytes' (M IIs) diameter at the stage where the first polar body is visible. Two measurements were taken for each oocyte and the average diameter as well as the ratio were calculated.

Main results and the role of chance: We didn't find any correlation between the diameter of the oocyte and the development to a top quality embryo. The diameter of oocytes that developed to a top quality blastocyst compared to the diameter of the oocytes that did not develop to the blastocyst stage was similar (112.3 vs. 112.5 micro)

We did find a correlation between the ratio of the oocytes' two diameters and the development to a top quality blastocyst. The oocytes with a ratio below 1.1 had higher probability to develop to a top quality blastocyst compared to oocytes with ratio above 1.2 (15% vs 5% p <0.001).The probability of oocytes to arrest prior to the blastocyst stage was increased in the group of oocytes with a ratio above 1.2 compared to the oocytes with the ratio below 1.1 (80% vs. 66% p< 0.01)

Limitations, reasons for caution: None.

Wider implications of the findings: The study shows that as the ratio of the oocyte diameters increases, the chances of it developing to a good quality embryo decreases. In other words, the lower the ratio, meaning the more spherical the shape of the oocyte the more likely it will develop to a good quality blast.

Trial registration number: Not applicable.

P-388 Age stratified Anti-Müllerian Hormone (AMH) reference range evaluation in polycystic ovary syndrome women at reproductive age using an automated AMH assay

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Study question: Do AMH levels differ significantly between age groups in PCOS women?

Summary answer: The AMH levels in PCOS women are substantially higher compared to age-matched healthy controls and decrease significantly with increased age.

What is known already: AMH has been proposed as a surrogate test for follicular count in the diagnosis of PCOS. Although there has been interest to define a universally agreed threshold of serum AMH in PCOS women, studies have shown AMH levels and age have a negative correlation in PCOS women. The age stratified AMH reference ranges for PCOS women at reproductive age has not been determined using the automated AMH assays.

Study design, size, duration: This study was a multi-center, prospective, cross-sectional study of 146 PCOS women enrolled from March 2016 to August 2016. The study used four geographically diverse sites in the United States.

Participants/materials, setting, methods: The study included PCOS women aged 18–45 years who met the Rotterdam criteria. The age stratified AMH reference ranges were established, and the difference of AMH distributions between age groups were further analyzed using the Kruskal-Wallis test. A comparison of PCOS reference ranges was made to age-matched healthy adult females. Serum samples were measured at a separate, single site using the Beckman Coulter Access 2 immunoassay system and the Access AMH assay.

Main results and the role of chance: Age stratified AMH reference ranges were established in 146 PCOS women with AMH levels ranging from 0.03 to 34.6 ng/mL. Age was categorized as 18-25 (n = 16), 26-30 (n = 51), 31-35 (n-41), 36-40 (n = 23), and 41-45 (n = 15) years. Median AMH levels categorized by age for PCOS women demonstrated a decreasing trend (7.11, 7.35, 6.77, 3.95 and 1.63 ng/mL, respectively) with increased age, but were statistically significantly higher than apparently healthy age-matched adult women. Overall, median AMH level for PCOS women was 3 times higher than apparently healthy adult women with a statistical significance (6.02 vs. 1.83 ng/mL, respectively, p-value<0.0001). Kruskal-Wallis test showed substantial difference in AMH distributions between the age groups (p-value = 0.0022) in PCOS women, and the Pearson's correlation analysis confirmed a negative relationship between AMH levels and age (r=-0.229, 95%CI=-0.379 – -0.069, p-value = 0.0054).

Limitations, reasons for caution: The small sample size represents a limitation.

Wider implications of the findings: To our knowledge, this is the first study that established the age stratified AMH reference ranges using the automated AMH assay in PCOS women. AMH distributions in PCOS women differ significantly between age groups, questioning the use of one universal AMH threshold for PCOS women at all ages.

Trial registration number: Not Applicable.

P-389 Mitochondrial DNA copy number in peripheral blood: a potential non invasive biomarker for female subfertility

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Study question: Does the mitochondrial DNA copy number (mtDNAcn) in peripheral blood have the potential to serve as a non invasive biomarker for female subfertility?

Summary answer: A mtDNA peripheral blood level below 105 mtDNAcn resulted associated with a five-folds increased risk of subfertility, providing a possible way of predicting this condition.

What is known already: Low mtDNA content in oocytes and in cumulus cells is a significant indicator of poor oocyte quality and may prevent the oocyte from completing the process of fertilization and embryo development. Furthermore, and of utmost relevance, is the recent evidence showing a correlation between mtDNA content in cumulus cells and mtDNAcn in peripheral blood cells.

Study design, size, duration: This is a nested case-control study drawn from a prospective cohort of pregnant women referred for routine first trimester screening for aneuploidies (from 11+0 to 12+6 weeks of gestation) between January 2012 and March 2013 at the "Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico" of Milan, Italy.

Participants/materials, setting, methods: Cases were subfertile women who attempted to become pregnant for 12–24 months. Controls were the two subsequently age-matched women who became pregnant in less than one year. Blood samples were obtained from all participating women. MtDNA was quantified using real time PCR and normalized to nuclear DNA.

Main results and the role of chance: One hundred and four subfertile women and 208 matched fertile controls were selected. The median (interquartile range) mtDNAcn was 95 (73-124) and 145 (106-198), respectively (p<0.001). The area under the receiver operating characteristic (ROC) curve was 0.73 (95% Confidence Interval-CI: 0.67-0.79) (p<0.001). The Youden index was 105 mtDNAcn. The crude odds ratio for subfertility in women with mtDNAcn below this threshold was 5.72 (95%CI: 3.43-9.55). We repeated these analyses in different age groups: the accuracy of mtDNAcn assessment in peripheral blood resulted higher in younger women and progressively decreased with increasing age.

Limitations, reasons for caution: We did not perform an infertility diagnostic work-up of the couples and we are thus unable to rule out other potential causes of subfertility. However, severe concomitant causes of infertility can be confidently ruled out since all women conceived within two years.

Wider implications of the findings: MtDNAcn assessment in peripheral blood appears to have all the features of the much sought non-invasive biomarker of female fertility. Noteworthy its performance resulted very good in young women. Our results may thus have future clinical applications. However, further independent studies including non-pregnant women undergoing high throughput screening are warranted.

Trial registration number: Not applicable.

P-390 Prognostic of IUI depends of the number of motile spermatozoa inseminated and patient age, however high concentrations are always detrimental: an analysis of 9618 cycles

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Study question: What is the impact of the number of motile spermatozoa inseminated (NMSI) on clinical pregnancy rates (CPR) in intrauterine insemination (IUI) according to patient age?

Summary answer: The NMSI positively influences the CPR of IUI for women between 30 and 40 years, while not for women under 30, or older than 40.

What is known already: Many authors have studied the effects of the NMSI on the success of IUI, but a real consensus has not yet been achieved. A minimum of 1 M (million) motile spermatozoa inseminated is often quoted as required to optimize IUI but several authors question this threshold. Some studies have shown that the pregnancy rates were higher if the NMSI exceeds 2 M or 5 M. However, according to a recent study, female age is the only variable significantly correlated with IUI success rates. NMSI and female age have rarely been studied together.

Study design, size, duration: This is a retrospective cohort study, in which 4394 couples completed 9618 IUI cycles with partner sperm, between January 2011 and December 2015, at a University-affiliated private ART clinic in Montreal.

Participants/materials, setting, methods: All couples had been trying to conceive without success for at least 1 year. Indications for IUI included moderate male factor, dysovulation and unexplained infertility. IUI was performed after a natural cycle (n = 180) or an ovarian stimulation by clomiphene citrate or letrozole (n = 7611) or gonadotropins alone (n = 218) or in addition to letrozole (n = 1609). The outcome measure was clinical pregnancy rates defined by an intrauterine gestational sac with heartbeat visible with ultrasonography at 7 weeks.

Main results and the role of chance: The 9618 cycles were divided into four groups based on the NMSI as follows: group 1, <1 M; group 2, 1 to 4 M; group 3, 5 to 10 M; group 4, ≥10 M. In women younger than 30 years (n = 1814 cycles), CPR between the four groups were not significantly different (6.4%, 14.8%, 13.7% and 14.1%, respectively; p = 0.20). Nevertheless, the CPR appeared clinically lower in group 1. In women 30 to 34 (n = 3499 cycles), CPR were significantly different between groups (2.9%, 10.2%, 15.6%, and 15.0%, respectively; p<0.001). In women 35 to 39 (n = 3083 cycles), CPR were also different between the groups (5.5%, 10.5%, 8.0%, 14.2%, respectively; p = 0.001). Thus, better results are observed if the NMSI is ≥5 M between 30 and 34 years, and if the NMSI is ≥10 M between 35 and 39 years. For patients ≥ 40 years (n = 1222 cycles), CPR were not different by groups of NMSI (4.7%, 5.4%, 6.3%, 9.7%, respectively; p = 0.21), although the CPR seemed to double between groups 1 and 4. Less pregnancies in the older age group could explain the lack of significance. Interestingly, we noted that an NMSI ≥150 M had a detrimental effect on CPR any age combined (10.0%, p<0.001).

Limitations, reasons for caution: Our population was heterogeneous; all indications of inseminations combined. Ovulation stimulation treatments varied and the therapeutic strategy was physician-dependent. However, the very high number of cycles adds strong value to our study and the NMSI is a standardized data.

Wider implications of the findings: The only study analyzing the NMSI by age category reported no difference in CPR in women older than 25, possibly due to a lack of statistical power. Our study suggests that in women 30-39 years with NMSI<5 M, IUI results in lower CPR, thus IVF should be considered a first option.

Trial registration number: Not applicable.

P-391 The effect of weight intervention on obstetric and neonatal outcome in obese women scheduled for IVF treatment

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Study question: What is the effect of a low calorie diet weight intervention in obese women prior to IVF on maternal and neonatal outcome?

Summary answer: No differences in maternal and neonatal outcomes between the study groups were found and most pregnancies were successful with healthy children and few maternal complications.

What is known already: Fertility, obstetric and neonatal outcome are negatively affected in obese women. A recent Dutch randomized trial of infertile women and lifestyle weight intervention with a weight loss of 3,3 kg, found no difference in live birth rate, obstetric or neonatal outcomes. In previously published data from our trial no effect on live birth rates were found despite large weight loss (9,44 kg). A difference was found in spontaneous pregnancies leading to live birth and was in favor of the weight intervention, 10,5% (16/152) versus 2,6% (4/153) in the control group.

Study design, size, duration: This is a secondary analysis of a prospective, multicenter, randomized controlled trial that was performed between 2010 and 2016 in the Nordic countries. 305 women were randomized to one of two groups; weight reduction intervention followed by IVF-treatment or IVF-treatment only. The weight reduction included 12 weeks of a low-calorie liquid formula diet (LCD), of 880 kcal per day followed by two to five weeks of diet re-introduction and weight stabilization before IVF treatment started.

Participants/materials, setting, methods: Nine infertility clinics in Sweden, Denmark and Iceland participated. Women under 38 years of age planning for IVF, and having a body mass index (BMI) ≥30 and <35 kg/m^2 were randomized to two groups: an intervention group (152 patients) with weight reduction before IVF, starting with 12 weeks of a low calorie liquid formula diet of 880 kcal/day and thereafter weight stabilization for 2-5 weeks, or a control group (153 patients) with IVF only.

Main results and the role of chance: There were 87 live births; 45 singletons in the intervention group and 41 singletons and one twin birth in the control group. The characteristics of the women having a live birth were comparable in the two groups. The mean age and infertility length were 31,4 years and 35,3 months in the intervention group and 30,7 years and 36,6 months in the control group. The infertility diagnoses were comparable. None of the maternal and neonatal outcomes differed significantly between the intervention and the control group: cesaerean section 28,9% vs. 24,4%, OR: 1.14 (CI: 0.45; 2.94); preeclampsia 11,1% vs. 9,8%, OR: 1.19 (CI: 0.30; 4.76); gestational diabetes 2.2% vs. 4.9%, OR: 0.45 (CI: 0.04; 5.21); postpartum hemorrhage >1000 ml 4.4% vs. 12.2%, OR: 0.34 (CI: 0.06; 1.88); preterm birth <37 weeks 4.4% vs. 2.4%, OR: 1.95 (CI: 0.17; 22.36); low birth weight <2500 g 4.4% vs. 2.4%, OR: 1.95 (0.17; 22.36). No children within the study were treated in a neonatal intensive care unit or had a major malformation observed within the first week of life. When comparing the women who achieved a live birth through a spontaneous pregnancy in the different randomization groups no significant differences were found for any variables.

Limitations, reasons for caution: The study was not designed or powered to detect differences in maternal or neonatal outcome nor was it blinded for patients or physician. Further, the intervention group had longer time to achieve a spontaneous pregnancy, but was slightly older than the control group at IVF.

Wider implications of the findings: Maternal and neonatal outcomes were satisfactory in both groups. Although estimates of absolute values and OR indicated no differences between groups in any outcomes, the 95% CI were generally wide meaning that clinical important differences may exist.

Trial registration number: ClinicalTrials.gov number, NCT01566929

P-392 The predictive value of pulsatility index for pregnancy after assisted conception: a systematic review

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Study question: Is there a difference between the uterine vascularity of women who get pregnant and the uterine vascularity of women who do not become pregnant after assisted conception.

Summary answer: The pregnant group had a significantly lower uterine pulsatility index when compared with the non-pregnant group [WMD -0.14; 95% confidence interval (CI): -0.22,-0.06; p = 0.000].

What is known already: Angiogenesis is critical for the development of the endometrium to enable successful embryo implantation. Pulsatility index is a marker of resistance to blood flow which can be used to quantify and compare downstream blood flow to tissues. Our study is the first systematic review to investigate whether the vascularity of the endometrium correlates with assisted conception outcomes.

Study design, size, duration: The electronic databases EMBASE and MEDLINE Were systematically searched, up to October 2017, for publications that studied the differences in pulsatility index measurements between women who became pregnant and women who did not become pregnant after assisted conception. Twenty-nine papers met the inclusion criteria, which included 4022 participants in total.

Participants/materials, setting, methods: Participants included infertile women who were undergoing either IVF, frozen embryo transfer or intrauterine insemination. 1292 became pregnant and 2730 did not become pregnant

after assisted conception. Causes of infertility included a range of male and female factors. The studies were performed in the fertility clinic setting. Pulsatility index measurements were taken using transvaginal ultrasound at various points prior to embryo transfer.

Main results and the role of chance: The search produced a total of 758 studies which were all retrieved. A total of 29 cohort studies were included in the review. A raised mean PI (>3) was present in 1 study within the group of pregnant women and present in three studies within the group of non-pregnant women. Overall the pregnant group had a significantly lower uterine pulsatility index when compared with the non-pregnant group [WMD -0.14; 95% confidence interval (CI): -0.22,-0.06; p = 0.000; 29 studies]. Pulsatility index measurements taken within a day of embryo transfer most reliably correlated with the primary outcome after assisted conception [WMD -0.18, 95% CI 0.26, -0.10); p = 0.065; 10 studies), however this could be due to chance. There was a high degree of heterogeneity (I^2 = 44%).

Limitations, reasons for caution: There was significant heterogeneity between studies, with variation in population characteristics and the treatment protocols given to participants. Using the Newcastle-Ottawa scale for assessing quality, the included studies were considered "satisfactory".

Wider implications of the findings: This review suggests that women with a lower uterine pulsatility index are more likely to achieve pregnancy, after assisted conception. Further research is required to determine whether there is a cut-off pulsatility index above which pregnancy is not possible and to determine the most reliable test of endometrial vascularity.

Trial registration number: CRD42017059529

P-393 Comparison of Cap-Score™, TUNEL, and semen analysis in couples undergoing timed intercourse and IUI

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Study question: What relationships exist among Cap-Score™ (test of sperm capacitation), TUNEL (test of DNA fragmentation) and traditional semen analysis parameters?

Summary answer: Cap-Score™ didn't correlate with semen analysis or TUNEL. However, TUNEL had a strong negative correlation with motility, making determination of cause of fertilization failure difficult.

What is known already: High sperm DNA fragmentation and diminished capacitation response may impair conception with timed intercourse or intrauterine insemination (IUI). Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) is widely used to assess breaks within sperm DNA. CapScoreÔ measures the proportion of sperm that respond to capacitation stimuli by assessing localization changes in GM1, a key regulator of capacitation and acrosome exocytosis. One prior study showed no relationship between Cap-Score™ and semen analysis, whereas multiple conflicting reports exist on the relationship of TUNEL and semen analysis. No one has previously assessed the potential correlation of Cap-Score™ and TUNEL.

Study design, size, duration: In the past 3 months, ejaculates were obtained from 41 consenting men for infertility screening. Preliminary pregnancy outcomes were also collected. Statistical relationships among semen analysis, TUNEL and Cap-Score™ were assessed. Potential linear associations between Cap-Score™ or TUNEL and traditional semen parameters were assessed by Pearson's Product-Moment Correlation. Any potential relationship between TUNEL and Cap-Score™ was evaluated similarly, and "normal vs abnormal" Cap-Score™ and TUNEL were examined by Chi-Square.

Participants/materials, setting, methods: To control for female confounding factors, inclusion criteria consisted of couples with female partners ≤ 35 years old with normal uterine cavity and patent fallopian tubes. Semen samples were processed by density gradient centrifugation according to WHO 2010 criteria. Post-wash specimens were sent to Androvia LifeSciences for Cap-Score™ assessment. Cap-Scores™ ≥27.6% were considered "normal". DNA strand breaks were assessed by TUNEL on at least 500 spermatozoa under fluorescent microscopy. TUNEL scores £15% were considered "normal".

Main results and the role of chance: Cap-Score™ was independent of ejaculate volume (r = 0.075; p = 0.571), concentration (r=-0.032; p = 0.810), percentage of motile sperm (r = 0.005; p = 0.967) and percentage of sperm having strict normal morphology (r = 0.010; p = 0.942). No relationship was observed between Cap-Score™ and TUNEL, either linearly (r=-0.091; p = 0.571), or when grouped into normal or abnormal bins and evaluated by Chi-Square (p = 0.524). Conversely, a very strong negative relationship was observed between TUNEL and the percentage of motile sperm (r=-0.930; p<0.001), meaning that as DNA fragmentation increased, motility decreased. Of the 41 men, TUNEL was abnormal in 11 (26.8%) and Cap-Score was abnormal in 19 (46.3%). In the 11 patients with abnormal TUNEL (mean = 24.1), no natural conceptions were yet reported, nor in the 2 cycles of IUI for which outcomes are known. Of the 30 having normal TUNEL, there were 3 natural conceptions and 5 conceptions out of 9 cycles of IUI for which outcomes are known. In the 22 men having normal Cap-Scores™, 3 natural conceptions occurred, whereas none occurred in the 19 men with low Cap-Scores™. In couples pursuing IUI with normal Cap-Scores™, there were 3 pregnancies out of 6 cycles for which outcomes are known; 2 of 5 cycles with a low result achieved pregnancy with IUI.

Limitations, reasons for caution: These data clearly indicate that the ability of the sperm to capacitate does not correlate with sperm DNA strand breaks. Clinical outcomes represent preliminary data from a limited number of couples. More clinical outcomes data are needed to determine the efficacy of either test at predicting conception by different means.

Wider implications of the findings: This is the first report showing that Cap-Score™ and DNA fragmentation are not related. As assessed by TUNEL, high DNA fragmentation correlated with decreasing motility. To better test and inform infertile couples, the addition of proven biomarkers to the standard semen analysis would help to identify those who can conceive.

Trial registration number: N/A.

P-394 A novel maternal age and blastocyst cohort size-based prediction model estimating the probability of obtaining at least one euploid blastocyst for transfer in IVF/ICSI cycles

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Study question: Can a statistical prediction model estimate the probability of achieving at least one euploid blastocyst as a function of female age and embryo cohort size?

Summary answer: Our novel predictive model estimates the minimum blastocyst cohort size needed to achieve at least one euploid blastocyst for transfer given a specified probability.

What is known already: Array comparative genomic hybridization data indicate a negative correlation between female age and embryo euploidy. Aneuploidy rates do not differ significantly by cohort size in women of same age, and the proportion of women with at least one euploid blastocyst is increased when more embryos are available. An individualized estimate of the couple's probability of having a euploid blastocyst and the number of blastocysts needed to achieve this goal before treatment can be used for counseling and treatment planning. Also, estimation of this

probability after the embryos are generated may aid doctors to set patient expectations.

Study design, size, duration: Retrospective analysis of 1,220 trophectoderm biopsy results from 436 infertile couples undergoing assisted reproductive technology (ART) cycles and preimplantation genetic testing for aneuploidy (PGT-A) between 2016 and 2017. PGT-A was used for reasons of advanced maternal age, severe male factor infertility, recurrent miscarriage, repeated implantation failure, as well as patients who were concerned about the euploidy status of their embryos.

Participants/materials, setting, methods: Biopsied trophectoderm cells were subjected to next-generation sequencing (NGS) analysis using the Ion PGM™ platform. Logistic regression was fit to the dataset, with female age as the predictor and the status of the embryos (euploid or aneuploid) as the response. The best model was chosen and validated by randomly splitting two portions of the data, 80% for training and the remaining 20% for testing. Computations were carried out using JMP 13 (www.jmp.com).

Main results and the role of chance: Given a blastocyst cohort, the number of euploid blastocysts (M) is a random variable with a binomial distribution. The quadratic model on female age was significant (Prob>ChiSquare = 0.028). A model-independent verification by aggregating the data in age bins revealed that the estimated probability distribution was verified in the validation dataset. The model was not biased as there was linearity in the relationship between the fitted probabilities and empirical data. The minimum number of blastocysts needed for obtaining at least one euploid blastocyst for transfer can be computed for different probabilities. At the age of 28 years, the model estimates that a total of three blastocysts is required to obtain at least one euploid blastocyst with 90% probability, whereas it is 4, 5, 6, 9, 16 and 29 for ages 35, 37, 39, 41, 43, and 45, respectively. Furthermore, an embryo cohort size of 2, 3, 5, 7, and 11 are required to obtain at least one euploid blastocyst with 80% probability for ages 28, 35, 39, 41, and 43, respectively.

Limitations, reasons for caution: External validation is currently being performed. Other covariates that could affect embryo genetic status were not analyzed. Furthermore, the model is not intended to be generalized to IVF patients undergoing cleavage-stage embryo transfer and should not be used for making decisions about whether or not patients should undergo treatment.

Wider implications of the findings: This novel resource will aid clinicians counsel and plan treatment of couples regarding the embryo cohort size needed to obtain at least one euploid blastocyst for transfer.

Trial registration number: Not applicable.

P-395 Women with polycystic ovary syndrome are at increased risk of postnatal depression

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Study question: Are women with polycystic ovary syndrome (PCOS) at elevated risk for postnatal depression (PND)?

Summary answer: For women with PCOS, a history of miscarriage and assisted conception interacted ($p = 0.06$ and $p < 0.05$, respectively), with 56% having PND.

What is known already: Maternal depression associated with pregnancy and childbirth, known as postnatal depression (PND), is a debilitating condition that can have a significant impact on the health and wellbeing of the woman, her child and her wider family. PND is likely to be under-diagnosed but is estimated to affect 10-20% of mothers in western countries.

Women with PCOS have increased susceptibility to depression and anxiety, which is an identified risk factor for PND. The potential for a link between PCOS and PND has not previously been published.

Study design, size, duration: The Lucina study is a community based retrospective birth cohort of female births from 1973-75 at a single maternity hospital in Adelaide, South Australia. Participants were traced and enrolled at age 30-31, with n = 566 providing data for the current study.

Participants/materials, setting, methods: Details of reproductive history, pregnancy, birth, and PND were obtained through structured face to face interview with study participants. PCOS was diagnosed using the Rotterdam criteria. Comparisons were made between women with and without PCOS using logistic regression analysis including the investigation of interactions.

Main results and the role of chance: Compared to their counterparts, women with PCOS were substantially more likely to have difficulty conceiving (OR = 5.2, 95% CI 2.9-9.4), to have conceived with medical assistance (OR = 11.6, 95% CI 5.5-24.4), and to have pregnancy complications (gestational diabetes, pregnancy-induced hypertension, or pre-eclampsia, OR = 2.0, 95% CI 1.1-3.5). A positive but statistically non-significant association was observed between PCOS and PND (OR = 1.6, 95% CI 0.9-2.9) for the overall group. However, where women with PCOS had a history of miscarriage or conceived with medical assistance, the combination interacted ($p = 0.06$ and $p < 0.05$, respectively), with 56% of these women having PND.

Limitations, reasons for caution: Susceptibility to PND for women with PCOS could be due to either their perturbed hormonal and metabolic profiles, or to distress created by PCOS symptoms and their management. Due to limitations in power, the study was not able to calculate risks for specific PCOS sub-phenotypes or treatments received.

Wider implications of the findings: As the first study to investigate postnatal depression (PND) in women with polycystic ovary syndrome, findings point to vulnerability inherent in PCOS being amplified, or by specific fertility treatment regimens. This highlights a potential need for targeted postnatal follow-up of women with PCOS.

Trial registration number: Not applicable.

P-396 Comparison of the treatment outcomes in women with unicornuate uterus undergoing ART versus controls with normal uterine anatomy: a nested case-control retrospective analysis

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Study question: Are the ART outcomes different in women with unicornuate compared to normal uterus and whether culture to blastocyst improves the outcomes in unicornuate uterus group?

Summary answer: In unicornuate uterus implantation rate following fresh embryo transfer and cumulative live-birth rate are lower than in normal uterus. Blastocyst transfer significantly improved clinical outcomes.

What is known already: Unicornuate uterus have been associated with a decreased risk of obstetrics complications with lower birth rate, mainly due to an increased risk of miscarriage and very preterm birth. It remains unclear, whether women with unicornuate uterus who undergo IVF-ICSI have different response to ovarian stimulation and whether implantation and pregnancy rates in these women are different from those in women with normal uterus.

Study design, size, duration: A retrospective nested case-control study was conducted at the Reproductive Medical Center of Peking University Third Hospital. The participants were recruited from the cohort of women who underwent IVF-ICSI cycles between 2012 and 2016, identified by using centralized computer database. Consecutive women diagnosed with unicornuate uterus and randomly selected controls with normal uterus were included. The controls were matched 1:3 by age, cause of infertility and number of embryos transferred.

Participants/materials, setting, methods: The study population comprised 342 women with unicornuate uterus (study group) and 1026 patients with normal uterus (control group). Demographic and clinical information was retrieved from the electronic records. Cumulative live birth rate upon one complete IVF cycle, including all resulting embryos was considered as a primary outcome measure. Other outcomes included number of oocytes retrieved, endometrial thickness, implantation rate, number of embryos obtained, clinical pregnancy, multifetal gestation, miscarriage rate.

Main results and the role of chance: The demographic characteristics, duration and causes of infertility were comparable between the groups. There was higher rate of primary infertility in unicornuate uterus group. Unicornuate uterus was associated with significantly lower endometrial thickness on day of hCG ($p<0.001$) and lower number of oocytes retrieved ($p = 0.016$), although

peak E2 levels did not differ between the groups. The number of embryos transferred per cycle, day-3 or blastocysts was similar between the study and control groups. Transfer of day-3 embryos in fresh cycle was associated with lower implantation rate (23.7% vs. 32.5%, p<0.001) and lower live-birth rate (26.9% vs. 34.4%, p = 0.031) per transfer. On contrast, frozen embryo transfer (FET) of day-3 embryos did not result in different implantation and live birth rate between the groups (p = 0.288 and p = 0.686, respectively). Blastocyst transfer led to comparable rates of implantation and live birth between the two groups in either fresh and frozen cycles. The cumulative live birth rate following one complete IVF-ICSI cycle including all the resulting FET was 42.4% in women with unicornuate uterus vs. 52.8%; p = 0.001 in controls. Subgroup analysis revealed that blastocyst transfer in women with unicornuate uterus led to significantly higher live birth rate than cleavage transfer (40% vs. 53%, p = 0.045).

Limitations, reasons for caution: Retrospective nature and no information on the obstetrics and neonatal outcomes are the main limitations of this study. Not all women treated in 2016 utilized their available embryos from the index stimulation cycle and were not included in estimation of cumulative live birth rate.

Wider implications of the findings: Lower implantation and pregnancy rates following fresh but not frozen cleavage embryo transfers and significantly improved outcomes with blastocyst culture were observed in unicornuate compared to normal uterus. This suggests considering blastocyst transfer or freeze all policy in women with unicornuate uterus, which warrants evaluation in prospective randomized setting.

Trial registration number: not applicable.

P-397 Should couples with low sperm count in the first intrauterine insemination (IUI) cycle continue IUI treatment?

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Study question: Should couples with low post-wash total motile count (TMC) in the first intrauterine insemination (IUI) cycle continue IUI treatment?

Summary answer: Couples with low post-wash TMC in the first IUI cycles can continue IUI treatment without compromising their fertility outcomes.

What is known already: IUI is the treatment of first choice for couples with unexplained or mild male infertility. The post-wash TMC in the first IUI cycle can be used to predict pregnancy, and at present many couples switch to IVF after a first poor post-wash semen analysis. However, it is unknown if a poor post-wash TMC in the first IUI cycle is predictive for future IUI outcomes. To study the association between post-wash TMC and outcomes of future IUI cycles, we compared live birth rates in women undergoing IUI in relation to their post-wash semen analysis.

Study design, size, duration: We performed a retrospective cohort study in the Reproductive Medicine Centre of Peking University Third Hospital.

Participants/materials, setting, methods: We included couples with unexplained or mild male infertility treated with IUI in two consecutive cycles with similar protocols. Women with polycystic ovary syndrome were not included. We compared live birth rates in the second cycle according to different groups in post-wash TMC in the first cycle.We also compared the baseline characteristics and clinical pregnancy between groups with different post-wash TMC. ANOVA and Chi square analysis were conducted in SPSS 19.0.

Main results and the role of chance: We studied 4,496 IUI cycles in 2,248 couples, of which 2,732 were unstimulated cycles and 1,764 were cycles stimulated with CC, letrozole or gonadotropins. We sorted data according to their post-wash TMC in the first cycle in ascending order and then divided all patients into eight equal groups. Medians of sperm count were 0.6, 1.3, 2, 3, 4, 5, 7, and 13. Live birth rates in the second cycle were 6.1%, 6.4%, 7.5%, 8.9%, 6.1%, 7.8%, 6.1%, and 7.5%, respectively. Likelihood ratios for various categories of the post wash TMC varied between 0.68 and 1.41, with most likelihood ratios being close to 1, indicating no predictive performance of the post-wash TMC in the first cycle for subsequent pregnancies.

Limitations, reasons for caution: Our study was retrospective, and we included only cycles in which the insemination was performed.

Wider implications of the findings: Our study shows that in couples treated with IUI, the post-wash semen analysis should not be used to select women who should continue IUI or switch to IVF.

Trial registration number: Not applicable.

P-398 Prediction models for IUI success incorporating male age are more accurate among older women

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Study question: What factors are important to predict who will not benefit from IUI and who should be recommended for IVF directly.

Summary answer: We identified clinical and laboratory parameters that predicted a low probability for pregnancy with IUI. These are patients who should be move directly to IVF.

What is known already: The decision of which assisted reproductive technology (ART) method is most suitable for specific subfertile couples is complicated. Intrauterine insemination (IUI) with or without ovulation induction is commonly offered to subfertile couples without bilateral mechanical factor or severe male factor. On average after three to six IUI cycles without pregnancy, couples will be recommended to undergo IVF. IUI success rate predictors have been suggested to be moderately accurate in the past, however older studies did not include ovarian reserve parameters and modern male factor parameters.

Study design, size, duration: In the current study we evaluated the predictive capacity of different parameters in predicting IUI success, and constructed a generalized linear regression model (LRM). A total of 467 patients (1342 IUI cycles) were studied retrospectively.

Participants/materials, setting, methods: Clinical and laboratory parameters significantly different between patients with clinical pregnancy and without clinical pregnancy were identified. The following parameters were significantly different: male age, duration of infertility, AMH, antral follicle count (AFC), DNA fragmentation index (DFI), number of follicles and E2 levels at HCG trigger, and total motile sperm count (TMC). These parameters were used to construct a two-step predictive model.

Main results and the role of chance: Our prediction model was able to identify couples with low probability for pregnancy upfront (less than 5% chance) that should be referred directly to IVF without any IUI cycle. This prediction could have saved going through failed IUI for 20.5% of patients. Later after the first IUI cycle a second predictive score was calculated and identified couples with low pregnancy probability who should be referred to IVF after one IUI cycle (26% of patients). Our predictive model was most accurate for females older than 40 years old (AUC = 0.775), with number of follicles before HCG trigger, male age, and DFI being the most important factors.

Limitations, reasons for caution: This is a single center retrospective study that should be validated prospectively.

Wider implications of the findings: Altogether, based on these results we conclude that after the first IUI cycle a predictive score can be calculated. Couples with a low score (pregnancy probability <5%) should be referred to IVF.

Trial registration number: REB #20120043-E.

P-399 Fresh or vitrified: comparing the number of oocytes necessary to achieve reproductive success in egg donation programme

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Study question: Does egg banking provide comparable outcome to fresh egg donation cycles, what are the oocyte-to-blastocyst and oocyte-to-baby rates per fresh and vitrified oocyte?

Summary answer: Compared to vitrified, fresh oocytes gain more available blastocysts, higher implantation rates and more babies born, higher number of surplus blastocysts increases cumulative success rate.

What is known already: Vitrification of oocytes represents an effective method resulting in pregnancy rates per embryotransfer similar to usage of fresh oocytes. Vitrification and banking of oocytes offers an advantage for both clinics and recipients in terms of management, timing and lesser treatment cancelations. However, due to losses during warming and fertilization failures, the number of available blastocysts declines. Metric methods calculating success rates per oocyte provide more accurate picture of the biological efficiency than standard pregnancy rate per embryotransfer. According to available data, the live birth rate per fresh donated oocytes, even with the best-prognosis group of young donors, is only 7.3%.

Study design, size, duration: A retrospective comparative analysis of 266 fresh egg donation cycles and 63 cycles using vitrified donor oocytes performed from January 2016 to December 2016.

Comparison parameters included age of oocyte donors, age of recipients, total gonadotrophin dose and average duration of stimulation. Total oocyte number and number of metaphase II oocytes, survival rate, fertilization rate, blastocyst rate, implantation rate and ongoing pregnancy rates per ET and per oocyte were calculated.

Participants/materials, setting, methods: All donors underwent gonadotrophin stimulation in antagonist protocol. Antagonist administration was initiated from day 6 of stimulation. GnRH-a (triptorelin 0.2) was used as a trigger and egg collection was performed 35–36 hours later. Oocytes were vitrified using Sage media and closed system. Both fresh and warmed oocytes were inseminated using ICSI. Both groups of fertilized oocytes underwent identical culture and handling conditions.

Main results and the role of chance: There was no statistically significant difference noted for age of donors, body weight or BMI, nor total gonadotrophin dose, duration of stimulation, no difference in age of recipients between both groups. There was no difference observed in terms of fresh oocytes and vitrified oocytes per recipient's treatment cycle (10,89 ± 2,00 vs 11,36 ± 3,14, p = 0,14).

Fertilization rate of fresh oocytes was higher (84,81% vs 72,97%, p<0,001), we have achieved significantly higher oocyte-to-blastocyst rate (32,11% vs 13,36%, p<0,001), Significantly higher numbers of fresh oocytes (6,11% vs 5,07%, p<0,001) increased implantation and live birth rate, oocyte-to-baby rate for fresh oocytes was 5,81% compared to 3,76%, p<0,001.

Average number of blastocyst per embryotransfer was 1,56 ± 0,56 for fresh oocytes and 0,81 ± 0,79 for vitrified ones, p<0,001. Pregnancy rate of 68,05% was significantly higher for fresh oocytes in comparison to 42,86% pregnancy rate for cycles using vitrified eggs (p<0,001).

Significantly higher number of surplus blastocysts per treatment cycle (1,93 ± 1,82 vs 0,60 ± 1,19, p<0,001) favors fresh vs frozen oocytes. Cumulative chance of reproductive success was estimated. If all available surplus blastocyst were used in the future and resulted to similar pregnancy rates, hypothetical cumulative oocyte-to-baby rate for fresh eggs would be 13,06% vs 6,87% for vitrified, p<0,001

Limitations, reasons for caution: Our results might be biased by legally enforced usage of close system for vitrification as majority of studies have included open vitrification systems. Contrary to published results, we have not confirmed the number of 8–10 vitrified eggs per ongoing pregnancy. Cumulative chance of success was based only on hypothetical estimation.

Wider implications of the findings: We assert that oocyte-to-blastocyst and oocyte-to-baby rates offer more equal methods for evaluating fresh and frozen egg donation programmes. Such data are easily interpreted to patients and help to understand better the efficacy of egg banking compared to fresh donation programmes from perspective of providers and patients.

Trial registration number: not applicable.

P-400 Odds and predictors for achieving successful pregnancy at two years follow-up in a cohort of treated and untreated subfertile couples in a secondary care hospital

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Study question: To estimate the chances of achieving live birth/ongoing pregnancy beyond twenty weeks for couples within two years and determine predictive factors for a successful pregnancy.

Summary answer: 60% in treated and 35% in the untreated cohorts achieved successful pregnancy in two years. Age and duration of subfertility were the most significant predictors.

What is known already: With fertility declining globally, more couples are seeking fertility treatment. Initial clinic investigation is key to determine the cause and future treatment regimen. A large cohort study across all UK in-vitro fertilisation (IVF) clinics identified key factors predicting success in assisted reproductive technology were younger maternal age and shorter duration of subfertility, with younger women being 66% more likely to achieve success. Reduced ovarian reserve, the primary measure of which is age, causes significantly decreased rates of treatment success. National statistics state that live birth rates following IVF and IUI treatment for women under 35 is 32% and 13% respectively.

Study design, size, duration: This observational cohort study evaluated all newly referred patients to a secondary care hospital Fertility Clinic of the UK between January 2013 and December 2015. These patients were followed up for 2 years using electronic records. This included 1257 couples, 1142 of which matched inclusion criteria and 1136 of whom were suitable for analysis.

Participants/materials, setting, methods: Inclusion criteria were heterosexual couples, with no prior fertility treatment. 677 patients were offered treatment based on the National Health Service (NHS) funding criteria. Data collected from electronic records was collated onto an excel database. Statistical analysis was carried out using IBM SPSS, reporting the median (IQR) as the data were skewed. Mann Whitney U Test or Chi Square Test and logistic regression analysis were done as appropriate.

Main results and the role of chance: The overall success rate was 50% among women who were referred. Patients offered treatment (N = 677) achieved 60% success, compared to 35% in untreated patients (N = 459). Across treated patients, those successful were younger (Median (IQR) = 31 (28-34) vs 32 (29-36), P<0.05), had higher rates of primary infertility (86.8% vs 80.6%, P<0.05) and shorter duration of subfertility (P = 0.001). Univariate regression analysis (OR, 95% CI) showed that younger patients (0.95, 0.92-0.98) with a reduced duration of subfertility (0.82, 0.75-0.91), and suffering from primary infertility (1.58, 1.04-2.40) were most successful. Anti-Mullerian Hormone (AMH) and Follicle Stimulating Hormone (FSH) levels were not predictive of successful outcome. Multivariate analysis (OR, 95% CI) showed that age (0.95, 0.90-0.99), duration of subfertility (0.85, 0.75-0.95) and primary infertility (2.03, 1.10-3.72) were still significant factors after adjusting for confounders. The most common cause was unexplained (44%), the least common was uterine factors (1.4%). The best success rates were with ovulatory cause (58%), the worst was uterine factors (25%). While 49% of successful outcomes were following IVF, ovulation induction, intrauterine insemination and spontaneous conception contributed to the success by 24%, 19% and 7.8% respectively.

Limitations, reasons for caution: While data of the treated patients were robust, some of the untreated patients may have been followed up for treatment elsewhere. Although difficult to quantify, this number is assumed to be low because of adherence to local patient referral pathways.

Wider implications of the findings: This study provides realistic chances of success for subfertile couples within two years and demonstrates that age and duration of subfertility are the most significant baseline predictors in achieving live birth/ongoing pregnancy at two years. These results are useful to explain success rates and counselling couples seeking fertility treatment.

Trial registration number: Not Applicable.

P-401 Oocytes quality in women with thalassaemia major

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Study question: Is the quality of the oocytes affected in women with thalassaemia major undergoing IVF-ICSI cycles?

Summary answer: The quality of oocytes obtained from women with thalassaemia major is not affected.

What is known already: Women with thalassaemia major commonly face reproductive disorders. The iron overload that is consequent to the lifelong transfusional therapy typically produces a toxic effect on the anterior pituitary, leading to hypogonadotropic hypogonadism, a condition that can be overcome with exogenous gonadotropins. However, recent studies showed that affected women have also reduced levels of AMH and AFC, suggesting that ovarian reserve may be damaged. Moreover, the elevated peripheral levels of free iron may directly harm the quality of primordial follicles and could perturb folliculogenesis. Evidence to support a detrimental effect on oocytes quality is however lacking.

Study design, size, duration: A retrospective case-control study was conducted in women with thalassaemia major referred to our infertility unit between January 2008 and December 2017 and undergoing at least one IVF or ICSI cycle. Controls were matched to cases in a 5:1 ratio by age and study period. Both cases and controls were included only for the first treatment cycle.

Participants/materials, setting, methods: Thirty-five infertile women with thalassaemia major were referred during the study period, of whom 21 (60%) underwent IVF-ICSI. These 21 cases were matched to 105 controls. Participants underwent IVF-ICSI according the local standardized protocols. Compared to controls, cases also received LH in a 1:2 ratio compared to FSH. The primary outcome was the proportion of top quality embryos per oocytes used.

Main results and the role of chance: The baseline clinical characteristics of the two study groups did not significantly differ, with the exception of serum AMH and AFC which were significantly lower among women affected. The medians (Interquartile-IQR) in cases and controls were 0.6 (0.2-1.8) and 1.5 (0.7-3.5) ng/ml ($p = 0.05$) and 4 (1-7.5) and 11 (5.5-16) ($p<0.001$), respectively.

Considering the IVF-ICSI cycle, despite the expected longer duration of stimulation and higher total doses of gonadotropins used for cases, the number of developed follicles and oocytes retrieved did not differ. Live births were 4 (19%) and 34 (32%), respectively ($p = 0.30$). Fertilization rate and cleavage rate were even higher in women with thalassaemia major. The medians (IQR) in cases and controls were 100% (76-100%) and 75% (50-100%) ($p = 0.03$) and 75% (39-100%) and 50% (29-64%) ($p = 0.04$), respectively. In contrast, the rate of top quality embryos (the main outcome of the study) did not differ. The median (IQR) was 20% (0-76%) and 25% (5-50%), respectively ($p = 0.98$). Finally, we correlated serum ferritin and the duration of transfusional therapy with the rate of top quality embryos but failed to detect any significant correlation.

Limitations, reasons for caution: Thalassaemia major is a rare condition and the sample size is inevitably small: a type II error cannot be excluded for some outcomes. Moreover, the primary outcome chosen is a surrogate marker of oocytes quality. Live birth rate would be more informative but would require a significantly larger sample size.

Wider implications of the findings: Fertility preservation in young age is not justified in affected women since the quality of the oocytes does not appear to be compromised. This conclusion is also strengthened by the recent progresses of iron chelation therapies that can reduce the injury to the pituitary, thus potentially consenting natural conception.

Trial registration number: Not applicable.

P-402 Freeze-all versus conventional approach in normal responders: comparison of cumulative live birth in a multicenter cohort study

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Study question: Does freeze-all assure a better outcome in terms of cumulative live birth rate (CLBR) in normal responder patients?

Summary answer: In the present analysis, freeze-all is not superior to the conventional strategy in terms of CLBR.

What is known already: To date some studies have suggested that cryopreservation of the entire embryonic cohort derived from a single cycle is associated with a higher live birth rate in selected patient categories, such as women at risk of developing ovarian hyperstimulation syndrome (OHSS), patients awaiting results of genetic tests or in cycles with supra-physiological levels of estrogen and progesterone. Since the experience with freeze-all has been limited to selected typologies of patients, this approach should be tested in a wider context in order to evaluate whether it can be proposed as the gold standard for IVF treatments.

Study design, size, duration: This is a retrospective matched cohort study involving completed IVF cycles conducted between 2012 and 2016, in 5 fertility centres. Cycle data were analyzed according to approach (fresh cycle or freeze-all) and then stratified according to the number of oocytes retrieved, age and embryo transfer day.

Participants/materials, setting, methods: This study analysed 564 completed IVF cycles in which 12-18 oocytes were retrieved[1]. In 435 cycles the conventional strategy was applied, involving first transfer of fresh embryo(s) and afterwards use of cryopreserved embryos; in 129 cycles the freeze all approach was performed, with elective cryopreservation of all viable embryos. Only patients who had transferred all available embryos and/or achieved a live birth, were included.

Main results and the role of chance: Our analysis showed a higher CLBR, but not statistically significant, in the freeze-all group (53.5% vs 45.5%, $p = 0.11$). Data stratification on the basis of age and number of oocytes retrieved suggested a trend towards a higher CLBR in the freeze-all group, although again in the absence statistical significance. In the sub-analysis focusing on the day of embryo transfer, no difference was found in the cleavage stage data segment, whereas in cycles where embryo transfer was carried out at the blastocyst stage the CLBR was significantly higher in the freeze-all group ($p<0.05$).

Limitations, reasons for caution: Since this is a retrospective study, the information obtained is based on previously recorded data. Consequently, certain parameters that could influence the outcome, such as BMI, markers of ovarian reserve and others, were not included in the analysis.

Wider implications of the findings: Our analysis ofCLBRshows that the freeze-all approach is not superior to the conventional strategy.However,its performance appears at least as competitive as the conventional modality of use of fresh and cryopreserved embryos.The statistically significant difference in CLBRin treatments where embryo transfer was carried out at the blastocyst stage warrants further investigation.

Trial registration number: Not applicable.

P-403 Atosiban Infusion (Oxytocin Receptor Antagonist) is effective in improving the pregnancy outcome of Blastocyst Transfer cycle in a Younger population (<35 yrs)

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Study question: Is Atosiban Effective in reducing myometrial contraction during Blastocyst transfer and improving pregnancy rate in a general population? (18-45 yrs)

Summary answer: Atosiban is more effective in younger population of women undergoing ART cycle (age<35 yrs)

What is known already: Implantation and pregnancy rates are inversely correlated with the frequency of uterine contractions. Supraphysiological concentration of oestradiol following ovarian stimulation has been shown to increase uterine contractility. Atosiban infusion prior to embryo transfer is known to reduce the uterine contraction but its effectiveness in a particular subset of age group is not known.

Study design, size, duration: This prospective non randomised study of infertile women (n = 150) undergoing Blastocyst transfer divided into two group A (Atosiban infusion) & B (no Atosiban) and further divided into subgroups of i (<35 yrs.) ii (>35 yrs.). Both groups received similar quality of blastocyst & were evaluated for myometrial contraction and clinical pregnancy rates by Transvaginal Ultrasound (TVS). Study was conducted between December 2016 and December 2017 at our private ART clinic.

Participants/materials, setting, methods: Group A(n = 75) received total dose of 22.5 mg atosiban in (100 ml Normal saline) @ 32 drops per min half hour before Blastocyst transfer while Group B(n = 75) received no Atosiban infusion. Group A evaluated for myometrial contraction (contraction /2 min) before transfer and after infusion and group B evaluated before transfer. Clinical pregnancy determined by presence of Foetal cardiac activity on TVS in both the groups and subgroups.

Main results and the role of chance: women undergoing Blastocyst transfer in group A and group B were subjected to student T test to determine the pregnancy rates, a significant difference was noted student t test p value 0.0013 between the two groups. A significantly higher rate of pregnancy was noted in subgroup i, student t test p value 0.0211. While there was no significant difference in group ii student t test p Value 0.780 in. There was an extremely significant decrease in the myometrial contraction in group A as compared to group B (p value <0.0001). Contingency analysis for group i (< 35 yrs) Fischer exact test p value was 0.048(significant), odds ratio 2.33, 95 % CI 1.001 to 5.7 sensitivity 21.8 %, specificity 89.2 %, Positive predictive value (PPV) 70 %, Negative predictive value (NPV) 50%, LR 2.04 while for group ii, Fischer exact test p value was > 0.999(non-Significant), odds ratio 1, 95 % CI 0.4002 – 2.499, Sensitivity 10.7%, specificity 89.2 %, PPV 50%, NPV 50% likely hood ratio of 1.

Limitations, reasons for caution: This prospective non randomized study has a small sample size which may cause bias in the Atosiban group hence a multicentre randomized control trial with a large sample size with follow up till delivery is to be done to determine the live birth rate and effectiveness of Atosiban.

Wider implications of the findings: Atosiban used at the time of blastocyst transfer in young age group(<35 yrs.) gives a favourable outcome in an ART cycle as compared to older age group patients in terms of decreased myometrial contraction and higher clinical pregnancy rates.

Trial registration number: not applicable.

P-404 Application of seminal plasma to female genital tract prior to embryo transfer in assisted reproductive technology cycles (IVF, ICSI and frozen embryo transfer)

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Study question: Does application of seminal plasma (SP) to the female genital tract prior to embryo transfer improve ART cycle outcome?

Summary answer: We found low quality evidence that SP application may increase clinical pregnancy rate. There was insufficient evidence for live birth or ongoing pregnancy rates.

What is known already: The female genital tract is not exposed to SP during standard assisted reproductive technology cycles. However, it is thought that the inflammatory reaction triggered by SP may be beneficial by inducing maternal tolerance to paternal antigens expressed by the products of conception, and may increase the chance of successful implantation and live birth. Studies have revealed inconsistent results.

Study design, size, duration: Systematic review and meta-analysis of randomised controlled trials (RCTs) comparing SP application versus no SP exposure were identified from Cochrane Gynaecology and Fertility Group Specialised Register of Controlled Trials, Cochrane Central Register of Studies Online, MEDLINE, Embase, CINAHL and PsycINFO, trial registers for ongoing trials, Web of Knowledge, OpenGrey, LILACS, PubMed, Google Scholar and the reference lists of relevant articles.

Participants/materials, setting, methods: Eleven RCTs (3215 women) were included.

Main results and the role of chance: SP may increase clinical pregnancy rates (RR 1.15, 95% CI 1.01 to 1.31; participants = 2768; studies = 10; I2 = 0%). If clinical pregnancy rate following standard ART is 22.0% it will be between 22.2% and 28.8% with SP application.

SP application makes little or no difference in live birth rates (RR 1.10, 95% CI 0.86 to 1.43; participants = 948; studies = 3; I2 = 0%). If the live birth rate following standard ART is 19% it will be between 16% and 27% with SP application.

SP application makes little or no difference in live birth or ongoing pregnancy rates (RR 1.19, 95% CI 0.95 to 1.49; participants = 1178; studies = 4; I2 = 4%). If the live birth or ongoing pregnancy rate following standard ART is 19.5% it will be between 18.5% and 29% with SP application.

There was insufficient evidence to determine whether there was a difference in miscarriage rate between the groups (RR 1.01, 95% CI 0.57 to 1.79; participants = 1209; studies = 4; I2 = 0%). If the miscarriage rate following standard ART is 3.7%, the miscarriage rate following SP application will be between 2.1% and 6.6%.

Limitations, reasons for caution: The quality of the evidence ranged from very low to low. The main limitations were risk of bias (associated with poor reporting of allocation concealment and other methods) and imprecision for the primary outcome of live birth rate.

Wider implications of the findings: We conclude that seminal plasma application is worth further investigation, focusing on live birth and miscarriage rates.

Trial registration number: N/A.

P-405 Reproductive strategy in young women with critical ovarian reserve

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Study question: Is Assisted Reproductive Technology (ART) indicated in young women aged ≤34 years old when ovarian reserve is critical?

Summary answer: After ART, the pregnancy rate in young patients with critical ovarian reserve (29.4%) is comparable to ≥35 years old women (30.7%) with normal ovarian reserve.

What is known already: The ovarian reserve reflects the oocyte quality and number. Anti-Müllerian hormone (AMH) and antral follicle count (AFC) are the

best predictive markers of the ovarian reserve and constantly decrease with age. AMH levels play a key role in the proper counselling of the infertile patients and allow the clinician to elect a personalized controlled ovarian stimulation (COS) protocol. AMH and AFC are the most important factor predicting ovarian stimulation response based on retrieved oocytes number. Pregnancy rate also depends on oocyte quality which is strictly related to age.

Study design, size, duration: Retrospective study: 2289 patients undergoing a COS cycle at the University of Bologna infertility and IVF center, from 2011 to 2017. Two groups were considered: Group A with 833 women aged ≤34, and group B with 1465 women aged ≥35. Both groups were divided based on AMH levels into Critical Ovarian Reserve (COR) AMH ≤ 0.5 ng/ml, Diminished Ovarian Reserve (DOR) AMH 0.5–1 ng/ml and Normal Ovarian Reserve (NOR) AMH ≥ 1 ng/ml.

Participants/materials, setting, methods: In group A there were 208 (25%) patients with COR, 51(6.16%) patients with DOR and 574(68.9%) patients with NOR. In group B there were 379(25.9%) patients with COR, 189 (12.9%) patients with DOR and 897(61.2%) patients with NOR. 3227 cycles of COS were performed. Duration of stimulation, total amount of gonadotropins used, number of retrieved oocytes, percentage of cycle cancellation and pregnancy rate were considered.

Main results and the role of chance: Patients of group A-COR compared to group A-NOR had a longer stimulation (11.4 ± 3.4 days vs 10.7 ± 2.8 days, p<0.0001) and a higher dose of gonadotropins used (2721 ± 1021 U.I. vs 1799 ± 927 U.I., p<0.0001). The number of retrieved oocytes decreases with the reduction of AMH level (3.4 ± 2.2 A-COR vs 5.3 ± 3.6 A-DOR vs 7.8 ± 4.6 A-NOR, p<0.0001), whereas the percentage of cycle cancellation was higher in group A-COR compared to group A-NOR (12.2% vs 0.5%, p<0.0001). The pregnancy rate in group A-COR was lower than in group A-NOR (29.4% vs 42,3%, p<0.0001).

Pregnancy rate of group A-COR was higher than that of group B-COR (29.4% vs 20,6% p<0.0001) but similar to that of group B-NOR (29.4% vs 30.7%, p=n.s.)

Limitations, reasons for caution: The scientific community has not determined a standardized cut off to define normal, diminished or critical ovarian reserve. There is not a recognized method to dose AMH level, therefore is not possible to compare values measured with different techniques. The cut-off used in this study are based on literature review.

Wider implications of the findings: Despite critical ovarian reserve, young patients seem to compensate the low number of oocytes with good quality gametes resulting in a satisfactory pregnancy rate.

Trial registration number: \

P-406 Oocyte accumulation as a strategy to optimize clinical outcomes in infertile poor ovarian responders meeting the Bologna criteria: A real-life non-randomized prospective study

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Study question: Does oocyte accumulation offer reasonable chances of pregnancy in infertile poor ovarian responders (PORs)?

Summary answer: Oocyte accumulation in PORs appears as a successful strategy for both treating their infertility as well as while preserving their fertility potential through embryo cryopreservation.

What is known already: In Assisted Reproductive Technology (ART), chances of live birth are highly correlated to the number of retrieved oocytes. PORs are particularly affected by this issue, since recent studies have set a threshold of 3 oocytes as a predictive factor of live birth. Moreover, repeating ART cycles in such patients represents a high risk of repeated failure, associated to an elevated drop-out rate. Hence, the management of PORs remains quite challenging, and the strategy of oocyte accumulation might constitute an interesting option to optimize clinical outcomes in these patients. However, data on this topic are still scarce.

Study design, size, duration: This single-center, prospective study has been in progress since January 2014. A total of 40 infertile couples have been enrolled in an oocyte accumulation program when female partner was diagnosed as POR according to Bologna criteria (2 out of the 3 following criteria: <4 oocytes in a previous cycle, abnormal markers of ovarian reserve, age >40 years) associated to any other infertility cause (male, female or both).

Participants/materials, setting, methods: Enrollment in the program required patients' written informed consent. Metaphase-2 (M2) oocytes were accumulated by vitrification during ≥1 controlled ovarian hyperstimulation cycle (s) (n = 85 cycles). Then, all vitrified oocytes were warmed and inseminated using ICSI simultaneously with fresh M2-oocytes retrieved during a last cycle of ovarian stimulation. Twenty-four couples underwent the whole procedure (oocyte-vitrification cycles, followed by oocyte warming +/- simultaneous fresh ICSI cycles). Biological and clinical outcomes of these cycles were analyzed.

Main results and the role of chance: Briefly, median patients' age was 36.0 years [28-42]. Their median serum AMH and FSH levels as well as antral follicle count were 1.2 ng/mL [0.2-2.8], 7.6 IU/L [3.2-15], and 6 follicles [3-14], respectively. Each couple underwent an average of 2.6 previous IVF/ICSI attempts before entering our program of oocyte accumulation. During the oocyte accumulation period, a mean of 2.3 cycles/couple (in total, 55 cycles) were performed, leading to the vitrification of 6.2 M2-oocytes/couple (standard deviation (SD) = 3.0). A total of 149 M2 oocytes were warmed (survival rate = 81.2%), and 121 M2-oocytes were micro-injected simultaneously with 81 fresh M2-oocytes (8.4 M2-oocytes injected/couple; SD = 4.6). Number of oocytes retrieved, maturation, fertilization rates and embryo quality were comparable between oocyte accumulation and fresh cycles. So far, oocyte accumulation strategy resulted in 23 embryo transfers (ET) (mean number of transferred embryos = 2.4): 10 ET arose from warmed oocytes only (43.5%), 4 from fresh oocytes (17.4%) and 9 from both warmed and fresh oocytes (39.1%). Furthermore, supernumerary embryos were cryopreserved for 10 couples (41.7%). Finally, clinical pregnancy rate (CPR) of 33.3%/cycle (8/24), and cumulative CPR of 37.5%/cycle (9/24) were achieved. Interestingly, surplus embryo cryopreservation was performed for 55.5% of pregnant patients and only 33.3% of non-pregnant ones.

Limitations, reasons for caution: This preliminary study lacks statistical power, and further investigations are required to confirm the efficiency of oocyte accumulation in the management of infertile PORs. Then, cost-effectiveness issues will have to be considered.

Wider implications of the findings: This study highlighted acceptable CPR for poor-prognosis patients. Thus, enrollment of infertile PORs in oocyte accumulation programs might optimize their success rates. Moreover, this strategy could offer an opportunity of fertility preservation for women whose ovarian reserve is inexorably decreasing, and enhance their chances of achieving a subsequent pregnancy.

Trial registration number: Not applicable.

P-407 Physical activity and sedentary time before and during in vitro fertilization: a longitudinal study exploring the associations with infertility treatment outcomes

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Study question: Do physical activity (PA) and sedentary lifestyle influence controlled ovarian stimulation (COS) and embryo implantation and pregnancy outcomes in in vitro fertilization (IVF) treatment?

Summary answer: Physically more active women obtained higher number of oocytes and embryos after COS, while PA and inactivity levels did not influence embryo implantation in IVF.

What is known already: Regular PA has positive effects on human health and several epidemiologic studies indicate an association between self-reported PA and fertility. There are studies demonstrating that physically more active women during IVF have higher probability of pregnancy, while others, on the contrary, report negative impact of PA on IVF. All these studies have assessed PA using self-reported questionnaires, which give only a rough estimate of PA. Only one published study has measured objectively PA, with accelerometry, in infertile women after embryo transfer, where no effect of PA was detected. We still are lacking objective recommendations of PA for women undergoing IVF.

Study design, size, duration: This longitudinal study was carried out among 108 infertile women receiving fresh embryo transfer (ET) in their first IVF cycle. The study was carried out at the Reproductive Unit at the University Hospital between 2013 and 2016.

Participants/materials, setting, methods: Each woman provided three objective measurements of PA using accelerometry (ActiGraph GT2X+) of 2 weeks: 1) the baseline PA level, 1-6 months before IVF; 2) embryo implantation period, from the day embryo was transferred; 3) early pregnancy establishment period, from the day of pregnancy test. Lifestyle questionnaire, body composition and IVF treatment outcomes were recorded for each patient.

Main results and the role of chance: In general, the group of infertile women undergoing IVF treatment significantly reduced their PA levels and increased sedentary time. Physically more active and less inactive women obtained significantly higher number of oocytes and embryos after COS, while PA and sedentary lifestyle did not seem to have any effect on embryo implantation and early pregnancy establishment in IVF. Further, we found that those women with a high screen time during the weekends (>3 h per day) have 4.7 oocytes and 2.8 embryos less after COS than women with low screen time. Furthermore, isotemporal analysis demonstrated that if a woman would increase her daily light PA for 1.5 h and thus by reducing their daily sedentary time for 1.5 h, she would obtain 2 more oocytes and 1 more embryo after COS.

Limitations, reasons for caution: Regardless of the clear results of PA effects on ovarian stimulation, bigger sample size would increase the power of the study.

Wider implications of the findings: We can suggest recommendations for infertile women undergoing IVF indicating that light and moderate PA is beneficial for ovarian stimulation outcomes, while sedentary lifestyle clearly is not. Additionally, IVF patients could continue with their normal lifestyle and there is no need to reduce PA levels after embryo transfer procedure.

Trial registration number: Not applicable.

P-408 Euploidy rates in donor egg cycles are significantly influenced by the number of accumulated ovarian stimulations

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Study question: Could ovarian stimulation protocols influence oocyte quality, embryo developmental potential and the euploidy status?

Summary answer: The total number of previous ovarian stimulations in donor egg cycles positively correlates with embryo aneuploidy rates.

What is known already: The presence of chromosome aneuploidy is one of the most significant causes of embryo loss. Studies performed in the last two decades reveal these abnormalities affect a remarkable number of ART produced human embryos (over 50%). Although the majority of aneuploidies are known to be maternal age related, this phenomenon has also been observed in young patients and donors at a considerable and wide ranged frequency (20-60%). Apparently, range dependent from center and/or doctors involved suggesting stimulation and/or culture conditions as possible explanations. To date, no detailed analysis of such factors has been performed.

Study design, size, duration: This observational retrospective study includes data from 102 donor-oocyte cycles undergoing Preimplantation Genetic Testing for Aneuploidy (PGT-A) between January 2017 and January 2018. A total of 2850 oocytes and 569 embryos were studied. Only cycles from couples who showed no clear male factor infertility (normozoospermic sperm according to WHO) were selected. The influence of donor characteristics, ovarian stimulation protocol, dose and number on oocyte count and quality and on embryo competence and ploidy was assessed.

Participants/materials, setting, methods: 67 donors aged 18-34 (24.86 ± 3.87) participated in the study. Donors followed a protocol for ovarian stimulation with rFSH (Puregon®, Merck) with variable doses (AMH, folliculometry and weight dependant). Oocytes collected were in-vitro fertilised, embryos cultured to blastocyst stage (equal conditions), biopsied and PGT-A tested by NGS (VeriSeq protocol, Illumina). Oocyte count and quality, fertilization rate, blastocyst formation rate, embryo quality and chromosome status depending on donor age, BMI, stimulation dose and number were statistically explored.

Main results and the role of chance: A significant positive correlation was found between donor age and the number of oocytes retrieved (Pearson correlation, R=-0.294 p = 0.008). Young donors ≤27 tend to produce significantly higher number of oocytes compared to older (>27) donors (28.74%±1.46 vs 22.35%±1.83, Student´s t-test, p = 0.011). No such age influence was found in embryo competence neither in terms of blastocyst formation rate, good quality blastocyst rate or euploidy rate. No correlation between oocyte count and BMI, stimulation dose or number of ovarian stimulation cycles was identified.

Interestingly, embryonic aneuploidy rates in the donor egg cycles studied were associated with the number of stimulation cycles performed by the donor. The number of previous stimulations negatively influenced embryonic euploidy rates (Spearman correlation, R=-0.222, p = 0.036). A significant increase in the proportion of embryos presenting chromosome abnormalities was found in donors that had undergone more than 5 stimulation cycles compared to donors experiencing 5 or less stimulation cycles (31.93%±3.64 vs 24.40%±2.78, U-Mann Whitney, p = 0.037). Neither age, BMI or r-FSH stimulation dose or hormone levels showed such an impact on embryo ploidy.

Limitations, reasons for caution: The retrospective and unicentric design of this study may be a reason of caution. The number of donors and donor egg cycles was limited; this should be increased to confirm the results ob-tained. Further prospective studies are needed to validate our results.

Wider implications of the findings: Our results show a strong association between the number of previous stimulation cycles and embryo aneuploidy in oocyte donors. These data indicate that other factors like age, previous stimulations or ovarian damages of oocyte maturation and genetic competence, may influence chromosome abnormalities in IVF embryos.

Trial registration number: not applicable.

P-409 Nodal deletion increases susceptibility to inflammation-induced preterm birth in mice and human

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Study question: How does inflammation combined with reduced Nodal gene expression increase susceptibility to Preterm Birth in mice and humans.

Summary answer: Nodal heterozygous mice are senstive to LPS which causes preterm birth. In humans,Nodal revealed that Nodal SNPs correlate with increased susceptibility to inflammation-induced preterm birth.

What is known already: Potential risk factors prior to conception, during pregnancy genetic predisposition have been identified to correlate with increase susceptibility to preterm birth. Our lab has been studying the role of Nodal signaling during pregnancy. Nodal is belonging to the Transforming Growth Factor-beta superfamily. Previously we have demonstrated that Nodal signaling is active in uterus during early pregnancy and required both for embryo implantation and for the timing of parturition. We have a uterine specific deletion using

a progesterone receptor-Cre system and observed a 75% reduction in pregnancy, 25% of Nodal mutant gave birth two days prior to term.

Study design, size, duration: In mouse we have injected a low dose of LPS (1.4 mg/kg) which resulted in 50% of pregnant *Nodal* heterozygous mice to give birth preterm. Furthermore, we have also verified the protein and RNA levels of several pro-inflammatory cytokines using immunofluorescence and real time PCR. In the human study, 613 women (189 preterm and 424 term) from the Montreal Prematurty Study were genotyped for *NODAL* polymorphisms and assessed for bacterial vaginosis and placental inflammation.

Participants/materials, setting, methods: We are using an RT^2 Profiler PCR Array, we examined the expression of 84 genes involved in innate and adaptive immunity. Critical cytokines in the parturition cascade were significantly up-regulated in maternal decidua tissue of *Nodal* heterozygous mice at day 16.5 of pregnancy. We have also used different antibodies to look to different immune cells in placental tissue in our mouse model.

Main results and the role of chance: Our mouse study demonstrated that absence of one allele of *Nodal* leads to increased susceptibility to preterm birth in response to low doses of LPS. We also provide evidence that NODAL may regulate the immune system during late pregnancy and labor. We suggest that the *Nodal* heterozygous knockout mouse is a suitable model to study gene-environment interactions in human preterm birth. Future experiments will address the mechanism of how NODAL affects the immune system.

In human, we identified 12 known SNPs in the *NODAL* gene of participants in the study population. Of these, four had VAF > 1%, three of the *NODAL* polymorphisms were not associated with preterm birth.in more details, the rs2231947 variant allele was associated with increased risk for preterm birth among women with bacterial vaginosis. Among women without placental inflammation, the rs1904589 variant allele was associated with increased risk of preterm birth. Among women with placental inflammation, the rs10999338 variant allele was associated with reduced risk of preterm birth.

Limitations, reasons for caution: Experiments were performed on a mouse model and the implication of the Nodal signaling pathway in humans is still unclear.

Wider implications of the findings: As Nodal appears to be involved in the timing of parturition, manipulation of its activity or of its downstream signaling components may lead to effective treatments in the prevention of preterm birth.

Trial registration number: Funded by March of Dimes Grant, Toronto Dominion Bank Post-Doctoral Fellowship for Child Health Research Excellence and The Ministry of Higher Education of Saudi Arabia.

P-410 BMI adversely affect embryo quality and morphokinetics

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Study question: What is the effect of BMI on embryo's morphokinetics as reflected by timelaps scores with regard to treatment protocol IVF cycle outcome.

Summary answer: Obesity has adverse effect on IVF treatment represented by less TOP quality embryo and a trend of lower pregnancy rate.

What is known already: It is well established that there is a strong correlation between obesity and reproductive pathology including anovulation, infertility and obstetrical complication.

Study design, size, duration: A retrospective cohort study.

179 infertile women, total of 2280 oocytes.
January 2016 to December 2017

Participants/materials, setting, methods: Records of all fresh cycles that their embryos were cultured in a time-laps incubator were evaluated. Embryos were scored by EmbryoScope™ and correlated with patients BMI and clinical pregnancy rates.

Main results and the role of chance: 179 infertile women, total of 2280 oocytes were divided according to their BMI into 3 groups: BMI < 25, BMI 25-29, and BMI ≥30. The duration of treatment was significantly longer for the BMI≥30 (10.2 ± 1.6 days vs. 9.3 ± 2.2 days, respectively). The endometrium was significantly thicker in the BMI≥30 group (10.2 ± 1.6 vs. 9.8 ± 2.4). The Estradiol level on ovulation induction day, the number of oocyte retrieved, the number of oocytes at the M2 phase and the number of the normal fertilization oocyte were significantly higher at the BMI<25 group. We found significantly more TOP quality embryos in the BMI< 25 compared with the other groups (58% vs 52%). Pregnancy and clinical pregnancy rate demonstrated a trend towards higher pregnancy in the BMI<25 group vs. the BMI≥30 group (49% vs 32%, NS and 46% vs 32%, NS; rspectively).

Limitations, reasons for caution: Since it is a retrospective cohort study, the results shoud be interprated with cuation.

Wider implications of the findings: Understanding the adverse effects of obesity, may contribute to infertility couples education and may contribute to the understanding of adverse effect of obesity on oocyte developement.

Trial registration number: N/A.

P-411 Association of weight change since age 18 years and probability of live birth among women undergoing assisted reproduction

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Study question: What is the association between female weight change from young adulthood (age 18) to time of ART and the probability of live birth?

Summary answer: Greater weight gain in adulthood was associated with lower probability of live birth following ART, particularly among women who were ≥22.5 kg/m^2 at age 18.

What is known already: Pre-pregnancy overweight and obesity in adulthood has been associated with lower natural fertility and lower success rates in ART. While short-term weight loss trials have not demonstrated improved live birth rates among obese women undergoing ART, the impact of longer-term weight change on ART outcomes, particularly throughout early adulthood has yet to be studied.

Study design, size, duration: Our analysis included 448 women (767 ART cycles) enrolled in the Environment and Reproductive Health (EARTH) study -a prospective cohort study- recruited between 2005-2016 at the Massachusetts General Hospital Fertility Center.

Participants/materials, setting, methods: At study entry, women were asked to recall their weight at age 18 and trained research nurses measured women's current height and weight. ART outcomes were abstracted from medical records. We used multivariate generalized linear mixed models with random intercepts to account for repeated observations adjusted for age, smoking status, race, height, and BMI at age 18.

Main results and the role of chance: At time of study entry, 62% of women had gained > 5 kg since age 18 and 33% were overweight or obese. The

adjusted percentage of initiated ART cycles resulting in live birth (95% CI) among women who gained 0-5 kg, 5-9.9 kg, 10-14.9 kg, and ≥15 kg since age 18 was 57.2% (49.8%, 64.2%), 58.9% (51.4%, 66%), 54.6% (45.2%, 63.7%), and 48.1% (40.5%, 55.9%), respectively (p-trend = 0.08). There was no difference in the probability of live birth between women who lost weight versus those who remained relatively weight stable (gained 0-5 kg). The inverse association between adult weight gain and probability of live birth was more pronounced among women who were ≥22.5 kg/m^2 at age 18 (p-interaction = 0.09). Specifically, among women who were ≥22.5 kg/m^2 at age 18 the adjusted difference in percentage of initiated ART cycles resulting in live birth was 17.6% (95% CI 2.9%, 28.0%) comparing women who remained weight stable versus those who gained ≥15 kg in adulthood. This similar comparison in women <22.5 kg/m^2 at age 18 was 6.6% (95% CI -2.5%, 14.6%).

Limitations, reasons for caution: Weight at age 18 was self-reported by participants, which may lead to misclassification. There were very few women who lost weight since young adulthood which limited the statistical power of those comparison. Despite our adjustment for a variety of confounders, residual confounding is possible.

Wider implications of the findings: Women who gained ≥15 kg in adulthood have lower probability of live birth per initiated ART cycle, particularly among women who were heavier at age 18. These results add to the growing literature supporting the benefits of minimizing weight gain in adulthood on female fertility.

Trial registration number: None.

P-412 Predictive models for pregnancy outcomes with non-IVF treatments reveal that protocol choice, not patient-specific factors, are the primary risk for multiple gestations

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Study question: Which factors are predictive of ongoing pregnancy and risk of multiple gestation with non–*in vitro* fertilization (non-IVF) treatment?

Summary answer: Personalized predictive models for OPR (ongoing pregnancy rate) and MGR (multiple gestation rate) highlight an increased risk of multiple gestation with non-IVF treatment using gonadotropins.

What is known already: Non-IVF treatments with OI using oral medications (clomiphene citrate or letrozole) or injectable gonadotropins with IUI or timed intercourse (TI) are frequently used as first-line fertility treatment due to the lower physical and financial burden. Treatments vary in pregnancy rate as well as MGR, and thus in associated maternal and fetal risks. Although previous studies have looked for factors predictive of pregnancy, they did not analyze the multiple gestation rate. Patient-specific predictions for both pregnancy and risk of multiples across treatment options and cumulatively over multiple cycle attempts can help with patient counseling and allow for informed treatment decisions.

Study design, size, duration: Predictive models were built using retrospective data collected from 173,916 cycles from 69,003 patients at 13 fertility centers in the United States between 2002 and 2017. We included cycles that received the OI medications clomiphene citrate or letrozole (Orals) and/or injectable FSH or hMG (Gnd) with or without IUI. We excluded cycles that utilized donor sperm.

Participants/materials, setting, methods: Outcomes evaluated were OPR (presence of fetal heartbeat upon discharge to obstetrical care) and MGR (presence of multiple fetal heartbeats relative to ongoing pregnancy). Patient data were divided into a training set (2/3) and a validation set (1/3). The OPR and MGR models were logistic regression models and used inverse probability-of-censoring weighting to compensate for informative censoring. Continuous patient factors were evaluated for nonlinear spline terms.

Main results and the role of chance: In a multivariate model for OPR, significant nonlinear patient factors included age, anti-Müllerian hormone, body mass index, basal antral follicle count, and day 3 FSH, estradiol, and luteinizing hormone. In addition, endometriosis (odds ratio [OR] 0.48), tubal factor (OR 0.57), and sperm total motile count <10 million (OR 0.55) and morphology <4% Kruger (OR 0.82) (p<0.001 for all) had a negative impact on OPR. Notably, we found that inclusion of Gnd or IUI in the protocol led to increased OPR (OR: Gnd+IUI, 2.80; Gnd+Orals+IUI, 2.13; Orals+IUI, 1.40; Gnd+TI, 2.04; Gnd+Orals+TI, 1.37; Orals+TI, 1.0). Modeling OPR over multiple cycles, we observed that the incremental probability with each additional cycle varied by treatment protocol, with a greater decrease for cycles including Gnd.

Additionally, a multivariate model for MGR showed that risk primarily depended on protocol choice, with Gnd inclusion significantly increasing risk of multiple gestation (OR: Gnd+IUI, 3.34; Gnd+TI, 3.09; Gnd+Orals+IUI, 1.86; Gnd+Orals+TI, 1.45; Orals+IUI, 1.23; Orals+TI, 1.0; p<0.001). The MGR was independent of cycle attempt number.

Evaluating the OPR model against a holdout validation dataset, we observed an AUC of 0.65 and a Brier score of 0.091. The MGR model had an AUC of 0.65 and a Brier score of 0.079.

Limitations, reasons for caution: The described predictive models were built and validated on retrospective data and should be independently validated using a prospective dataset. In addition, differences in clinic treatment and monitoring protocols may influence both OPR and MGR.

Wider implications of the findings: Personalized probabilities for both OPR and the associated MPR are important for helping patients make treatment choices to maximize their likelihood of a healthy outcome. The >3-fold increase in MGR with Gnd compared to oral medications highlights the importance of balancing this significant risk with the incremental increase in OPR.

Trial registration number: N/A.

P-413 Comparative study of the Poseidon subgroups 1a and 1b low responder IVF cycles

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Study question: What are the comparative trends of the Poseidon group 1a and group 1b low responder IVF cycles?

Summary answer: Low responder cycles increased with time and age with group 1a carrying a significantly higher no embryo transfer rate and lower clinical pregnancy rate.

What is known already: The 4 group Poseidon stratification of low responders was introduced in 2016. Unexpected poor or suboptimal responders, 34 years and younger with a normal ovarian reserve are classified as Poseidon group 1. Subgroups 1a and 1b are based on the number of oocytes retrieved after standard ovarian stimulation. Low responders with fewer than 4 oocytes are classified as subgroup 1a. Those with 4-9 oocytes are classified as subgroup 1b. Since this is a new classification, there is a need for fertility centres to apply it to their own population of women to enable the development of reproducible and comparable research.

Study design, size, duration: Retrospective comparative study of 777 IVF cycles of Poseidon subgroups 1a and 1b low responders. The IVF cycles were undertaken between Jan 2007 to Dec 2017.

Participants/materials, setting, methods: First IVF cycle low responders, 34 years and younger with normal ovarian reserve parameters (AFC >5, AMH>1.2 ng/ml). This group was divided into subgroups 1a and 1b. Subgroup 1a were low responders with fewer than 4 retrieved oocytes. Subgroup 1b were low responders with 4-9 retrieved oocytes. The data was extracted from an electronic register of IVF cycles that were undertaken at a university hospital fertility centre. Chi square test for statistical analysis. Significance: p <0.05.

Main results and the role of chance: There was a 4-fold and 7-fold increase in Poseidon subgroup 1a and subgroup 1b first IVF cycles respectively. Poseidon subgroup 1a consisted of 114 women (14.6%) and subgroup 1b consisted of 663 women (85.4%). An upward trend with age was found in both subgroups. The mean (SD) number of oocytes retrieved per cycle was 2.2 ± 0.8 in subgroup 1a, and 6.7 ± 1.7 in subgroup 1b. The mean (SD) number of embryos transferred per cycle was 1.1 ± 0.5 in subgroup 1a and 1.1 ± 0.6 in subgroup 1b.

Embryo transfer was not achieved in 111 women. The no embryo transfer rate was significantly higher in subgroup 1a (29.8% v/s 11.6%, p<0.05). Of the 111 women, 18 (16.2%) undertook further IVF treatment. Of these 18 women, there was a cumulative pregnancy rate of 44.4% over three cycles.

The clinical pregnancy rate of subgroup 1b was significantly greater than subgroup 1a (41.8% v/s 22.8%, p<0.05). A sub-analysis of single embryo transfers found that the clinical pregnancy rate of subgroup 1a was lower than subgroup 1b (30.1% v/s 47.1%, p<0.05). For double embryo transfers, the clinical pregnancy rate in subgroup 1a was significantly higher than subgroup 1b (57.1% v/s 40.0%, p<0.05).

Limitations, reasons for caution: This was a retrospective study which is historically known for its limitations. However, there was no missing data in this study.

Wider implications of the findings: Research is warranted to determine parameters that can identify Poseidon type 1 women prior to ovarian stimulation. This will enable optimisation of treatment and reproductive outcomes. Double ET can be considered for group 1a women based on low oocyte numbers, difficulty creating embryos and low pregnancy rates with single ET.

Trial registration number: Not applicable.

P-414 Antibodies to chlamydial heat shock protein 60 kDa in sera and follicular fluid of women undergoing IVF

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Study question: To determine whether past or persistent chlamydial infection is associated with the results of infertility treatment with *in vitro* fertilization (IVF)

Summary answer: No relation between antibody positivity to chlamydial heat shock protein 60 kDa (cHSP60) and IVF outcome was found.

What is known already: Recent studies have shown that serum antichlamydial antibodies are found in almost every second woman seeking treatment for tubal infertility with IVF. However, seropositivity of follicular fluid to chlamydial antigens and its diagnostic value is described only in several publications. Even more limited is the knowledge on the prevalence of persistent infection with *Chlamydia trachomatis*, defined by anti-cHSP60 antibody positivity, in women undergoing infertility treatment.

Study design, size, duration: A prospective comparative cohort study of 242 women with tubal factor of infertility was conducted at the IVF department of a state institution between January 2012 – January 2016.

Participants/materials, setting, methods: Serum (obtained at the first day of stimulation) and follicular fluid (aspirated at the day of oocyte pick-up) samples were analyzed using enzyme-linked immunosorbent assay for immunoglobulins (Ig) to major chlamydial antigens (IgG, IgA) and for anti-cHSP60 IgG. Study participants were divided to 2 groups according to the results of serum anti-*C.trachomatis* IgG test: 122 seropositive (main) and 120 seronegative subjects (comparison). Primary study outcome was clinical pregnancy rate.

Main results and the role of chance: Prevalence of anti-*C.trachomatis* IgG, IgA and anti-cHSP60 IgG was 50.4%, 10.7% and 5.8% in sera; 37.1%, 4.6% and 5.6% – in follicular fluid. Major stimulation and in vitro stage parameters did not differ between the study groups. 'Poor response' to ovarian stimulation was found to be 2 times more frequent in the main group (22.9% vs. 13.3%, p = 0.039), however was not associated with anti-cHSP60 antibody detection both in sera (OR: 2.56, 95%CI 0.87-3.34) and follicular fluid (OR: 1.93, 95%CI 0.99-3.87). Seropositive and seronegative women demonstrated similar clinical pregnancy (20.5% vs. 25.0%) and life birth rates (13.1% vs. 20.0%). Incidence of missed abortion after IVF was higher in the main group (28.0% vs 3.3%, p = 0.018) and showed no relation with anti-cHSP60 antibody positivity.

Limitations, reasons for caution: Despite the relatively sufficient initial size of the study, the subpopulation of anti-cHSP60 positive women appeared to be rather small. In addition, only women with tubal factor of infertility were assayed during the trial. All of this expectedly limits the generalizability of the obtained results.

Wider implications of the findings: The possibility of *Chlamydia trachomatis* persistence in women with tubal factor of infertility should be investigated with the employment of additional microbiological methods. Further trials may estimate the diagnostic and prognostic value of antichlamydial antibody testing for women undergoing IVF.

Trial registration number: Not applicable.

P-415 Acquisition of a next baby using a frozen sibling embryo(s): a proposal for advanced aged infertility couples to build a desired family

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Study question: To evaluate the predictability of the acquisition of a next baby using a frozen sibling embryo(s) from the successful single embryo transfer (SET) cycle.

Summary answer: Our data indicate that 38.8% couples that keep at least one sibling embryo for the next baby may have another success without additional oocyte retrieval.

What is known already: The essential aim of ART is the birth of one single healthy child. SET has been introduced to avoid multiple pregnancy that is a major complication of ART. However, in advanced ages, usually from the latter half of 30's, pregnancy rate is gradually decreasing, and miscarriage rate is markedly increasing. Thus, it is difficult for advanced aged subfertility couples to build a desired family using high potential embryos with safety. Improvement of cryopreservation of embryos may contribute to maintain the reproductive results at the age of the embryo freezing.

Study design, size, duration: This is a retrospective clinical study in our institute from 2007 to 2017. We included 121 patients who gave birth to a baby through a timelapse-confirmed embryo transfer, keeping a sibling frozen embryo(s) and used the frozen embryo(s) for another baby.

Participants/materials, setting, methods: Patients were divided into acquisition and non-acquisition group (AG and non-AG) according to the final results of the new baby acquisition through the sibling embryo transfer(s). Main outcome was the ratio of AG. Furthermore, we newly defined the original embryo score based on the quality of the embryo at any developmental stage. The total score of the frozen embryos at the time of cryopreservation (F score) was evaluated.

Main results and the role of chance: The number of AG and non-AG were 47 (38.8%) and 74 (61.2%), respectively. There was one monozygotic twining in AG. No sibling embryo was remained in non-AG. Female age (32.3 ± 2.8 *vs.* 32.3 ± 3.5; *P* = 0.85) and body mass index (21.3 ± 2.2 *vs.* 21.4 ± 4.2; *P* = 0.15) were similar between the two groups. Common etiological factors of subfertility were also similar between the groups. The number of retrieved oocytes (15.9 ± 7.4 *vs.* 13.4 ± 7.4; *P* = 0.03), that of MII oocytes (12.5 ± 5.9 *vs.* 9.8 ± 5.2; *P* = 0.006), that of fertilized oocytes (10.4 ± 4.7 *vs.* 8.0 ± 4.4; *P* = 0.002), that of frozen embryos (6.4 ± 4.1 *vs.* 4.2 ± 3.3; *P* = 0.0003), F score (9.9 ± 6.5 *vs.* 5.6 ± 4.1; *P* < 0.0001) were higher in AG. The total amount of administrated FSH/HMG and the peak estradiol concentration of each group (AG *vs.* non-AG) were 2,210 ± 1,007 IU *vs.* 1,965 ± 915 IU (*P* = 0.06) and 3,680 ± 2,262 pg/ml *vs.* 2,956 ± 2,627 pg/ml (*P* = 0.009), respectively.

Limitations, reasons for caution: Limitations of this study include the smaller sample size and the retrospective study from a single institute. Consideration for the next baby was not given into the stimulation protocol in this study.

Wider implications of the findings: This study indicate that much higher acquisition rate for the next baby may be expected if we utilize information on the number of children of couple's desire, and if more precise developmental prognosis of each embryo is available in the near future.

Trial registration number: Not applicale.

P-416 Does starting day of progesterone intake really matter in frozen blastocyst transfer cycles?

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Study question: This study aims to evaluate whether a 1-day delay in the starting day of progesterone intake can change the clinical outcome in frozen blastocyst transfers.

Summary answer: Both P+0 and P+1 can be taken as the starting day of progesterone supplementation resulting in similar clinical outcome in frozen blastocyst transfers.

What is known already: Due to recent improvements in human embryo cryopreservation, deferred embryo transfer strategies involving frozen cleavage and blastocyst-stage embryos are increasingly adopted in IVF clinics worldwide. On the other hand, this continuous increase in the number of frozen embryo transfer (FET) cycles also necessitates successful endometrial preparation. Although encouraging clinical outcomes have been reported in the literature, optimal timing of progesterone intake during FET cycles still need to be established. Since serum progesterone levels start to rise before ovulation, it can be argued that progesterone administration before FET should also start earlier.

Study design, size, duration: This retrospective study consisted of 1407 consecutive FET cycles involving single blastocysts performed between September 2013 - August 2017. In all cycles, same endometrial preparation strategy was used except the starting day of progesterone which was either P+0 or P+1 depending on the clinician's preference. All embryos were warmed in the morning of embryo transfer day and FET was performed in the afternoon.

Participants/materials, setting, methods: In 749 FET cycles, progesterone intake was found to be started as P+0 (Group I) and in 658 cycles, it was commenced as P+1 (Group II). During the study period, embryo culture media as well as vitrification/warming media were purchased and used from the same producer (Irvine Scientific). FETs were performed either on the fifth or sixth day of progesterone supplementation, which was given either from vaginal or intramuscular route.

Main results and the role of chance: In both groups, general patient- and cycle-related demographics and distributions such as female age, BMI, number of previous ART trials, reason of infertility as well as quality scores and distribution of transferred embryos scores were found to be similar (p>0.05). Comparison analysis also displayed high but similar biochemical pregnancy (74.2% vs. 69.6%) and clinical pregnancy rates (68.6% vs. 65.3%; p>0.05). Twenty-eight percent of these pregnancies have already resulted in live birth in Group I and 30.2% in Group II respectively.

Limitations, reasons for caution: The main limitation of our study was its retrospective design. Also, patients were not blinded about the exact duration of progesterone supplementation.

Wider implications of the findings: Our study shows that setting of starting time of progesterone intake either as P+0 or p+1 does not create any significant difference in clinical outcomes in frozen single blastocyst transfer cycles. Further randomized studies involving single euploid blastocysts with a single progesterone supplementation route are needed to confirm these results.

Trial registration number: Not applicable.

P-417 Impact of Body Mass Index on outcome of the first pregnancy after referral among women with recurrent pregnancy loss

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Study question: Does Body Mass Index (BMI) impact outcome of the first pregnancy after referral among women with recurrent pregnancy loss (RPL) referred to a tertiary center?

Summary answer: BMI is not a prognostic factor for the chance of live birth among women with RPL referred for evaluation and treatment in tertiary center.

What is known already: Obesity has been reported to affect the chance of a live birth in women with RPL. One study reported that BMI, maternal age, number of previous losses, and ethnicity were associated with pregnancy outcome. They found an increased risk of another pregnancy loss in in obese women with unexplained RPL but not in overweight women. We have previously reported that BMI is not a significant prognostic factor for live birth in women with RPL and a normal menstrual cycle. As concluded in the new ESHRE guideline the impact of BMI on the prognosis of live birth has not been determined.

Study design, size, duration: A retrospective cohort study including 1068 Danish women with RPL, defined as three consecutive pregnancy losses, referred for evaluation and treatment at the National Danish RPL Unit between 2000-2010

Participants/materials, setting, methods: Anonymized data on age, number of pregnancy losses prior to referral, outcome of first pregnancy after referral, BMI and menstrual cycle (normal vs. oligomenorrhea) were retrieved from the Danish National RPL database. Chi-square testing and logistic regression analysis were performed. A p-value of <0,05 was considered significant. Women, whose first pregnancy outcome after referral were ectopic pregnancy or induced abortion, were excluded from the analysis.

Main results and the role of chance: Normal weight women with BMI between 18.5–24.9 kg/m^2 constituted the largest group (n = 623, 59.4%) followed by overweight women with BMI between 25.0-29.9 kg/m^2 (n = 242, 23.1%), obese women with BMI ≥30 kg/m^2 (n = 165, 15.7%), and the smallest group being underweight women with BMI <18.5 kg/m^2 (n = 18, 1.7%). Live birth rates in the BMI groups were 60.3% (n = 356); 58.6% (n = 136); 55.3% (n = 89); 64.7% (n = 11), respectively. There was no impact of BMI on chance of a live birth, neither as a grouped variable (<18.5; 18.5-24.9; 25-29.9; ≥30), OR 0.93 (CI 0.79-1.09), $p = 0.37$, nor as a continuous variable OR 0.992 (CI 0.97-1.02), $p = 0.56$ adjusted for menstrual cycle (normal vs. oligomenorrhea), maternal age, and number of previous pregnancy losses. We did not find a poorer prognosis for live birth among obese women compared with non-obese women (% live birth 55.3 vs. 60.2, $p = 0.26$). Maternal age ranged from 20 to 45 years (mean age 33 years). Increasing maternal age reduced the chance of a live birth, OR 0.97 (95% CI 0.94-1.00) $p = 0.05$, as did number of prior losses (OR 0.80 95% CI 0.69-0.92), $p = 0.002$.

Limitations, reasons for caution: We did not adjust for ethnicity, karyotype, autoimmune and endocrine diseases. Especially karyotyping of prior losses could change our results as a higher number of euploid losses are found when BMI >25 kg/m^2, suggesting that high BMI or associated conditions could be the cause in overweight and obese women.

Wider implications of the findings: Our study in women with RPL cannot confirm that high BMI is a negative prognostic factor for birth in the first pregnancy after referral. We confirm that maternal age and number of losses are important prognostic factors. These findings merit further scrutiny and additional studies are needed.

Trial registration number: N/A.

P-418 The influence of subfertility, type of counsellor and educational level on women' choices regarding first trimester prenatal screening

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Study question: Does subfertilit, type of counsellor and education level influence the choice for first trimester prenatal screening?

Summary answer: subfertility and type of counsellor have limited impact on the choice of women for prenatal screening, while education level does.

What is known already: Prenatal screening is performed between 11 and 14 weeks of gestation and provides a risk assessment concerning congenital abnormalities. Since 2017 a non-invasive prenatal test is accessible to all pregnant women, screening for trisomy 13, 21 and 18. In case of an increased risk for congenital abnormalities, termination of pregnancy is permitted till 24 weeks of gestation.

In the Netherlands parental counselling is practiced by both midwives and gynaecologists. Women with healthy pregnancies, defined as a low-risk pregnancy without co-morbidities, are counselled by a midwife. Women with high-risk pregnancies (i.a. previous preeclampsia) are counselled by a gynaecologist.

Study design, size, duration: For a period of 10 months, a questionnaire regarding prenatal screening was given to all women with a new pregnancy, by midwives and gynaecologist in Maastricht, the Netherlands. Prior to the questionnaire, women were informed about the prenatal screening, impact of outcomes). Survey questions focused on social and medical maternal characteristics, socioeconomic. status and choice and motivation for prenatal screening. A total of 82 questionnaires were completed and returned.

Participants/materials, setting, methods: The questionnaire consisted of three parts. The first part focused on the choice of first trimester screening, the second part on screening at 20 weeks of gestation (ultrasound) and the third part on paternal influence regarding (shared)decision making. Questions were formatted into statements and provided with an answer scale from 0 till 4 (0 = no influence, 4 = highly influential). Data on maternal, obstetric and socioeconomic status were recorded in an electronic database.

Main results and the role of chance: In total 258 women returned a completed questionnaire The average age was 31 ± 4 years and 56% of all women were multiparous. Subfertile were 10%. Neither subfertility itself, nor factors related to it (type, cause, duration) affected the choices women made regarding prenatal screening, there was a non-significant trend among subfertile women to more towards NIPT 4% vs 1%, and less towards combined test 43% vs 59%, and invasive tests (0% vs 5%), compared to control women. The type of counsellor did not affect the choice for FTS, 68.6% in the midwife-counselled group vs 69.2% in the gynaecologist-counselled group ($p = 0.66$). Education level was defined as "low educated" (completed primary school), "secondary trained" (completed any secondary education) and "high educated" (bachelor' or masters' degree) according to the Dutch Statistic Registry. The percentage of women that declined versus those that choose FTS were 5% vs 9%, 64% vs 25% and 32% vs 66%, respectively. A significant difference was found between the "low educated" and "high educated" group with a p-value of 0.006.

Limitations, reasons for caution: The educational level of the subjects does not match the Dutch society, more low educated people compared to this study. Furthermore, counselling was not performed in a controlled setting.

Wider implications of the findings: These results show that subfertility and type of counsellor have limited impact on the choice of women for prenatal screening, while education level does Further research should focus on the reason for this difference and eventually may lead to more intensive counselling in lower educated women.

Trial registration number: NA.

P-419 RCT of Single versus double intrauterine insemination in ovarian hyperstimulation cycles

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Study question: To find out any difference in sucess rates between single and double IUI groups in Ovarian Hyperstimulation cycles with multi-follicular development?

Summary answer: Double IUI offers no clear benefit in the overall clinical pregnancy rate in Ovarian Hyperstimulation cycles with multi-follicular development.

What is known already: Correct timing of intrauterine insemination (IUI) might optimize the success rate in infertile couples.

Nonetheless, timing of ovulation varies and might depend on the ovarian stimulation protocol used.

Away to bypass this problem in IUI cycles is to increase the frequencyof IUI in the same treatment cycle.

Early randomized trials revealed higher clinical pregnancy rates during double IUI treatmentcycles. IUI is an evidence-based, first-step approach for the management of patients with unexplained infertility (Verhulst et al., 2006), and it is a generally preferred treatment for mild male factor infertility, ovulatory dysfunction,cervical factor infertility and endometriosis (Duran et al., 2002).

Study design, size, duration: 426 women who underwent IUI from 2015 to 2017.under age of 40 yrs.

Women were randomly assigned to single (n = 213) or double (n = 213) IUI groups.

Women in the single group underwent IUI performed 36-38 hours after human chorionic gonadotropin (HCG) administration.

Women in the double group underwent two IUIs performed 18–20 and 40-42 hours after HCG administration. The main outcome was clinical pregnancy Rate by evidence of fetal cardiac activity.

Participants/materials, setting, methods: 426 women who underwent IUI from 2015 to 2017.under age of 40 yrs. Women were randomly assigned to single (n = 213) or double (n = 213) IUI groups. Women in the single group underwent IUI performed 36-38 hours after human chorionic gonadotropin (HCG) administration. Women in the double group underwent two IUIs performed 18–20 and 40-42 hours after HCG administration.

The main outcome was clinical pregnancy Rate.

Data were analyzed by SPSS software.

Main results and the role of chance: Pregnancy rate was 10.2% in the single IUI group and 11.4 % in the double IUI group. difference between the two groups was not statistically significant.

There were no significant difference between the groups regarding women's age, their husbands' age, women's BMI,duration of infertility, causes and types of infertility, the level of AMH, FSH and LH, the number of dominate follicles, endometrial thickness, endometrial pattern, and the sperm character ($P > 0.05$)

Limitations, reasons for caution: Despite the 36th hour being the preferred timing for IUI, there was no difference regarding pregnancy rates between single and double inseminations.

Wider implications of the findings: There is consensus that controlled ovarian hyperstimulation(COH) combined with an IUI is a much more effective treatment for infertility than single applications of IUI or COH. Limited data exist on the optimal timing of an IUI and the number of inseminations needed to improve pregnancy rates.

Trial registration number: Not applicable.

P-420 The clinical potential of "random start" ovarian stimulation of fertility preservation for Japanese breast cancer patients

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Study question: Is "Random start" ovarian stimulation for Japanese breast cancer patients effective?

Summary answer: The "Random start" ovarian stimulation protocol is effective method for breast cancer patients who need to start their cancer treatment immediately.

What is known already: The traditional approach of ovarian stimulation as fertility preservation depends on their menstrual cycle. As a result, some of the patients may give up fertility preservation, because there are the possibilities of a significant delay of cancer treatment and increasing psychologic stress for the

patients. For that reason, to prevent any delay in cancer treatment, the "Random start" ovarian stimulation has been reported as effective for cryopreservation at any point during the menstrual cycle in 2013.

Study design, size, duration: Data were retrospectively obtained from the clinical records of 44 breast cancer patients who were referred to the Oncofertility Unit at the Center for Reproductive Medicine of St. Marianna University Hospital from March 2014 to December 2017.

Participants/materials, setting, methods: The breast cancer patients who hope to preserve own fertility were received controlled ovarian hyperstimulation regardless menstruation cycle for oocyte or embryo cryopreservation. We checked back the clinical records of patients and figured out their backgrounds, the way of stimulation, the phase of menstruation (follicular, periovulatory, luteal), and the level of serum anti-Müllerian hormone (AMH), the number of oocytes per cycle and the mature oocytes rates.

Main results and the role of chance: Mean age of 44 participants was 34.9 (±3.8) years-old, and 18 of them were unmarried. They had normal menstrual cycles prior to treatment and their average level of serum AMH was 5.1 (±2.8) ng/ml. The period from consultation to the first visit to our hospital was 7.4 (±5.1) days. And the surplus period, which was period for fertility preservation from visiting our hospital to starting the cancer therapy, was around 1.4 (±0.6) months. Nineteen of them used GnRH antagonist for ovarian stimulation, 24 of them were stimulated with short protocol. In addition, 33 of them used aromatase-inhibitor combined FSH agents. Altogether 8.9 (±6.8) oocytes per cycle were retrieved, the mature oocytes rate was 0.86(±0.2), which outcomes were comparable to previous reports. The phase of menstruation did not affect either the number of oocytes extracted ($p = 0.34$) or maturation oocytes ($p = 0.10$). Also, no complications (e.g. OHSS, infection,etc.) resulted from the procedure.

Limitations, reasons for caution: This study was only data which are the numbers of oocytes per cycle and the mature oocytes rates, but the fertilization rates, pregnancy rates are not evaluated enough, because we have few embryo transfer cases. Therefore, they are needed to evaluate, intend for verification the efficacy of "Random start" method.

Wider implications of the findings: Present research outcome which is consistent with published data may promote "Random start" ovarian stimulation for fertility preservation in Asian countries, too.

Trial registration number: This study received Institutional Review Board approval from St. Marianna University of Medicine. (approval No. 1588 2873 3464 3486)

P-421 Asymptomatic bacterial colonization during embryo transfer and its impact on ICSI pregnancy rate

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Study question: What's the effect of bacterial contamination of the embryo transfer catheter and the vagina on ICSI outcomes.

Summary answer: This study shows that the contamination of the embryo transfer catheter by cervical-vaginal bacteria is associated with significantly decreased pregnancy rates during ICSI.

What is known already: As the procedure of ICSI involves the replacement of embryos into the uterine cavity using a catheter that passes through the cervix, bacterial contamination during embryo transfer is possible. Studies have demonstrated a 50% reduction in pregnancy rate when a contamination of the embryo catheter tip occured. Furthermore, a decreased live birth rate has been reported in patients in whom pathogenic bacteria were isolated from the embryo transfer catheter tip. Dominant microorganisms involved were B and D groups *Streptococci, E.coli* and *Enterococcus.*

Study design, size, duration: A prospective, longitudinal and analytical study during eight months (from February 1st to October 31st, 2017)

Participants/materials, setting, methods: The study included patients who attended the ART center for embryo transfer and who matched the following criteria: age ≤ 40, normal uterine ultrasound and hysteroscopy, first or second attempt of ICSI, use of fresh semen and transfer of at least one good quality embryo. At the time of embryo transfer, three samples were done for microbiological analysis: a vaginal swab, a cervical mucus sample and the tip of the transfer catheter.

Main results and the role of chance: The study included 40 patients. The overall pregnancy rate was 52.5%. Catheter contamination occurred in nine cases (22.5%). The most frequently isolated bacteria were Streptococcus anginosus (37%), Gardnerella vaginalis (27%) and Streptococcus agalactiae (18%). In all cases of contamination, the same isolated bacteria were also found at in the vagina and in the cervical mucus. In cases of contamination with Gardnerella vaginalis, the study of vaginal flora based on the calculation of the Nugent score showed bacterial vaginosis. No cases of pregnancy have been obtained when a contamination of the transfer catheter occurred. However, in the absence of contamination, the success rate was 61.2% ($\rho = 0.001$). Patients' characteristics, stimulation protocols, embryology, number and quality of embryos transferred and partners sperm parameters did not vary significantly between the two groups (patients with catheter contamination and patients without catheter contamination). We can then speculate that the presence of these bacteria on the cervix at the time of embryo transfer could affect the pregnancy rate during ICSI.

Limitations, reasons for caution: Even if the results of this study were statistically significant, they need to be confirmed on large scale since the population size was relatively small.

Wider implications of the findings: If the negative effects of bacterial contamination during embryo transfer are confirmed on larger population, systematic antibiotic prophylaxis may be effective to limit this impact. After the inclusion of additional subjects, we could study the cervical vaginal flora before and after antibiotic administration.

Trial registration number: not applicable.

P-422 Embryo development, aneuploidy and clinical outcome in different isolated female infertility indications: Do our expectations meet the reality?

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Study question: This study aims to evaluate the realistic contribution of female infertility on embryonic development, aneuploidy and clinical outcome in non-male infertility couples undergoing PGD-A cycles.

Summary answer: Our results show that embryonic development, aneuploidy and clinical outcome are all variably affected by the nature of female infertility diagnosis.

What is known already: It is nowadays well-known that female age and the severity of male infertility are one of the biggest and direct contributors of human embryonic aneuploidy leading to poor clinical outcome. Although selection and uterine replacement of euploid embryos by preimplantation genetic diagnosis (PGD-A) greatly contributes in successful clinical outcome, recent studies show that its efficiency and possible benefits in IVF are usually hindered by biological and technical limitations related to the complex nature of infertility diagnosis, making it very difficult to predict or assess the true impact and success of the approach.

Study design, size, duration: This retrospective study includes 668 patients undergoing 755 PGD-A and 348 subsequent euploid frozen embryo transfer (FET) cycles performed between January 2016 - August 2017. All PGD-A cycles involved trophectoderm biopsy of developing blastocysts on day 5 or day 6 and subsequent vitrification. Embryonic aneuploidy was analyzed by next generation sequencing (NGS). All embryo transfers were performed as FET.

Participants/materials, setting, methods: Only cycles with a single female infertility diagnosis and non-male infertility were included in the study and grouped as follows: Group I consist of cases with diminished ovarian reserve (DOR; 257 patients/311 cycles), Group II includes cases with endometriosis (55 patients/61 cycles); Group III includes cases with PCOS (66 patients/69 cycles). Group IV consists of unexplained infertility cases (UEI; 290 patients/314 cycles). Laboratory and clinical outcome were compared among groups.

Main results and the role of chance: Female age, laboratory parameters such as mean number of oocytes collected, fertilized and mean number of embryos biopsied in Group I was significantly lower than the others ($p<0.01$). Although blastocyst development rate was found to be comparable among groups (50.9%, 53.4%, 46.9% and 40.6%), the rate of embryo euploidy was found to be significantly lower in Group I than the others (34.0%, 52.4%, 57.4% and 52.2% respectively; $P<0.01$). Cases in Group III and IV had the lowest cycle cancellations compared to Group I and II (13.0%, 29.9%, 58.8% and 36.1% respectively). Once an euploid embryo was found and transferred in the uterine environment, Group I, III and IV resulted in comparable clinical pregnancy rates (64.1%, 61.9% and 67.2% respectively). Although the highest clinical pregnancy rate (90.9%) and lowest miscarriage rate (6.9%) were observed in cycles involving endometriosis (Group II), the differences were not found to be statistically significant ($p>0.05$).

Limitations, reasons for caution: The main limitations in our study were the number of cases included and its retrospective design. Prospective studies with higher number of cases included are needed in order to validate these findings.

Wider implications of the findings: Our results first show that transfer of a euploid embryo results in successful clinical outcome in all non-male infertility groups evaluated in the study. However, quantitatively poor laboratory outcome prior to embryo biopsy is found to be the major determinant which leads in high cycle cancellations (>50%) in DOR cases.

Trial registration number: None.

POSTER VIEWING

Implantation and early pregnancy

P-423 Oxidative stress induced autophagy dysfunction in human decidual cells: a novel mechanism involved in early pregnancy loss

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Study question: To examine whether autophagy is associated with the pathogenesis of early pregnancy loss(EPL)?

Summary answer: We found in EPL decidual cells, endoplasmic reticulum stress-induced(ER stress) autophagy through increasing Ca^{2+} release and activating of CaMKKβ-AMPK signaling pathway.

What is known already: Our previous study first reported that ER stress existed in maternal-fetal interface, and sustained ER stress could cause decidual cells apoptosis. Autophagy as an essential, conserved lysosomal degradation pathway, is involved in pathological processes including neurodegenerative diseases, infection and cancer. Many studies have demonstrated that ER stress triggers autophagy. And the possible mechanism of ER stress induced autophagy might be the release of Ca^{2+} from the ER into the cytosol, which can subsequently activate various kinases and proteases involved in autophagy signaling.

Study design, size, duration: Normal and EPL decidual tissues were collected from patients with normal pregnancies undergoing elective termination of gestation, and from patients with EPL, respectively.

Participants/materials, setting, methods: From January 2015 to may 2016, 15 pregnant women with a diagnosis of EPL and 30 age-matched women with normal pregnancy were selected in the international peace maternity and child health hospital, school of medicine, shanghai Jiao tong university. The technology of western blot, immunohistochemical and transmission electron microscopic were performed on deciduas from women with EPL, and then ER stress markers, autophagy associated protein and autophagic features were analyzed in decidual cells (DCs).

Main results and the role of chance: This present study found that EPL deciduas were characterized by up-expression of microtubule-associated protein (MAP) 1 light chain 3II(LC3II), extensive dilation of ER, and morphological features of autophagosome. To explore the function and regulation mechanism of autophagy in the pathogenesis of EPL, we developed a cell model of oxidative stress(OS) using hydrogen peroxide(H_2O_2) administrated normal decidual cells. In normal decidual cells, we found that H_2O_2-induced ER stress could lead to autophagy through increasing Ca^{2+} release and activation of CaMKKβ-AMPK signaling pathway. However, vitamin E could reverse the concentration of cytosolic Ca^{2+} and the expression of LC3II. These findings indicated the occurrence of ER stress-induced autophagy in EPL decidual cells, which might play an important role in the development of EPL.

Limitations, reasons for caution: This study focused on the function of LC3II in EPL, however, numerous other autophagy related proteins may also be responsible for the pathogenesis of EPL. Therefore, further studies are required to elucidate the mechanism in EPL.

Wider implications of the findings: Our findings indicate that autophagy may contribute to the development of EPL, and the inhibition or disruption of autophagy may be a potential therapeutic target to prevent EPL in the future.

Trial registration number: no.

P-424 A systematic review and meta-analysis of chronic endometritis and recurrent miscarriage

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Study question: Is their an association between chronic endometritis (CE) and recurrent miscarriage (RM)? Does CE predict miscarriage? Do antibiotics reduce CE and prevent miscarriage?

Summary answer: Despite a high prevalence of CE in RM, there is insufficient evidence to recommend screening for and treating CE in recurrent miscarriage.

What is known already: Chronic Endometritis (CE) is defined as long-term inflammation of the endometrium and possibly driven by infection. The diagnosis is based on the identification of plasma cells within the endometrium by morphology with Haematoxylin and Eosin or mere recently using immunohistochmestry to CD138 or hysteroscopy. CE has been associated with potential pathogenic organisms, bacterial dysbiosis. Numerous studies have reported a high prevalence of CE in the recurrent reproductive failure population and antibiotics have been used to reduce CE and prevent miscarriage. A systematic review of these studies is needed to provide clarity as to the status of the current evidence.

Study design, size, duration: Search terms were identified and searches were run in the MEDLINE, EMBASE, Web of Science, CINAHL and Cochrane databases. Studies were excluded if large potential bias existed. Two independent reviewers selected 17 studies, which met predefined criteria. Manuscript quality and analysis of bias were assessed using the Critical Appraisal Skills Programme.

Participants/materials, setting, methods: Studies were included if they included women with RM and/or; investigated a link between CE and RM, investigated CE prevalence in women with and without RM, compared treatment of women with RM with antibiotics or no antibiotics, looked for an outcome of live birth rate, tested for CE using endometrial biopsy or hysteroscopy. There were no language restrictions. 173 studies were identified of which 17 met the inclusion criteria.

Main results and the role of chance: Several studies reported a high incidence of CE in recurrent miscarriage, up to 57%. However, there were no studies with adequate controls to establish the prevalence in the general population.

Two studies found a higher live birth rate in women with RM and CE than without CE (OR 3.34 (1.67, 6.87) N = 159).

Based on assessment of a repeat endometrial biopsy, a single course of Doxycycline was reported to "cure" CE defined histologically in 92%, 94%, 75% and 70% of patients (4 studies). Although no randomised controlled trials were identified, non-randomised observational studies found that:

- Published comparisons of livebirth rate in women with untreated CE compared to treated CE were small OR 1.12 (0.27,4.67) N = 71
- An increase in livebirth rate in CE patients who responded to antibiotic treatment compared to those who did not. OR 12.40 (6.74, 22.81) N = 244
- A comparable livebirth rate in women with CE who were actively treated with antibiotics compared to subjects without CE OR 1.48 (0.83, 2.65) N = 540.

Limitations, reasons for caution: The incidence of CE in the normal population was not published. No randomised control trials were identified assessing the efficacy of antibiotics in the prevention of miscarriage for women with CE and RM.

Wider implications of the findings: This review has found insufficient evidence to recommend screening for and treating CE in recurrent miscarriage at the present time. However, the data do suggest that CE may be a treatable cause of recurrent miscarriage. Randomised, controlled trials are urgently needed.

Trial registration number: NA.

P-425 Lipidomic profile as a non-invasive tool to predict endometrial receptivity in freeze-all cycles

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Study question: Is the endometrial fluid lipidomic an useful approach to predict endometrial receptivity in freeze-all cycles?

Summary answer: The endometrial fluid lipidomic is a valuable tool to predict endometrial receptivity in freeze-all cycles.

What is known already: The embryo implantation depends on the proper embryo development and the acquisition of a receptive endometrium. The embryo is unable to adhere to it through most of the menstrual cycle, except during a short, self-limited period, the window of implantation (WOI). Inadequate uterine receptivity has been estimated to contribute to one third of implantation failures, whereas the embryo itself is responsible for two thirds of them. Predictors of the uterine receptive are needed to better understand the causes of endometrial-based infertility, and help women with recurrent implantation failure (RIF) due to possible dyssynchrony, between embryo and endometrium, to achieve pregnancy.

Study design, size, duration: For this prospective cohort study, endometrial fluid samples, were collected immediately before embryo transfer, using a soft catheter, attached to a 5mL-syringe, with which a slight suction was made to bring the fluid into the catheter. Samples (n = 41) collected between Jan/2015 and Dez/2016 were split into two groups depending on the pregnancy outcome: Positive-Group (n = 24) and Negative-Group (n = 17). The study included exclusively freeze-all cycles in which one or two top quality blastocysts were transferred.

Participants/materials, setting, methods: Collections were performed in university-Affiliated IVF-center. Lipid extraction was performed according with the Bligh and Dyer method and spectra were acquired in the positive mode by MALDI-TOF mass spectrometer method. The principal component analysis (PCA) and Partial Least square discriminant Analysis (PLS-DA) were applied to the dataset. A list of potential ions ratios biomarker was obtained, the values were used to build a ROC curve to predict pregnancy success, and the lipid categories were identified.

Main results and the role of chance: Patient's and cycle's characteristics did not differ among the groups. The raw data was processed and 265 ions were used for statistical analysis. Twenty ions ratios were established according with their correlations and those variables were used for the further analysis. The fold-change analysis detected 13 ratios with two-fold increased representation in the Positive and 84 in the Negative-Group. According with the t-test, 16 ratios were differentially represented among the groups ($p<0.05$), and the volcano plot analysis detected five ratios two-fold differentially represented among groups with statistical significance. The PCA analysis showed a tendency of separation between the studied groups, while the PLS-DA was able to clearly distinguish the groups. Fifteen ratios (13 hyper-represented in the Negative and two in the Positive-Group) were selected considering their importance for the model prediction. These ratios were used to build the ROC curve, which presented an area under the curve of 81.8% (CI 95%: 62.7–94.7%, $p = 0.009$). Ions identified by the lipidmaps database were: phosphoethanolamine, phosphatidic-acid, diacylglycerol, triacylglycerol, glycosyl diacylglycerol, phosphatidylcholin, neutral-sphingolipidium, lysophosphatidylglycerol. Functional enrichment analysis revealed that increased triacylglycerol and phospholipids ratio leads to low density lipoproteins remodeling, which may be associated with changed steroid syntheses, and therefore, WOI displacement.

Limitations, reasons for caution: The model was not validated because the MS/MS experiment was not yet performed.

Wider implications of the findings: Our findings demonstrated that endometrial fluid lipidomics may be a powerful approach to define the exact time of the WOI. This would be extremely important for determination of the right time for embryo transfer and, consequently, diminish the incidence of RIF, a substation challenge in assisted reproduction.

Trial registration number: None.

P-426 Endometrial thickness and oocyte quality affect perinatal outcomes in intracytoplasmic sperm injection cycles

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Study question: Which factors affect perinatal outcomes in intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: Perinatal outcomes are positively affected by embryo quality and endometrium thickness and negatively affected by the presence of oocyte polar body fragmentation.

What is known already: Pregnancies derived from assisted reproduction techniques (ART) cycles have been correlated with worse perinatal outcomes, such as preterm birth, low birth weight, small size for gestational age, and perinatal mortality. Although these complications are commonly attributed to the higher rate of multiple births with ART, assisted singleton pregnancies had also worse perinatal results than natural ones. Nevertheless, the role of ART itself on poorer obstetric and perinatal outcomes has not been demonstrated convincingly and is still to be determined which aspects of ART pose greater risks of perinatal complications and how these risks can be minimized.

Study design, size, duration: This cohort study included 402 babies born to 307 patients undergoing ICSI cycles from January/2014 to December/2015. The number of gestational weeks (GW), baby weight (BW) and length (BL) at birth were correlated with: number of follicles and retrieved oocytes, mature and immature oocytes, fertilization rate, number of high-quality embryos, transference stage and endometrial thickness using linear regression models. In single embryo transfer (SET) cycles, oocyte dysmorphisms and embryo quality were also evaluated.

Participants/materials, setting, methods: The study was performed in a private university-affiliated in vitro fertilization center. After childbirth, the GW, BW, BL, baby sex, and presence of malformations at birth were recorded by the patient gynecologist and a written reported. All analysis were adjusted for

mother's age, body mass index (BMI), number of transferred embryos, gestational sacs, and born infants.

Main results and the role of chance: The endometrial thickness was positively correlated with GW (95%CI OR: 0.069; 0.327 p = 0.003) and BW (95% CI OR: 0.770; 55.932 p = 0.044). Blastocyst transference was negatively correlated with the GW in comparison to cleavage-stage embryo transfer (95%CI OR: -1.899; -0.057 p = 0.037). The number of follicles (95%CI OR: -0.127; -0.012 p = 0.018), that of mature oocytes (95%CI OR: -0.175; -0.001 p = 0.050), and that of immature oocytes (95%CI OR: -0.668; -0.141 p = 0.003) were negatively correlated with BL. In SET cycles, the transfer of embryos classified as high-quality was positively correlated with the number of GW (95%CI OR: 0.633; 5.685 p = 0.014) and BW (95%CI OR: 56.394; 720.323 p = 0.022). The only oocyte dysmorphism that negatively impacted all the perinatal outcomes was the presence of polar body fragmentation. Babies born from oocytes with this dysmorphism were characterized by a lower GW (95%CI OR: -2.907;-0.083 p = 0.038), a lower BW (95%CI OR: -814.697; -85.140 p = 0.016), and a smaller BL (95%CI OR: -5.801; -2.346 p<0.001).

Limitations, reasons for caution: Although the analysis was adjusted for a number of important confounders, the dataset included no information on education level, ethnicity, and medical history of the women before and during pregnancy to allow for additional adjustments. Moreover the small samples size may limit the relevance of the study.

Wider implications of the findings: Ovarian stimulation, oocyte and embryo quality may impact perinatal outcomes. Better perinatal outcomes may be achieved through the transfer of high-quality embryos to a proper endometrium. The presence of polar body fragmentation highly impacts the perinatal outcomes, suggesting that the fertilization of oocytes with this dysmorphism must be further evaluated.

Trial registration number: None.

P-427 Embryo transfer medium containing granulocyte macrophage colony-stimulating factor improved implantation and ongoing pregnancy rates in patients who have experienced miscarriage

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Study question: How does embryo transfer medium containing granulocyte macrophage colony-stimulating factor (GM-CSF) affect clinical outcomes in vitrified-warmed embryo transfer cycles in patients who have experienced miscarriage?

Summary answer: Embryo transfer medium containing GM-CSF produced high implantation and ongoing pregnancy rates in vitrified-warmed embryo transfers in patients who had experienced miscarriage.

What is known already: Human embryonic development and implantation *in vivo* is regulated precisely by various cytokines including growth factors. Granulocyte macrophage colony-stimulating factor (GM-CSF) is a cytokine that plays an important role in reproductive function. Previous studies have reported that GM-CSF is increased in normal pregnancy and is very low during pregnancy before abortion. It suggests that increasing the concentration of GM-CSF in implantation environment may be beneficial for previous miscarriage patient. However, the effect of GM-CSF on the cleavage stage in vitrified-warmed embryo transfer in patients who have experienced at least one miscarriage remains unclear.

Study design, size, duration: A prospective quasi-randomized controlled study was performed in a single *in vitro* fertilization (IVF) center between January 2015 and December 2017. This study included patients with nulliparity who had experienced at least one miscarriage. All study participants provided informed consent, and the study design was approved by the ethics committee of IVF Nagata Clinic, Fukuoka, Japan.

Participants/materials, setting, methods: We examined 243 cycles of vitrified-warmed embryo transfer. Embryos were vitrified at the 4-cell stage (day 2). Warmed embryos were cultured for 24 hours and transferred on day 3. Patients were allocated at random to two groups. Embryos were cultured and transferred to control medium containing no GM-CSF (Global; Lifeglobal) (control group) or to test medium containing 2 ng/ml GM-CSF (EmbryoGen; ORIGIO) (test group). Embryo transfer was performed at hormone replacement therapy cycle.

Main results and the role of chance: Clinical pregnancy, implantation, miscarriage and ongoing pregnancy rates after embryo transfer were compared between the two groups. The mean patient age was 38.8 ± 4.1 years (± SD; range 23–45). There were no significant differences in patient characteristics (age, endometrial thickness, number of miscarriages, and number of embryos transferred) between the two groups. Among the 243 vitrified-warmed embryo transfer cycles, 120 were allocated to the control group and 123 to the test group. The clinical pregnancy rate in the test group was higher than that in the control group (p = 0.10, 30.9% vs. 21.7%). The implantation rate in the test group was higher than that in the control group (p = 0.09, 20.3% vs. 13.9%). The miscarriage rate in the test group was lower than that in the control group (p = 0.269, 28.9% vs. 42.3%). The ongoing pregnancy rate in the test group was higher than that in the control group (p = 0.05, 22.0% vs. 12.5%).

Limitations, reasons for caution: The study was limited by the study size and lack of data about live birth rates after embryo transfer. In addition, the mechanism of action of GM-CSF on implantation is unknown. Further functional studies are needed to explain the molecular mechanism of GM-CSF.

Wider implications of the findings: Our findings indicate that medium containing GM-CSF improves implantation and ongoing pregnancy rates. The results suggest that the use of embryo transfer medium containing GM-CSF may be effective as a treatment strategy not only for patients who have experienced miscarriage but also for patients with repeated implantation failure.

Trial registration number: Not applicable.

P-428 Lipidomic analysis reveals increased TXA2 presence in non-receptive endometrium of recurrent miscarriage and repeated implantation failure patients

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Study question: Would the endometrial prostanoids profile of unexplained infertility, recurrent miscarriage (RM) and repeated implantation failure (RIF) patients differ from fertile controls?

Summary answer: We have identified the increased endometrial presence of TXA2 in RM and RIF patients; TXA2 is a prostanoid linked with platelet aggregation and vessel contraction.

What is known already: The endometrium is a dynamic tissue that undergoes growth, differentiation, and regression periods throughout the menstrual cycle in response to hormonal regulation to prepare the uterus for embryo implantation. In recent years, several studies in animal models have shown the importance of lipids at the time of embryo implantation. Lipidomics studies have enabled the identification and characterization of these lipids at the time of endometrial receptivity. Lipid molecules such as prostaglandins are some of the most widely studied mediators of embryo implantation. Prostaglandins modulate the activity of endometrium and their functional role can be appreciated through tissue concentration of prostanoids.

Study design, size, duration: In this prospective cohort study, endometrial samples were collected from 18 unexplained infertility patients, 16 RM patients, 15 RIF patients and 23 fertile controls during the window of implantation (LH +7 to LH+10) at gynecology and infertility clinics of Istanbul University School of Medicine. Tissue samples were homogenized and extracted. Then lipidomic analysis of prostanoids were carried out by LC-ESI-MS/MS in the analytical chemistry laboratory of the Istanbul University.

Participants/materials, setting, methods: 18 prostanoids were identified according to literature: 6-keto 6-keto PGF1α, PGF3α, TXB2, PGE3, PGD3, PGF1α, PGF2α, PGE2, PGE1, 13,14-dihydro-PGF2α, PGD1, PGD2, 13,14-dihydro-PGF1α, 13,14-dihydro PGE1, 13,14-dihydro-15-keto PGF2α, 13,14-dihydro-15-keto PGE2, 13,14-dihydro-15-keto-PGE1, 15-deoxy-Δ 12,14 PGJ2. Unexplained infertility was defined as normal hormone panel, hysteroscopic findings and sperm parameters. RM was defined as at least 2 miscarriages at 20 weeks or less. RIF was defined the failure to conceive after at least 2 cycles of IVF.

Main results and the role of chance: When prostanoid concentration in endometrium samples of unexplained infertility patients were compared with fertile controls, there was no statistically significant differences (p>0.05). When prostanoid concentration in endometrium samples of RM patients was compared with fertile controls TXA2 (measured as TXB2 in line with literature due to the unstable nature of TXA2) was significantly higher (p<0.001). Similarly, TXB2 was higher in endometrium samples of RIF patients again in comparison to fertile controls (p<0.001). Median values of TXB2 are as follows: in fertile controls 133.5 pg/mg; in RM patients 858.7 pg/mg; in RIF patients 843.1 pg/mg. When TXB2 was further compared between samples of RM and RIF patients, there was no statistically significant difference (p>0.05).

Limitations, reasons for caution: Recent studies have used lipidomic analyses to study pregnancy outcome, but receptive versus non-receptive endometrium in humans were not analyzed. For the first time in literature, we attempted to compare receptive versus non-receptive endometrium using lipidomic approach via 18 chosen prostanoids. However, a comprehensive map of prostaglandin expression is lacking.

Wider implications of the findings: Using lipidomic analysis we have identified the increased endometrial presence of TXA2 in RM and RIF patients, while no such presence in unexplained infertility patients. Interestingly, TXA2 has well-recognized platelet aggregating and vessel-contracting activities. This is clinically relevant because it may shed light on events leading to successful embryo implantation.

Trial registration number: not applicable.

P-429 Ectopic pregnancy and endometrial thickness in assisted reproduction cycles: is there a link?

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Study question: Can a thick endometrial lining measured prior to embryo transfer be considered a protective factor against ectopic pregnancy (EP)?

Summary answer: An endometrial thickness (ET) of > 12 mm is associated with a 68% decreased chance of EP in assisted reproduction technology (ART) cycles.

What is known already: EP is more common in pregnancies resulting from ART compared to those from spontaneous conception, with some peculiar risk factors like number and day of embryos transferred, volume and depth of transferred material, among others. However only one study so far assessed the link between ET and EP.

Study design, size, duration: This is a retrospective case-control study comparing all cases (n = 45) of EP (study group) to 119 cases of documented viable intra-uterine pregnancies (control group) between August 2009 and December 2016 at a private fertility clinic (OVO clinic, Montreal, Canada). Pregnancies resulting from both fresh embryo transfer and frozen embryo transfer (FET) were included.

Participants/materials, setting, methods: The study group consisted of 29 EP in the fresh cycles and 16 in the FET cycles. The control group cases were selected based on a random number generator model on a year-to-year basis. Bivariate analysis was conducted to assess the effect of all collected variables on EP. A multivariate analysis was then used to calculate the odds ratio (OR) for EP with an ET > 12 mm, adjusted for known confounders.

Main results and the role of chance: The two groups did not differ significantly in factors traditionally associated with EP (previous EP, endometriosis, tubal disease, history of pelvic infection, and abdominal surgery). Patients with EP were more likely to have a thinner endometrium (9.7 vs 10.2 mm, p = 0.009), had day 3 rather than a day 5 transfer (p <0.001), had double rather than a single embryo transfer (p <0.001), and finally were more likely to have had a difficult transfer (p = 0.002).

When analyzed separately, the association between a small ET and EP was preserved in FET cycles (8.9 vs 9.8 mm, p = 0.015) but not in fresh cycles (10.0 vs 10.4 mm, p = 0.116).

In multivariate analysis, ET > 12 mm was found to be a significant protective factor against EP with an OR of 0.32 (95% CI 0.12-0.84). However, difficult transfer and day 3 embryo transfer remained as statistically significant independent risk factors for EP with OR's of 18.04 (95% CI 2.36-137.62) and 7.07 (95% CI 2.62-19.07) respectively.

Limitations, reasons for caution: The retrospective nature of the study and the inter-observer variability in the measurement of the ET might weaken the conclusion. It is also possible that some other confounders have not been accounted for.

Wider implications of the findings: It is the first study to show that a higher ET could have a risk-reducing effect on EP, which if confirmed by future prospective observations, can act as a counseling cornerstone by providing reassurance for patients at risk undergoing ART.

Trial registration number: Not applicable.

P-430 Danaparoid is effective and safe for patients with obstetric antiphospholipid syndrome

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Study question: It was unclear whether danaparoid sodium (Orgaran[R]) is effective in improving the live birth rate in patients with obstetric antiphospholipid syndrome (oAPS).

Summary answer: The live birth rate in patients who received danaparoid was similar to that in patients who received unfractionated heparin.

What is known already: Unfractionated heparin and low-dose aspirin (LDA) is known to improve the live birth rate in patients with oAPS. Alternatively, anticoagulation with danaparoid was reported to be effective and safe in 83 patients with a history of thrombophilia and/or intra-uterine growth retardation complicated by HIT or intolerance or resistance to low molecular weight heparins.

Study design, size, duration: A case-control study was conducted between 2006 and 2017. It examined 86 pregnancies in 62 patients with oAPS diagnosed according to criteria of the International Congress on APS. Patients who suffered two miscarriages were included based on ESHRE recurrent pregnancy loss guidelines.

Participants/materials, setting, methods: APS was diagnosed by measuring lupus anticoagulant (LA), by the activated partial thromboplastin time and Russell's viper venom time and/or the presence of b2glycoprotein I dependent anticardiolipin antibodies. Live birth rates, and adverse pregnancy and perinatal outcomes were compared among patients given danaparoid and LDA (danaparoid group), unfractionated heparin and LDA (heparin group) and LDA and/or prednisolone (LDA group).

Main results and the role of chance: Live birth rates were 63.6 % (14/22) for the danaparoid group, 79.5 % (31/39) for the heparin group and 56.0 % (14/25) for the LDA group. After excluding 11 miscarriages with abnormal embryonic chromosomes and one ectopic pregnancy, live birth rates were 87.5 % (14/16) for the danaparoid group, 86.1% (31/36) for the heparin group and 63.6 % (14/22) for the LDA group. Frequencies of abnormal embryonic karyotypes were 75.0% (6/8), 25.0% (2/8), 33.3% (3/9), respectively, because the mean (SD) age of the danaparoid group tended to be higher (p = 0.014). The live birth rate of patients given danaparoid and unfractionated heparin was similar and tended to be higher than that of patients given LDA (OR 4.0, 95%

confidence interval 0.72-22.2 and 3.5, 0.98-12.8). One patient given unfractionated heparin developed HIT and this case resulted in stillbirth.

Limitations, reasons for caution: This study was not a randomized control trial (RCT). The sample size of patients with danaparoid was relatively small because Japanese medical insurance could cover only treatment with unfractionated heparin. Further RCTs are necessary to confirm these results.

Wider implications of the findings: Danaparoid is safe and effective in improving the live birth rate in patients with oAPS.

Trial registration number: UMIN000031012

P-431 Serum 17-alpha-hydroxyprogesterone (Sr. 17-α-OHP): a precocious early indicator of implantation and gestational status in double embryo-transfer fresh IVF cycles

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Study question: To evaluate if serum level of 17-alpha-hydroxyprogesterone (17-α-OHP) in the peri-/post-implantation period may be early indicator of gestational status in double embryo-transfer fresh IVF cycles.

Summary answer: Ratio of Day of Embryo-transfer(dET):d7ET serum 17-α-OHP and its pattern of rise/fall post-ET is a robust marker of implantation and gestational status in IVF cycles.

What is known already: The complications of maternal morbidity and neonatal care associated with high-risk multiple-gestations that often occur in IVF cycles due to transfer of supernumery embryos has prompted the practice of single embryo-transfer. However, several countries, with no IVF funding/insurance cover, continue to transfer ≥2 embryos on day3/day5 to enhance pregnancy rates in minimal number of cycles and to complete family structure (one boy/one girl) in single cycle. Under such circumstances, it is imperative and advantageous to know gestational status well in advance so that necessary care and precautions may be taken. Currently, no such early indicator of gestational status is available.

Study design, size, duration: Prospective cohort study of IVF cycles (n = 780) carried out from July 2014 to March 2017 in consenting women undergoing antagonist stimulation protocol followed by double embryo-transfer on either day3 or day5 in a suitably prepared endometrium. Similar luteal phase support was provided to all women. Serum 17- α-OHP level was measured on dET and subsequently on days 7,11,14,19,23 post-ET. Serum estradiol, β-hCG and progesterone levels were also measured on all these days using diagnostic kits.

Participants/materials, setting, methods: β-hCG measurement on d7ET was considered indicator of pregnancy. Implantation was main outcome measure with singletons (n = 131), twins (n = 43), miscarriages (n = 18), ectopic pregnancy (n = 9) and biochemical pregnancy (n = 70) as offshoot measures of implantation. Ratio of 17-α-OHP levels on dET:d7ET in no-implantation cycles (n = 509) were compared with those in different categories of implantation. Statistical analysis involved Student's-t test, Chi-square test, Correlation, One-Way ANOVA/Post-test, ROC analysis, as applicable, using Graph-pad Prism VI software.

Main results and the role of chance: The ratio dET: d7ET serum 17-α-OHP levels differed significantly between no-implantation (3.2 ± 0.16) versus singletons (1.18 ± 0.05;p<0.0001,), twins (0.67 ± 0.05;p<0.0001), early miscarriages (1.23 ± 0.1;p = 0.02), ectopic pregnancy (0.82 ± 0.1;p = 0.04) and biochemical pregnancy (1.87 ± 0.1;p = 0.002).

Day7 serum 17-α-OHP levels strongly correlated with implantation (Spearman r = 0.63,p<0.0001, 95%CI 0.58-0.67). The likelihood for all kinds of pregnancies increased above the D7 17-α-OHP median value of 3.2 ng/ml; whereas likelihood of singletons and twins was high above the 75th percentile value of 5.4 ng/ml and 95th percentile value of 9.98 ng/ml respectively. Post-test for linear trend in 17-α-OHP levels serially every 4 days from day7 to day23 post-ET showed a linear rise in singleton and twins cycles (Slope 0.77, p<0.0001 and 2.3, p = 0.0002 respectively); displayed non-significant steep rise in ectopic cycles (slope 4.97,p = 0.33) whereas declined in biochemical pregnancies (Slope -0.06, p = 0.45). Indication for IVF, day of ET (Day3/Day5), endometrial thickness and echo-pattern on ultrasonography, all remained non-significantly comparable throughout the study population.

Thus, transition from dET to d7ET and thereafter maintenance of linear rise in 17-α-OHP levels decides the fate of implantation/gestational-status. Minimum sample size of 108 was required for 17-α-OHP to have >85% predictive power for no-implantation versus 5 different types of implantation/gestational status. The power of our study (n = 780) is therefore very strong and predictive.

Limitations, reasons for caution: Not exactly a limitation but although the sample size of this study is sufficiently large with robust results and strong predictive power; a multi-centric Randomized Controlled trial is warranted for establishing serum 17-α-OHP as an early indicator and a gold standard for implantation/ gestational status in multiple embryo-transfer IVF cycles.

Wider implications of the findings: This study assumes immense significance especially in Asian sub-continent where multiple-embryo-transfer is done for various reasons. Risks associated with multiple-gestations/ectopics are well documented and it is often too late to take necessary steps. An early indicator can facilitate timely clinical management and help circumvent the complications to a large extent.

Trial registration number: Not Applicable.

P-432 Endometrial transcriptomic pathways analysis in recurrent miscarriages and unexplained infertility

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Study question: Would endometrial genes and their transcriptomic pathways in patients with recurrent miscarriage (RM) and unexplained infertility (UI) differ from fertile controls?

Summary answer: In RM and IU, respectively 8 and 20 pathways based on 730 and 2171 differentially expressed genes are significantly altered when compared to fertile controls.

What is known already: The endometrium is a complex structure undergoing significant transcriptomic transformations during menstrual cycle. This constantly changing feature support the adherence, then invasion and growth of an embryo. Any disturbance in this fragile system results with infertility or negative pregnancy outcomes. Beginning with Carson's paper in 2002, genomic analysis have been broadly used in different subgroups of infertility. RM and UI have been studied in many papers using microarray analysis in order to find 'target' genes responsible of the disease. Despite the high number of microarray studies, a consistency between results is still lacking, making this topic still interesting for researchers.

Study design, size, duration: This prospective cohort study involved 24 patients in each group: RM, UI and control. Recruited between August 2014-August 2015, all patients were <35 years old. RM was defined as ≥2 consecutives pregnancy losses in women with normal RM workup (hormon profile at Day 3, hysterosalpingogram, spermiogram, karyotype, antiphospholipid syndrome antibodies, inherited trombophilias). Women with no evident reason for infertility were in UI group. Women with ≥1 live birth(s) and no other comorbidities were controls.

Participants/materials, setting, methods: Endometrial samples were taken in Istanbul University School of Medicine, during the window of implantation (LH+7 to LH+10). They were put in RNAlater for one night at +4°C, and then snap frozen in liquid nitrogen. In our molecular biology and genetics laboratory, RNA extraction and hybridization with microarray slides were performed. Slides were scanned and hybridization signals were extracted using a sotfware program. R[10] package limma and PANOGA were used for statistical analysis.

Main results and the role of chance: Analysis for RM compared to controls revealed 730 differentially-expressed genes (DEGs) clustered in 8 representative pathways: pathways in cancer (p value = 3.11E-12), regulation of actin cytoskeleton ($p = 2.81E-09$), NF-kappa B signaling pathway ($p = 5.89E-11$), adherens junction ($p = 9.99E-09$), complement and coagulation cascades ($p = 6.23E-09$), SNARE interactions in vesicular transport ($p = 5.60E-06$), citrate cycle (TCA cycle) ($p = 2.98E-07$) and sulfur relay system ($p = 1.90E-06$).

Analysis for UI compared to controls revealed 2171 DEGs clustered in 20 representative pathways: complement and coagulation cascades ($p = 1.22E-19$), morphine addiction ($p = 6.49E-19$), PI3K-Akt signaling pathway ($p = 3.25E-14$), SNARE interactions in vesicular transport ($p = 2.33E-13$), adherens junction ($p = 4.43E-11$), p53 signaling pathway ($p = 6.15E-11$), spliceosome ($p = 3.02E-10$), endocytosis ($p = 3.02E-10$), TGF-beta signaling pathway ($p = 3.15E-10$), amyotrophic lateral sclerosis (ALS) ($p = 2.30E-09$), notch signaling pathway ($p = 5.36E-09$), viral myocarditis ($p = 1.76E-08$), renin-angiotensin system ($p = 2.64E-08$), maturity onset diabetes of the young ($p = 4.40E-08$), proteasome ($p = 4.97E-06$), dilated cardiomyopathy ($p = 1.08E-05$), non-homologous end-joining ($p = 3.28E-05$), basal transcription factors ($p = 3.83E-05$), folate biosynthesis ($p = 5.74E-05$) and amoebiasis ($p = 6.03E-05$).

Limitations, reasons for caution: Some limitations should be addressed: endometrial samples were not taken on the exact same menstrual day for all patients, the commercially available microarray slides used in our study can have differences from other brands, and complete disclosure of genes in others studies is lacking making a fair literature comparison difficult.

Wider implications of the findings: Instead of being limited to one gene or a group of genes, pathways analysis provides us a better understanding of the ethiology beneath RM and UI. Different genes may impact one common pathway: this could be the reason why a consistency can not be achieved across microarray studies.

Trial registration number: not applicable.

P-433 Prostaglandin E2 receptor 3 (EP3) signaling is induced in placentas of patients with unexplained recurrent pregnancy losses (uRPL)

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Study question: Is prostaglandin E_2 receptor 3 (EP3) signaling involved in the mechanism of unexplained recurrent pregnancy losses (uRPL)?

Summary answer: Elevated expression of EP3 signaling in first trimester placentas plays an important role in the fetal-maternal interface of patients with uRPL.

What is known already: An inflammatory microenvironment is required for successful implantation, however an inflammatory overreaction is one of the main causes for uRPL. Prostaglandin E_2 (PGE_2) plays a pivotal role in regulating immune balance and angiogenesis during early pregnancy and it is able to stimulate inflammatory reactions via EP3.

Study design, size, duration: A total of 19 women with uRPL and 19 healthy pregnancies were included. For *in vitro* studies, JEG-3 and HTR-8/SVneo cells were used and stimulated by PGE_2, sulprostone (an EP1/EP3 agonist) and L-798,106 (an EP3 antagonist) with different concentrations.

Participants/materials, setting, methods: We analysed the expression of cyclooxygenase-2 (COX-2), EP3 and G protein alpha inhibitor 1 (G_{i1}) in first trimester placentas of women with uRPL and healthy pregnancies via immunohistochemistry and double immunofluorescence. The production of β-hCG, progesterone and plasminogen activator inhibitor type 1 (PAI-1) in JEG-3 and HTR-8/SVneo cells was examined by enzyme-linked immunosorbent assay (ELISA). The expression of EP3 and G_{i1} of JEG-3 and HTR-8/SVneo cells was evaluated by western blotting.

Main results and the role of chance: The expression of COX-2, EP3 and G_{i1} was upregulated in the placenta of women with uRPL in comparison to healthy controls. Sulprostone inhibited the secretion of β-hCG and progesterone in JEG-3 cells and the secretion of β-hCG in HTR-8/SVneo cells, while it stimulated the expression of PAI-1 in JEG-3 cells *in vitro*. In addition, PGE_2/sulprostone was able to induce the expression of G_{i1} in JEG-3 and HTR-8/SVneo cells, respectively. L-798,106 (an EP3 specific antagonist) suppressed EP3 expression in both trophoblast cell lines.

Limitations, reasons for caution: Further studies are required to understand the role of the additional membrane receptors of PGE_2 (EP1, 2 and 4) and other members of the G_i family (G_{i2} and G_{i3}) in the placenta of uRPL patients.

Wider implications of the findings: These mechanisms give a better understanding of how PGE_2 may regulate placental inflammatory status in uRPL patients. As an EP3 antagonist, L-798,106 might quantify a 'potential therapeutic candidate' for the treatment of uRPL.

Trial registration number: NA.

P-435 Utilization of dydrogesterone in programmed FET cycles allows the determination of serum progesterone of exclusively trophoblastic origin in pregnancy- an update on the luteoplacental shift

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Study question: What are the precise kinetics of placental progesterone production in early pregnancy and how do these relate with the type and course of pregnancy?

Summary answer: Progesterone remains virtually undetectable until the +16-22 days (d) after embryo transfer. The increase is nonlinear, depends on the type of pregnancy and predicts miscarriage.

What is known already: The onset of placental progesterone production is termed the luteo-placental shift (LPS). Previous studies on pregnancies after oocyte donation in ovarian failure patients map the LPS to around the 5th (Devroey et al. 1990) or 9th gestational week (GW) (Scott et al. 1991), whereas the classical oophorectomy studies of Csapo *et al.* report the LPS between the 8th and 9th GW. Dydrogesterone does not cross-react with antibodies in ELISAs. The utilization of dydrogesterone in a programmed frozen-thawed embryo transfer cycle (in which no corpus luteum is present) therefore allows the determination of serum progesterone of exclusively trophoblastic origin in pregnancy.

Study design, size, duration: Prospective analysis of pregnancies resulting from programed FET cycles with oral E2 (6 mg/d for minimal intake 13 days) followed by E2 (6 mg/d) and 30 mg/d oral dydrogesterone up to the 12th GW. The sample collection started in 7/2015 and is currently ongoing. Progesterone levels are expressed in µg/l (±standard deviation).

Participants/materials, setting, methods: All patients were transferred one (singletons) or two embryos. Patients were divided into groups by pregnancy type (singletons, n = 17; dichorionic twins, n = 7; triplets, n = 1, abortion, n = 20). The type of pregnancy was determined by ultrasound starting +16-22d following embryo transfer (ET). Progesterone, E2 and hCG was measured in serum samples by the Roche Elecsys ELISA before dydrogesterone intake on day 13-15 (to confirm absence of ovulation) and weekly in early pregnancy up to +65-71d following ET.

Main results and the role of chance: Endogenous progesterone was not increased in all groups on first blood analysis on day 9-15 days following ET compared to baseline levels on day 13-15 (singletons after eSET: 0.25 ± 0.20; n = 16; twins: 0.25 ± 0.15; n = 6, triplet: 0.33; n = 1) and increased only marginally up to +16-22d post ET independently from the type of pregnancy (singleton: 0.33 ± 0.11; n = 13; twins: 0.51 ± 0.27; n = 6, triplet: 0.33). Progesterone levels started to rise above 1.0 µg/l within 23-29d post ET (singleton: 1.43 ± 0.89; n = 16; twins: 1.96 ± 0.83; n = 6, triplet: 3.44). From

+30-36d on the twin group and the triplet pregnancy (n = 1) showed an elevated progesterone increase versus singleton pregnancies (singleton: 3.67 ± 1.53; n = 14; linear slope: y = 2.8465× - 6.912; twins: 6.45 ± 2.29; linear slope: y = 4.6047× - 10.821; n = 6, triplet: 11.9; linear slope: y = 6.5007× - 15.602) which was aggravated even further on following analysis and resulted in markedly enhanced progesterone levels on last analysis during +65-71d (singleton: 23.33 ± 7.51; n = 11; twins: 36.57 ± 6.57, n = 4; triplet: 60.9;) in parallel with estradiol levels. While hCG plateaus around +37-43d, placental progesterone production steadily increases throughout the first trimester. Miscarriage was reliably predicted by progesterone levels measured +23-29d after embryo transfer (ROC analysis AUC = 0.85, 95% CI: 0.69–1.00, p = 0.004).

Limitations, reasons for caution: Minimal cross-reaction of the antibodies used in the Roche ELISA between endogenous progesterone and dydrogesterone may have slightly distorted the values. The sample size is too small to reliably establish the predictive performance of endogenous progesterone values for miscarriage or adverse events related to abnormal early placental development.

Wider implications of the findings: For the first time in human reproduction, early pregnancy trophoblastic progesterone production was assessed without the background "noise" caused by either a corpus luteum graviditate or exogenous progesterone supplementation. The findings fire new studies on the LPS, prediction of pregnancy viability, placental activity/pathology with wide implications (fetal growth etc.).

Trial registration number: n.a.

P-436 Local Endometrial injury before frozen-thawed embryo transfer (FTET) cycles? A review and meta-analysis

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Study question: Does endometrial injury before a frozen-thawed embryo transfer (FTET) cycle increases chances of pregnancy or live birth?

Summary answer: Currently there is limited evidence to recommend endometrial injury before FTET cycles.

What is known already: Randomised studies and meta-analysis suggest that local endometrial injury (LEI) in the cycle preceding IVF treatment appears to increase pregnancy rate particularly in women with recurrent implantation failure. A further meta-analysis concluded that while results must be interpreted with caution due to low quality evidence, endometrial injury before intrauterine insemination or when trying for a natural conception could be associated with higher live birth rate. It is not known whether the same benefit could be observed after endometrial injury for FTET cycles.

Study design, size, duration: We searched the MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials (CENTRAL), as well as reference lists of relevant reviews and included studies. We performed the search from inception to January 2018. We included randomised controlled trials (RCTs) that evaluated any kind of intentional endometrial injury in women planning to undergo a FTET compared to no intervention or a mock intervention. The risk of bias was judged according to Cochrane Handbook's recommendations.

Participants/materials, setting, methods: We identified 4 RCTs that included in total 476 FTET cycles; three studied patients who had FTET with hormone replacement treatment (HRT) and one patients during a natural cycle. In all studies the intervention group had endometrial injury with an endometrial biopsy catheter (Pipelle, Pipette or an Endocyte® sampler) while the control group in one of the three studies had endocervical manipulation and in the remaining three no uterine manipulation.

Main results and the role of chance: Meta-analysis of two RCTs that evaluated LEI in women undergoing FTET showed no significant difference in pregnancy rate between the LEI and control group (OR 0.89; 95% CI 0.41-1.94; p = 0.77).

Studies that investigated FTET during natural and HRT cycles were pooled due to evidence suggesting the outcome is similar and also due to the small number of studies.

Four RCTs evaluated the role of LEI in women undergoing FTET (1 with natural cycles and 3 studies with HRT) showed no difference rate in clinical pregnancy rate and ongoing/live birth rate versus the control group (OR 1.17; CI 0.68-2.01; p = 0.57 and OR 1.40; CI 0.63-3.11; p = 0.40, respectively).

A subgroup analysis of two RCTs including unselected women for FTET and two RCTs including women with at least 2-4 failed embryo transfers didn't suggest a higher LBR for women having EI for either of the two subgroups (OR 0.95; CI 0.54-1.66; p = 0.85 and OR 2.61; CI 0.44-15.51; p = 0.29, respectively).

Limitations, reasons for caution: This is the first attempt to systematically review the evidence for endometrial injury before FTET. However, this was based on a small number of RCTs, which did not allow for the analysis of subgroups such us natural vs HRT or number of previous failed embryo transfers for all outcomes.

Wider implications of the findings: While there is evidence to suggest that endometrial injury could increase the success rate for women having fresh IVF treatment, IUI or when trying to conceive naturally, there is currently limited evidence to indicate that LEI could improve FTET outcome.

Trial registration number: Not applicable.

P-437 Identification of the human uterine acetylome

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Study question: Can we identify the breadth of lysine residues modified by acetylation in the human uterine proteome?

Summary answer: Almost 2,000 lysine residues were acetylated in the human myometrium proteome. >300 were potentially modulated by treatment with a lysine deacetylase inhibitor TSA.

What is known already: There are many possibilities for post-translationally modifying proteins and regulating uterine cell function during menstruation, early and established pregnancy - e.g, acetylation of lysine residues, regulated by acetyltransferases (KATs) and deacetylases (KDACs), plays a prominent role in cellular biology via modification of histones and non-nuclear proteins. We have previously shown that protein acetylation is important for regulating human uterine contractility. Treatment of ex vivo biopsies with KDAC inhibitors changed the acetylation of selected proteins – e.g. the cytoskeletal protein HSPB6 - and altered contractility. These observations suggest protein acetylation to be a potential mechanism for initiation/regulation of uterine function.

Study design, size, duration: Uterine biopsies were obtained, following written informed consent, from pregnant women undergoing elective Caesarean section (38 subjects in total). 20 samples were treated with TSA and 18 were used as controls. To reach the required amount of material per replicate, samples were randomly pooled into 5 treated and 6 control pools.

Participants/materials, setting, methods: The collected tissues were immediately treated with 3.3uM TSA for 6 hours and stored frozen. Tissues were homogenized, digested to peptides with tripsin/endolyse C and subjected to immunoprecipitation with anti-Acetyl Lysine Antibody. The eluates, containing enriched acetylated peptides, were analysed using LC-MS based approach (Thermo Q-Exactive). The individual peptides and acetylated residues were identified and quantified using MaxQuant 1.5.3.8 comparisons to a Uniprot–derived human proteome database.

Main results and the role of chance: We identified (with FDR<0.01) 1951 acetylation sites (localization probability >0.75) related to 821 protein groups. 342 acetylation sites were uniquely identified in TSA-treated samples. Acetylation of 1254 sites was quantifiable between experimental conditions, of which 127 differed significantly (t-test, FDR<0.05) and 45 were increased by TSA treatment. A combination of quantitative (t-test and clustering) and interrogative analysis allowed us to identify that many of the acetylated proteins were involved in cellular processes related to energy utilisation (TCA cycle and

oxidative phosphorylation), cell motility (MYH9, MYH10, ACTB, TPM4) and adhesion (COL6, ZYX, VCL) and chromatin structure (histones).

Limitations, reasons for caution: The process of immunoprecipitation targeted for acetylated lysine residue-containing peptides enriches a small pool of total digested peptide and cautions the interpretation of subsequent quantifications.

Wider implications of the findings: This study provides a platform of identification of almost 2,000 protein lysine acetylation sites in human uterine tissue. Future experiments designed to expand the breadth of acetylated protein identification (the acetylome) will facilitate the examination of the regulatory influences of protein acetylation on human uterine function.

(Supported by MRC grant MR/L0009560/1)

Trial registration number: not applicable.

P-438 Is Endometrial Receptivity Array (ERA) screening relevant to increase pregnancy rates in patients with failed IVF cycles?

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Study question: Is Endometrial Receptivity Array (ERA) screening relevant to increase pregnancy rates in patients with previous negative IVF results?

Summary answer: There is no difference in pregnancy rates for patients with previous fails followed by personalized ERA embryo transfer if compared to controls with standard preparation.

What is known already: Onset of pregnancy is given when a proper embryo has the ability to attach in a receptive tissue. Endometrial receptivity is accomplished on day 19-21 in normal cycle, also known as window of implantation (WOI). Usually, endometrial thickness and progesterone serum levels are parameters considered to evaluate the endometrium receptivity, but it remains a lack of accurate methods. The endometrial receptivity array (ERA) is a transcriptomic signature test aiming to provide profiles of biopsied tissue in pre/post non-receptive or receptive endometrium. According to this profile, an adjustment in endometrial stimulation protocol could be performed to better synchronize an embryo transfer.

Study design, size, duration: A retrospective study, including 418 patients divided into two groups: 209 had at least 1 previous IVF fail, followed by an ERA biopsy, proceeded to a frozen embryo transfer (FET) where the endometrial stimulation was adjusted according to the ERA result in a later cycle (personalized embryo transfer, pFET), named ERA group, and 209 age-matched controls, at the same time, not-biopsied for ERA with a FET with a standard endometrial preparation, named non-ERA group.

Participants/materials, setting, methods: Endometrial biopsies samples were obtained after 120 hours of progesterone introduction and classified into Receptive, Pre-Receptive, Post-Receptive or Non-Receptive by ERA. In both groups, transfers were performed on blastocyst stage (embryo day 5-6). Patients from pFET and FET groups (38 ± 5.26 years old) were also subdivided into 2 subgroups: those with euploid biopsied embryos (ERA/PGD and Non-ERA/PGD, n = 92 each, CGH or NGS) and those with no biopsied embryos transferred (ERA/non-PGD and non-ERA/non-PGD, n = 117 each).

Main results and the role of chance: The majority of patients, with at least one IVF fail and submitted to an ERA test, were found to displace a receptive endometrium (n = 137, 65.55%) followed by pre-receptive (n = 53, 25.35%), non-receptive (n = 11, 5.26%) or post-receptive (n = 8, 3.82%). The average pregnancy rates were similar comparing both groups (51.67% vs 47.85%, p = 0.4935; ET number 1.75 ± 0.62 vs 1.56 ± 0.52, p = 0.0011). Either in euploid biopsied embryos or not biopsied embryos subgroup: ERA/PGD vs Non-ERA/PGD (48.91% vs 43.47% p = 0.5561; ET number: 1.60 ± 0.50 vs 1.43 ± 0.57, p = 0.02), ERA/No PDG vs Non-ERA/No PGD (55.55% vs 51.28% p = 0.6002; ET number: 1.88 ± 0.72 vs 1.65 ± 0.51 p = 0.009). Receptive and pre-receptive endometrium showed similar pregnancy rates (54.01% vs 49.05%, p = 0.6274, ET number: 1.77 ± 0.68 vs 1.72 ± 0.49, p = 0.8607). Non-receptive and post-receptive patients were in low numbers for relevant purpose (n = 11 and 8 respectively). For statistical analysis, paired T-test or Fisher´s test was applied as appropriated.

Limitations, reasons for caution: In the cases where ERA result was pre, post or non-receptive, adjusted endometrial stimulation protocol was applied in a later cycle as suggested, but the array was not repeated to confirm the adjusted receptivity. Male factor and primary infertility reason were not considered in this study.

Wider implications of the findings: Using ERA adjustment indiscriminately, is not relevant to increase patient's pregnancy rates when compared to standard endometrial stimulation protocol. However, ERA should be considered for patients with specific cause of infertility, such as repeated implantation failure.

Trial registration number: Not applicable.

P-439 The inflammatory response during the implantation period: association with the Reticular Stress and the Unfolded Protein Response

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Study question: Could the decidualization program of endometrial cells induce a sterile inflammatory response through the induction of reticular stress (RS) and unfolded protein response (UPR)?

Summary answer: Decidualized cells display a physiological RS and UPR that induce the inflammasome activation generating a sterile inflammatory response with production of IL-1b.

What is known already: Embryo implantation in humans requires the generation of a sterile inflammatory response for blastocyst invasion; however, it is still unclear how it could be induced. During decidualization, endometrial stromal cells suffer RS and trigger UPR, allowing the expansion of the endoplasmic reticulum and the production of immunomodulators. The physiological RS generates the activation of sensing proteins, which induces kinase/Rnase-TXNIP expression, activating the inflammasome. This multiprotein system allows caspase-1 activation, which catalyzes the cleavage of inactive pro-form IL-1b to secretory mature IL-1b. This pro-implantaoty cytokine is associated with an inflammatory response that should be control in favor of a tolerogenic microenvironment.

Study design, size, duration: Human endometrial stromal cell line (HESC) was decidualized or not with medroxiprogesterone+dbcAMP during 8 days, and was used to evaluate RS and UPR. An *in vitro* implantation model based on co-culture of blastocyst-like spheroids (BLS) from Swan-71 trophoblast cells line over decidualized-HESC cells was used to evaluate decidual functionality. Finally, we evaluated the UPR-inflammatory pathway in endometrial biopsies obtained from fertile women, with recurrent spontaneous abortions (RSA) or with *in vitro* fertilization failures (RIF).

Participants/materials, setting, methods: RS sensors (IRE1a, ATF6 and PERK) and UPR markers (sXBP1 and CHOP) were evaluated by RT-qPCR. Inflammasome activation was measured by TXNIP and NLRP3 expression. Caspase-1 activity was evaluated by the fluorescent inhibitor probe FAM-Flica and IL-1b production by FACS analysis, ELISA and Western Blot. Assays were performed also in the absence/presence ofSTF083010 (an Ire1α inhibitor) and Thapsigargin, Tg (RS-inducer). These pahtways were evaluated in endometrial samples from RSA, RIF and fertile women by RT-qPCR.

Main results and the role of chance: The decidualized cells (Dec) increased the expression of RS-sensors (ATF6, PERK and IRE1a) and UPR markers (sXBP1 and CHOP) in comparison with non-Dec cells. Tg-treated non-Dec cells were used as RS +control. Then, we evaluated the UPR inflammatory

pathway, which activates the inflammasome. Interestingly, while TXNIP expression remains unchanged, we oberved an increased NLRP3 expression in Dec cells compared with non-Dec cells, while STF083010 prevented this increase. Downstream, we detected increased levels of active caspase-1 on Dec cells compared with non-Dec uding the fluorescent inhibitor probe-FAM-Flica Caspase-1, suggesting the NLRP3 inflammasome activation. According to this, decidualized cells significantly increased IL-1b protein expression compared with non dec cells. This multiprotein complex increases IL-1b production in Dec cells. In fact, the inhibition of IRE1α-UPR by STF083010 treatment prevented IL-1b increase in Dec cells and decreased the invasion index evaluated in the *in vitro* model of implantation, suggesting that the induction of IL-1b is partially dependent of a RS-UPR process. Finally, the present results were validated using endometrial samples. We found an increase expression of IRE1α, sXBP1, TXNIP, NLRP3 and IL-1b on RSA-endometrial biopsies in comparison with fertile women; while biopsies from RIF patients showed a significantly lower expression than fertile women.

Limitations, reasons for caution: The present results were obtained using immortalized cell lines and *in vitro* decidualization and implantation models. Even the results were validated in endometrial samples from fertile women, recurrent spontaneous abortions and in recurrent in vitro fertilization failures, further studies are necessary to elucidate whether these mechanisms operate similarly *in vivo*.

Wider implications of the findings: The present results suggest that human decidualization process is accompained by a physiological RS/UPR, which induces a physiological and sterile inflammatory response associated with an increase of IL-1b production. These processes were differentially affected in RSA and RIF patients in comparison with fertile women, suggesting their relevance on reproductive pathologies.

Trial registration number: n/A.

P-440 HLA-C haplotypes distribution among gametes donors in our population

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Study question: HLA-C haplotypes distribution among gametes donors in Caucasian population.

Summary answer: Among oocyte donors, 37.5% have an HLA-C1C1 haplotype, and very similar among sperm donors 32%.

What is known already: Increased risk of recurrent miscarriage (RM), preeclampsia and fetal growth restriction has been described in mothers KIR AA when the fetus has more HLA-C2 genes than the mother with a higher incidence in oocyte donation pregnancies compared to spontaneous conception or IVF pregnancies. In oocyte donation, the oocyte HLA-C behaves as the paternal HLA-C increasing the number of non-self-antigens presented to the mother´s uterine immune cells. This KIR-HLA-C combination is still not considered nowadays in the process of donor selection. KIR AA patients have lower live birth rates (LBR) after double embryo transfer (DET) oocyte-donation, especially with HLA-C2 partner.

Study design, size, duration: Between April 2015 and October 2017, we performed a prospective study that included 783 gametes donors matched for couples with recurrent miscarriage or recurrent implantation failure of unknown etiology. We performed HLA-C typing for 683 oocytes donors and for 100 sperm donors.

Participants/materials, setting, methods: All the donors were selected from IVIRMA Clinics, and showed a normal karyotype, negative serology and fulfilled our standards for gamete donation. We did genetic typing for HLA-C after obtaining signed informed consent. HLA-C haplotype distribution among oocytes and sperm donors in our Caucasian Spanish population was analyzed.

Main results and the role of chance: In our cohort of 683 oocytes donors, we observed a 37.5% HLA-C1C1 haplotype (N = 256), 47.7% HLA-C1C2 and 14.8% HLA-C2C2. Among the sperm donor cohort, HLA-C haplotype distribution was 32% for HLA-C1C1, 50% for HLA-C1C2 and 18% for HLA-C2C2.

Knowing the HLA-C distribution in our gametes donors might be helpful as LBR is lower when women KIR AA received a C2 donor, especially when they have a HLA-C2 partner and the embryo has more HLA-C2 antigens than the mother. This first study, observed a 32-37% of HLA-C1C1 frequency among gametes donors in our population. Those KIR-HLA-C mismatched couples affected by recurrent miscarriage or recurrent implantation failure may benefit from this approach.

Limitations, reasons for caution: The study's objective was to describe the HLA-C distribution among our donors cohort and the frequency of what we may consider the "best" haplotype, HLA-C1C1. This is the first study observing the HLA-C distribution among gametes donors and represent useful data.

Wider implications of the findings: It's believed that completing a normal pregnancy is possible only for KIR AA mothers who carry a baby with a least one non—self HLAC1. During oocyte donation, the KIR-HLA-C mismatch increases compared to own oocytes. Therefore, selecting HLA-C1 among donors could be more efficient and safer, for specific couples.

Trial registration number: not applicable.

P-441 In-cycle prediction of the outcome after fresh embryo transfer in IVF/ICSI: a molecular analysis of follicular endometrial biopsies

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Study question: Can molecular analysis of an endometrial biopsy harvested during ovarian stimulation (OS) predict the outcome of fresh embryo transfer (ET) in that cycle?

Summary answer: Early follicular phase endometrium harvested during OS is significantly different in patients having a live birth compared to an implantation failure after fresh ET.

What is known already: The endometrium adapts from cycle to cycle thanks to tissue renewal and decidualization. Endometrial biopsies taken during OS have been shown not to harm the chances of implantation in case of fresh ET. At the molecular level, differential gene expression (DGE) has been reported between women that achieve pregnancy versus those who do not. In luteal phase endometrial biopsies, a transcriptional signature predictive for repeated implantation failure (RIF) was shown to be associated with reduced cell proliferation, an essential function of the proliferative phase. Furthermore, a disordered endometrial stromal cell (EnSC) secretome has been linked to failed implantation.

Study design, size, duration: This study was part of a RCT investigating the effect of scratching during OS on clinical pregnancy rates after IVF/ICSI. Patients in the intervention arm underwent an endometrial biopsy in the early follicular phase of OS. Of the 85 biopsies available for this study, after matching to minimize the risk of potential confounding, 18 (9 versus 9) were used for transcriptome analysis and 9 (5 versus 4) for EnSC isolation and in vitro decidualization (IVD).

Participants/materials, setting, methods: RNA-sequencing was performed for individual endometrial biopsies on an Illumina HiSeq1500 machine (BrightCore, Brussels). DGE was analyzed using 3 software systems (DESeq2 in R, EdgeR, Wilcoxon rank-sum test) and gene set enrichment analysis (GSEA) was performed using the GOrilla tool.

EnSC isolation and IVD (for 2 and 4 days) was performed, after which media from undifferentiated and decidualized cultures were harvested, stored at

-80°C and later assayed using a multiplex suspension bead immunoassay (Ebioscience, Singapore).

Main results and the role of chance: After correction for multiple hypothesis testing, DGE analysis of whole tissues revealed no differences between patients having a live birth and patients having an implantation failure after fresh ET (lowest adjusted p-value = 0.15). However, after GSEA (false discovery rate < 0.05) several enriched ontologies were identified, the most affected biological process being protein targeting to the endoplasmic reticulum and the cell membrane.

Secretome analysis after EnSC isolation and culture, analyzed by partial least squares regression modelling, showed 2 distinct clusters that corresponded to the two clinical groups. Increased secretion of MCP-1, LIF and eotaxin in undifferentiated EnSC was associated with implantation failure. Upon IVD, the secretome profile clearly shifted from that of undifferentiated cells, although it remained different between the 2 groups. VEGF-A was highly secreted in the live birth group whereas a preponderance of IL-8 secretion characterized the implantation failure group. The differences in secretomes between the groups became muted upon prolonged IVD, suggesting convergence of cytokine profiles upon full differentiation.

Limitations, reasons for caution: Caution is warranted because of the limited sample size of the study and the in vitro nature of the second part of the experiment.

Wider implications of the findings: If confirmed on a larger scale, an in-cycle predictive test could be developed to guide clinical decision-making between a fresh ET immediately after OS or a freeze-all approach with a frozen ET in a subsequent cycle, possibly linked to higher success rate in case of a sub-optimal test result.

Trial registration number: This study was part of a clinical RCT: the REFRESH trial, NCT02061228.

P-442 Serum vitamin D and calcium levels in ectopic pregnancies

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Study question: what is the place of vitamins and calcium metabolism in ectopic pregnancy?

Summary answer: this is the first study that reports the decreased serum levels of vitamin D, calcium and magnesium in patients with ectopic pregnancy.

What is known already: According to recent evidences, vitamin D was found to have important roles in female reproductive system, both in physiological and also pathological mechanisms. Since vitamin D is tightly related with calcium metabolism, both molecules were reported to be associated with some pregnancy complications including preeclampsia, gestational diabetes, low birth weight, preterm delivery, and cesarean section. The aim of the present study was to evaluate the vitamin D levels in ectopic pregnancies, which may have clinical importance in the etiology of this disorder.

Study design, size, duration: Between October 2015 and June 2017,50 women with ectopic pregnancy and 61 women with normal 1st trimester pregnancy were included. Blood samples were taken for biochemical analyses including vitamin D, calcium, magnesium, parathormone, thyroid stimulating hormone (TSH), T3, T4, hemoglobin, hematocrite, thrombocyte, C-reactive protein, beta-human chorionic gonadotropin, and procalcitonin. Study groups were compared for these biochemical markers.

Participants/materials, setting, methods: In the study, 50 women with ectopic pregnancy and 61 women with normal 1st trimester pregnancy were included. Blood samples were taken for biochemical analyses including vitamin D, calcium, magnesium, parathormone, thyroid stimulating hormone (TSH), T3, T4, hemoglobin, hematocrit, thrombocyte, C-reactive protein, beta-human chorionic gonadotropin, and procalcitonin. Study groups were compared for these biochemical markers.

Main results and the role of chance: The distribution of the serum levels of vitamin D, calcium, thyroid hormones, magnesium, and hemoglobin are presented in Table 1. Accordingly, vitamin D (p = 0.006), Ca^{++} (p<0.001), T3 (p = 0.003), magnesium (p = 0.001), and hemoglobin (p = 0.040) levels were significantly lower in the ectopic pregnancy group than those in the normal pregnancy group. On the other hand, TSH (p = 0.029) and thrombocyte (p = 0.018) levels were significantly lower in the normal pregnancy group than those in the ectopic pregnancy group.

Limitations, reasons for caution: study was conducted in a single center and with low number of participants. Te number should be extended for the future studies.

Wider implications of the findings: This study reports the decreased serum levels of vitamin D, calcium and magnesium in patients with ectopic pregnancy. The molecular interactions at ultrastructural level, altered tubal fluid content, or an inflammatory process that affects ciliary motility might all play a role in the etiology of tubal implantation of the fetus.

Trial registration number: 'not applicable.

P-443 Chemokines in relation to human Choriogonadotropin (hCG) after four weeks of gestation are significantly altered in women with miscarriage

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Study question: Are chemokine levels at the time of pregnancy testing relative to human Choriogonadotropin (hCG) altered in patients who will experience a miscarriage in this pregnancy?

Summary answer: Chemokines in relation to hCG after four weeks of gestation are significantly altered in women with miscarriage, promising potential as a prognostic biomarker.

What is known already: Miscarriage is one of the most common complications in early pregnancy and affects 10-20% of all pregnancies. A predictive test to identify women who will experience a miscarriage even before first symptoms occur is not established. Activation of maternal immunological tolerance seems to be essential for early fetal development and various cytokines have been described in different stages of pregnancy. However, none of these cytokines alone has been described as a useful biomarker in asymptomatic women. To screen for promising biomarkers, we tent to enhance the possible predictive effect for later abortion by examining a chemokines/hCG correlation in maternal plasma.

Study design, size, duration: The prospective pilot study was approved by Heidelberg University Ethical Committee (protocol S-243/2015). Blood samples were obtained at the time of pregnancy testing after informed consent from women who underwent ovarian hyperstimulation to perform in vitro fertilization by IVF or ICSI during the period 05/15-05/16 at the University Hospital in Heidelberg, Germany. Exclusion criteria were autoimmune diseases, essential hypertonia, diabetes mellitus or the intake of confounding medication.

Participants/materials, setting, methods: We obtained blood samples from n = 39 women. Dependent on the follow-up, patients with a positive pregnancy test were subsequently divided in two groups: ongoing pregnancy (n = 22, group 1) and 1st trimester miscarriage (n = 17, group 2) in this pregnancy. Immunological and endocrine profiling of maternal blood was performed using Multiplex and ELISA assays. Statistical analysis using student's t-test was performed with SPSS® Version 24, IBM, Armonk, USA. $p \leq 0.05$ was considered to be significant.

Main results and the role of chance: There was no significant difference in age (33.64 ± 6.49 years vs. 33.94 ± 4.4 years), body mass index with 23.81 ± 4.29 kg/m^2 in group 1 vs. 26.48 ± 5.27 kg/m^2 in group 2 or transfer day. hCG levels, despite normal overall levels, were significantly decreased in patients with abortion compared to those with ongoing pregnancy (151.75 ± 25.29 IU/l vs. 351.27 ± 111.02 IU/l, p<0.05) whereas levels of IL-1ra (655.80 ± 78.80 pg/ml vs. 398.69 ± 32.73 pg/ml, p<0.01), MIP-1a (73.74 ± 9.91 pg/ml vs. 34.20 ± 8.25 pg/ml, p<0.01) and TNF-alpha (5.11 ± 0.40 pg/ml vs. 4.00 ± 0.26 pg/ml, p<0.05) were significantly increased. GCSF/ IL-1ra-ratio was 1.66-fold increased in patients with ongoing pregnancy. TGF-beta /MIP1a-ratio was

significantly 3,45-times higher in patients with miscarriages. Comparing patients with ongoing pregnancy to patients experiencing a miscarriage, we could demonstrate significant alterations of the ratios MIP1a/hCG (0.16 ± 0.04 pg/mIU vs. 1.02 ± 0.38 pg/mIU, p<0.05), IL-1ra/hCG (2.22 ± 0.72 pg/mIU vs. 7.83 ± 2.30 pg/mIU, p<0.05), TNFalpha/hCG (0.02 ± 0.01 pg/mIU vs. 0.07 ± 0.02 pg/mIU, p<0.05), MCP1/hCG (0.50 ± 0.16 pg/mIU vs. 1.44 ± 0.40 pg/mIU, p<0.05), IL-6/hCG (0.0681 ± 0.002 pg/mIU vs 0.016 ± 0.003 pg/mIU, p<0.05), TPO/hCG (2.50 ± 0.70 pg/mIU vs 5.47 ± 1.31 pg/mIU, p<0.05) and TGF-beta1/hCG (68.04 ± 22.37 pg/mIU vs. 149.35 ± 33.89 pg/mIU, p<0.05). The strongest effects were seen for the ratio MIP1a/hCG, IL-1ra/hCG and TNFalpha/hCG.

Limitations, reasons for caution: The limitations of this study are its small sample size and the fact, that we only detected protein in maternal plasma with no further validation technique.

Wider implications of the findings: Biomarkers screening for an upcoming miscarriage in asymptomatic women of the first trimester can be a helpful instrument to reassure patients with expected normal pregnancy outcome. We think that these established ratios are better predictors for pregnancy outcome than cytokine levels alone.

Trial registration number: na.

P-444 Impact of resveratrol supplementation on implantation and early pregnancy during embryo transfer cycles: a cross-sectional study

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Study question: To determine whether resveratrol supplementation impacts on implantation and early pregnancy following transfer of morphologically competent embryos.

Summary answer: Resveratrol supplementation during the luteal phase was associated with lower implantation and clinical pregnancy rates and higher miscarriage rate following embryo transfer.

What is known already: Resveratrol is a polyphenolic compound and natural activator of SIRT1. Based on animal experiments, resveratrol has been mooted as a potentially therapeutic agent preventing infertility associated with ovarian aging due to its inhibition of senescence. Recent reports have shown a relationship between decidualization of human endometrial cells and cell senescence. Furthermore, inhibition of decidual senescence had also been reported to suppress decidual changes in the endometrium. However, clinical trials involving resveratrol supplementation for infertility treatment have not been performed in humans.

Study design, size, duration: The present study was approved by the local ethics committee. This single-centre, cross-sectional retrospective study (2012 to 2014) was designed to compare the outcomes of ET between women receiving resveratrol supplementation (200 mg/d) during the luteal phase (RES group) and a control group (non-RES).

Participants/materials, setting, methods: Of 8,686 ET cycles, we excluded 1,097 cycles with poor prognostic factors, including those from women ≥44 years old and those with poor-quality embryos (Veeck grade 4–5 or Gardner grade CC). The RES group (205 cycles, 102 women) was then compared with the non-RES group (7,382 cycles, 3024 women). Odds ratios (OR) and 95% confidence intervals (CIs) were calculated using multivariate logistic regression after controlling for potential confounders; patient age, embryo grade and cleavage speed.

Main results and the role of chance: After adjusting for age and embryo quality, statistical analysis showed a lower hCG positivity rate (OR = 0.68; 95% CI: 0.46–1.00), lower clinical pregnancy rate (OR = 0.51; 95% CI: 0.32–0.80) and higher miscarriage rate (OR = 2.68; 95% CI: 1.11–6.51) in the RES group compared to the non-RES group.

Limitations, reasons for caution: We focused on ET cycles with morphologically competent embryos. However, resveratrol supplementation may have a positive impact on the development of follicles and production of high-quality embryos.

Wider implications of the findings: In line with the adverse effects of senescence inhibitors on endometrial decidualization *in vitro*, resveratrol supplementation during the luteal phase appears to be detrimental for pregnancy outcomes during IVF treatment. Nonetheless, analysis of the supplementation period and additional studies are required.

Trial registration number: None.

P-445 Mid-luteal serum progesterone levels determine the reproductive outcome following controlled ovarian stimulation. A prospective study of 602 IVF/ICSI cycles

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Study question: Is the chance of on-going pregnancy following IVF treatment and fresh embryo transfer affected by early and mid-luteal serum progesterone (P_4) levels?

Summary answer: Low as well as high s-P_4 levels during the early and mid-luteal phase reduce the chance of on-going pregnancy following IVF-treatment and fresh embryo transfer.

What is known already: Data from non-human studies and studies of frozen/thawed embryo transfer cycles indicate that low as well as high P_4-levels during the early/mid-luteal phase decrease the chance of pregnancy. A suboptimal P_4-level may disrupt the endometrial maturation and lead to asynchrony between embryonic development and the endometrial receptivity, thereby compromising implantation and early pregnancy development.

Study design, size, duration: Prospective multicenter cohort study of 602 women undergoing IVF treatment. Patients were recruited from four Danish public Fertility Centers from May 2014 to June 2017. The study population was unselected, thus representing a normal everyday Danish patient cohort. Patients were treated in a long GnRH-agonist protocol or a GnRH-antagonist protocol and triggered for final oocyte maturation with either hCG or a GnRH-agonist. The same vaginal luteal support regime was applied in all patients.

Participants/materials, setting, methods: Serum P_4 levels on luteal day 2/3 or luteal day 5 were correlated to the rate of positive hCG and to on-going pregnancy rates (> gestational week 10). Patients were divided into four P_4-groups based on raw data of P_4-concentrations and reproductive outcome on luteal days 2/3 (P_4<60 nmol/l, P_4 60-100 nmol/l, P_4 101-400 nmol/l and P_4>400 nmol/l) and luteal day 5 (P_4<150 nmol/l, P_4 150-250 nmol/l, P_4 251-400nmol/l and P_4>400 nmol/l).

Main results and the role of chance: The optimal chance of pregnancy was achieved with s-P_4 levels 60-100 nmol/l on luteal day 2/3 whereas the optimal P_4-level on day 5 was 150-250 nmol/l. Below, but most distinctly above these levels, the chance of pregnancy was consistently reduced. Following cleavage-stage embryo transfer and an early luteal P_4-level of 60-100 nmol/l, the chance of a positive hCG- test was 65%, CI [51; 77%]. In contrast, with a cleavage-stage embryo transfer and a P_4-level > 400 nmol/l, the chance of a positive hCG-test was significantly reduced to 34%, CI [17; 56%], thus, a reduction of -31%, CI [-54;-10%]. A similar negative association between early luteal P_4 and on-going pregnancy rate was found, although it did not reach statistical significance. Following blastocyst transfer and a mid-luteal P_4 level of 150-250 nmol/l, the chance of on-going pregnancy was 57%, CI [41;73%] compared to 38%, CI [20;59%] with a P_4-level > 400 nmol/l, thus, a reduction in chance of -19%, CI [-40;2%], p = 0.078. With blastocyst transfer and P_4 < 150 nmol/l, the chance of an on-going pregnancy was reduced to 41%, CI [43;59%]. All estimates were adjusted for maternal age, BMI, smoking, final follicle count and late follicular P_4 levels.

Limitations, reasons for caution: This study is the first to explore a threshold and ceiling level for luteal P_4 following IVF treatment and fresh embryo transfer, and the optimal P_4-ranges found in this study must be corroborated in future clinical trials.

Wider implications of the findings: Future studies need to be performed to establish whether additional exogenous luteal P_4-supplementation in the low P_4 -group could increase the chance of on-going pregnancy following fresh embryo transfer and whether patients with luteal P_4-levels > 400 nmol/l would benefit from segmentation followed by subsequent transfer in frozen/thawed cycles.

Trial registration number: NCT02129998 (Clinicaltrials.gov).

P-446 The role of sub-chorionic blood flow measured by 3D power Doppler ultrasonography in the prediction of pregnancy outcome: a preliminary study

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Study question: Does sub-chorionic blood flow at 6 weeks of gestation analyzed by 3D power Doppler ultrasonography predict pregnancy outcome?

Summary answer: The sub-chorionic blood flows at 6 weeks of gestation analyzed by 3D power Doppler ultrasonography have the potential of predicting miscarriage.

What is known already: Transvaginal ultrasound has been considered as a reliable technique to monitor early pregnancy. Present ultrasonography features at the time of 6-8 weeks of gestation have been applied as a diagnostic tool of early pregnancy; while the predictive role of pregnancy outcomes were seldom developed.

Study design, size, duration: This is a prospective cross sectional preliminary study in a setting of assisted reproductive technology (ART) center. So far, 37 cases were recruited, in which 4 lost follow up of pregnancy outcome, 5 excluded from this study for twin pregnancy, and 2 excluded for biochemical pregnancy loss. Therefore, in total, there were 21 term live birth and 5 miscarriages (miscarried during 7-12 weeks of gestation) included in the analysis.

Participants/materials, setting, methods: After embryo transfer and positive hCG test, transvaginal ultrasound scan was scheduled for confirmation of clinical pregnancy for patients recruited for this study on the day of 6 weeks of gestation. After the routine confirmation of clinical pregnancy by 2D ultrasound, a 3D power Doppler dataset were taken and stored for VOCAL® (Virtual Organ Computer-Aided Analysis) histogram analysis to measure the gestational sac volume and indices of sub-chorionic blood flows.

Main results and the role of chance: There seems no difference in the comparison of the miscarriage and term live birth group in demographic information and general pregnant conditions, including patient age, body mass index, embryo quality, hormone levels (hCG and P4). It is also not significant different in the comparison of 2D ultrasound parameters between the two groups, including crown-rump length, diameters of gestational sac, yolk sac and presentation of heart beat. For 3D power Doppler VOCAL analysis, vascularization index (VI), and vascularization flow index (VFI) were likely higher in the live birth group compared with those of the miscarriage group (3.02 ± 1.03 vs 0.74 ± 0.58, $p = 0.08$; 1.26 ± 0.23 vs 0.23 ± 0.10, $p = 0.07$, respectively); whilst flow index (FI) might be not different between the two groups (32.92 ± 2.14 vs 28.60 ± 2.41, $p = 0.14$).

Limitations, reasons for caution: This is a preliminary prospective study with a current small group of patients. As our clinical routine monitoring of patients conceived by ART were scheduled in 6 weeks of gestation, we are recruiting more cases in this cohort for a more robust interpretation of the 3D parameters.

Wider implications of the findings: VI is considered to indicate the vascularity in the sub-chorionic area; FI indicating the average intensity of blood flow; while VFI is a combination of vascularity and flow intensity. Our preliminary data suggested that miscarriage may have some angiogenesis abnormalities early on, before the miscarriage occur.

Trial registration number: None.

P-447 Reference intervals of gestational sac, yolk sac, embryonic length, embryonic heart rate at 6-10 weeks after in vitro fertilization-embryo transfer

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Study question: What are the ultrasound reference intervals of early pregnancies after in vitro fertilization-embryo transfer (IVF-ET)?

Summary answer: Reference intervals and centile charts for early pregnancy biometry after IVF-ET have been established in this study.

What is known already: To accurately determine the normal range of early pregnancy biometry was the basis for predicting adverse pregnancy outcomes. Some studies have constructed such kind of reference intervals, mostly, depending on natural conceptions. However, the ovulation day were changeable even in regular menstrual cycle, which makes the uncertainty of the accuracy for reference intervals in natural pregnancies. While during the IVF-ET procedure, the days of oocytes retrieval and ET were certain, and thus, the gestational age (GA) was accurate. Then, we speculate the reference intervals deriving from IVF-ET data would be more accurate than natural conceptions.

Study design, size, duration: The data of 3763 singleton pregnancies, including embryonic length (or crown-rump length, CRL), embryonic heart rate (HR), gestational sac diameter (GSD) and yolk sac diameter (YSD) at 6–10 gestational weeks from January 2014 to December 2015 after IVF-ET were retrospectively analyzed. The ultrasound measurements conformed to uniform standards by experienced sonographers. The GA was calculated by subtracting the date of embryo transfer from the date of birth and the addition of 17 days.

Participants/materials, setting, methods: This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). All included patients conceived singleton pregnancies via IVF-ET and resulted in phenotypically normal live neonates after 36 complete gestational weeks, with birth weight above the 5th centile for GA. All patients had full records of early ultrasound measurements of CRL, HR, GSD and YSD. Regression analysis was used to establish normal ranges of CRL, HR, GSD and YSD with gestation.

Main results and the role of chance: At 6–10 weeks, there were significant quadratic associations between CRL, HR, GSD, YSD and GA. The corresponding quadratic models were $CRL = -0.445 - 0.559GA + 0.015GA^2$ ($R^2 = 0.986$, $F = 128300.664$, $P < 0.001$); $HR = -382.279 + 16.623GA - 0.123GA^2$, ($R^2 = 0.927$, $F = 23740.394$, $P < 0.001$); $GSD = -26.547 + 0.991GA + 0.001GA^2$, ($R^2 = 0.851$, $F = 10768.859$, $P < 0.001$); $YSD = 1.709 + 0.079GA + 0.000GA^2$, ($R^2 = 0.986$, $F = 128300.664$, $P < 0.001$). The normal ranges of CRL, HR, GSD and YSD were successfully derived from these models.

Limitations, reasons for caution: This study was confined to a reproductive center, territorial limitation may exist. A future study with multi-center samples are necessary to establish a nationwide or worldwide reference interval. In addition, whether the references coming from IVF population are suitable for natural conceptions needs further elucidation.

Wider implications of the findings: The normal ranges of early pregnancy biometry provide clinicians a reliable reference to analyze the development of early embryos after IVF-ET and also give a possibility for predicting pregnancy outcomes at an early stage.

Trial registration number: None.

P-448 Virtual screening and molecular dynamics of FDA drugs into the beta-2-glycoprotein I's fifth domain for treatment of pregnancy complications with antiphospholipid antibody syndrome

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Study question: What drugs can be used for treatment of pregnancy complications with antiphospholipid antibody syndrome?

Summary answer: Some drugs prescribed for recurrent miscarriage, RIF and preeclampsia and some FDA drugs might be useful candidates for pregnancy complications with antiphospholipid antibody syndrome.

What is known already: Antiphospholipid antibody syndrome (APS or APLS), is an autoimmune disease, caused by antiphospholipid antibodies. APS is associated with pregnancy complications, including preeclampsia, thrombosis, fetal growth restriction, fetal loss and recurrent implantation failure. B2GPI is the major antigen for APL antibodies and associated with thrombosis. B2GPI exists in different conformations, an open J-shaped conformation, a circular conformation and a S-shape conformation. B2GPI is a glycoprotein by a single polypeptide chain of 326 amino acids with five repeated domains. B2GPI's fifth domain is highly effective in binding to negative charge phospholipids and binding to oxidized LDL in inflammatory and blood clotting reactions.

Study design, size, duration: In this study calculation of binding free-energy change (ΔG) of drugs that are prescribed during pregnancy, and especially recurrent miscarriage, RIF, preeclampsia and all of the FDA drugs, were evaluated using virtual screening to b2GPI's fifth domain. The starting coordinates of b2GPI were obtained from PDB (PDB code: 1C1Z). The next step were Molecular dynamics simulation studies of protein and drugs/ protein complex using gromacs software in the Linux environment.

Participants/materials, setting, methods: Bioinformatics software, such as, auto dock vina, molegro virtual docker, pyrx 0.8, linux, gromacs software and online resources like NCBI, RCSB and Drug bank were used in this study. The best drugs were chosen based on binding free energy value and hydrogen bonding interaction. Regarding the mechanism of action for vorapaxar and antrafenine, as well as the optimal ΔG for these two drugs, vorapaxar and antrafenine were selected for more accurate studies using molecular dynamics.

Main results and the role of chance: In this study calculation of binding free-energy change (ΔG) of drugs that are prescribed during pregnancy, and especially recurrent miscarriage, RIF and preeclampsia, were evaluated using the Auto Dock Tools method on b2GPI's fifth domain. Heparin, folate and warfarin have highest ΔG. Also in the present study, the molecular docking and MD simulations were performed to explore the possible binding mode of beta-2-glycoprotein I's fifth domain with FDA drugs. The best drugs were chosen based on binding free energy value and hydrogen bonding interaction. The conclusion drawn from the docking analysis was that eight FDA drugs had the highest binding affinity with b2GPI. Vorapaxar and antrafenine are anti-platelet and anti-inflammatory drugs. Regarding the mechanism of action for vorapaxar and antrafenine, as well as the optimal ΔG for these two drugs, vorapaxar and antrafenine were selected for more accurate studies using molecular dynamics. B2GPI exists in different conformations, an open J-shaped conformation, a circular conformation and a S-shape conformation. In this study using molecular dynamics for protein, it was shown that the J-shaped was transformed into a s-shaped during the 10 ns. RMSD, RMSF, and H-bond results reveal that two complexes were stable.

Limitations, reasons for caution: Findings of this study is based on in silico method. Studies in vitro and in vivo can help us complete this study.

Wider implications of the findings: This study for the first time evaluated the effect of FDA Drugs on antiphospholipid antibody syndrome (APLS) based on in silico method. Vorapaxar and antrafenine might be useful candidates for antiphospholipid antibody syndrome and pregnancy complications with APLS.

Trial registration number: This investigation is based on in silico method.

P-449 Novel insights into 3D endometrial tissue and cellular architecture during preimplantation, in women with recurrent miscarriage

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Study question: Can whole-mount confocal microscopy be used to analyse three-dimensional spatial relationships between multiple endometrial cell types, providing a platform to individualise reproductive health diagnostics?

Summary answer: 3D endometrial tissue provides informative insight on the interaction between cell populations and tissue architecture in women with recurrent miscarriage versus controls.

What is known already: The endometrium forms the first point of contact for a conceptus during implantation, acting as the barrier between the uterine tissue and uterine cavity milieu. Immunohistochemistry is widely used to study the role endometrial cell types with respect to reproductive health. However, the 2D nature of these samples means that much of the information about the relationships between these cells is lost.

Study design, size, duration: An observational study comparing three main patient groups; (1) egg donor controls, (2) recurrent miscarriage patients with ≤ 4 consecutive miscarriages and (3) ≥ 4 consecutive miscarriages.

Participants/materials, setting, methods: Endometrial biopsy samples were collected at mid-luteal phase (LH +7-10). Patients were excluded if they were on hormonal contraceptives, had a history of subfertility or uterine pathology. Multi-marker whole mount immunohistochemistry was carried out for all endometrial samples, including markers for uterine natural killer cells (CD56), macrophages ($CD163^+$), and endometrial fibroblasts (anti-vimentin). Quantification methods were developed to investigate inter and intra-cellular relationships.

Main results and the role of chance: An informative approach to imaging the endometrium in 3D has been developed. Uterine natural killer cells (CD56), macrophages ($CD163^+$) and fibroblasts were imaged simultaneously in 3D endometrium, and the relationship between these cells identified at the mid-luteal phase. Tissue and cellular morphology was quantified in relation to uterine glandular structures and the luminal epithelial surface. With regard to patient groups, results are indicative of differences in morphological spatial relationships.

Limitations, reasons for caution: This is an observational study with a small sample size, larger studies are required. Further, 3D analysis of endometrial samples is currently time consuming in comparison to traditional 2D immunohistochemistry, thus more applicable for research studies.

Wider implications of the findings: This 3D approach can be later applied to answer questions in reproductive health and implantation.

Trial registration number: not applicable REC ref: 08/H0502/162

P-450 Interaction among embryos in blastocyst double embryo transfer: better alone than in bad company

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Study question: How does the quality of an additional blastocyst transferred with a top-quality one affect pregnancy rates?

Summary answer: The addition of a good-quality blastocyst to a one top-quality blastocyst transfer does not increase substantially pregnancy rates, while increasing twin gestations.

What is known already: It is well stablished that increasing the number of transferred embryos increases pregnancy rate; this is however associated with a substantial rise in multiple pregnancies. The morphology of transferred embryos is linked to IVF success and, combined with extended culture to blastocyst stage, it has been considered effective in selecting top-quality (TQ) blastocysts for transfer. Double embryo transfer (DET) is commonly used as a strategy to increase pregnancy rates. On the other hand, it is not clear how the morphological scores of any additional embryo affects the implantation and pregnancy rates of each embryo transfer.

Study design, size, duration: Retrospective cohort study of 1149 fresh IVF-cycles with single (SET) or double embryo transfer (DET) between 2014-2016. Cycles with donated oocytes and PGS cycles were excluded. Blastocysts were classified as good-quality (GQ; B and C scores according to Gardner) or top-quality (TQ; at least one A score). Patients received one TQ blastocyst (1-TQ, n = 62), one GQ (1-GQ, n = 158), two TQ (2-TQ, n = 235), two GQ (2-GQ, n = 488) or one TQ and one good blastocysts (1-TQ+1-GQ, n = 206).

Participants/materials, setting, methods: Ovarian stimulation, oocyte pickup and embryo culture followed routine protocols. Fresh blastocyst transfers were done at day 5 following routine protocols, and clinical pregnancy (CP) was confirmed by the presence of gestational sac with fetal heart-beat. Student's t-test and Chi-square were used as appropriate. Multivariate analysis was performed to evaluate the effect of transferring additional embryo, of same or different quality, on clinical pregnancy, adjusted for women age. Differences were considered significant if $p<0.05$.

Main results and the role of chance: The groups were comparable in women age (1-TQ:34.0 ± 3.6, 1-GQ:36.0 ± 4.2, 2-TQ:33.6 ± 3.9, 2-GQ:34.5 ± 4.0, 1TQ+1GQ:33.7 ± 3.8; $p<0.001$), number of MII oocytes recovered (1-TQ:7.1 ± 4.7, 1-GQ:5.2 ± 3.4, 2-TQ:10.2 ± 4.2, 2-GQ:8.3 ± 3.8, 1TQ +1GQ:8.1 ± 3.9; $p<0.001$), and of blastocysts available for transfer (1-TQ:2.6 ± 2.8, 1-GQ:1.3 ± 1.1, 2-TQ:5.3 ± 2.4, 2-GQ:2.9 ± 1.6, 1TQ+1GQ:3.5 ± 2.0; $p<0.001$). Both CP (1-TQ: 41.9%, 1-GQ: 18.4%, 2-TQ: 61.3%, 2-GQ: 43.9, 1TQ+1GQ: 51.5%; $p<0.001$) and multiple pregnancy rate (1-TQ: 3.8%, 1-GQ: 0.0%, 2-TQ: 40.3%, 2-GQ: 20.6%, 1TQ+1GQ: 26.4%; $p<0.001$) were much higher when two TQ blastocysts were transferred compared to all other categories. When 1TQ+1GQ were transferred, the CP was still satisfactory and multiple pregnancy rate was approximately half of that observed in 2-TQ. Multivariate analysis adjusted for woman age was performed to evaluate the interaction between different quality embryos and its effect on CP. Compared to a SET of 1-GQ embryo (OR = 1.0), having a SET of a TQ embryo increases the CP threefold (OR = 3.0). A second TQ embryo would give an OR = 6.3 and a second GQ embryo an OR = 3.96; this difference is due to the lower quality and the moderate negative interaction of the GQ embryo, meaning that when transferring 1-TQ, the addition of 1-GQ embryo only improve CP 4%. Since multiple PR is still high, SET of 1-TQ should be preferred.

Limitations, reasons for caution: The main limitations of this study reside in the fact that most SET were not elective, and in the small size of the 1-TQ group. Nevertheless, the multivariate and interaction analysis allows for generalizable results.

Wider implications of the findings: The higher clinical pregnancy rate was observed in the 2-TQ group, as did multiple pregnancy. However, the presence of a second GQ blastocyst didn't significantly improve the clinical pregnancy, but significantly increased multiple gestations. When at least one TQ-blastocyst is available, SET should be considered.

Trial registration number: not applicable.

P-451 Non-classical progesterone signaling may be sufficient to induce decidualization in Human Endometrial Stromal Cells (ESC)

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Study question: Is it progesterone (P4) entry into the human endometrial stromal cells (ESC) necessary to produce a correct decidualization?

Summary answer: P4 entry in ESC is not necessary to induce in vitro decidualization as long as PGRMC1 is inhibited by AG205.

What is known already: PGRMC1 is a membrane receptor that mediates non-genomic action of P4.It has been implicated in a diversity of biological events,including apoptosis and folliculogenesis(1,2).PGRMC1 mediates non-classical P4 signaling in endometrium but its mechanism of action is still unknown.PGRMC1 protein is down-regulated in receptive endometrium but its expression is higher in stromal compartment compared to epithelial cells(3). We previously published that it localizes in membrane, cytosol and nucleus of non-decidualized ESC,but accumulates in the nucleus of decidualized ESC (dESC).Its overexpression inhibits in vitro decidualization,co-localizing with SERPINE 1 mRNA binding protein(SERBP1) throughout the menstrual cycle and in the cytosol of non-decidualized and decidualized ESC(4).

Study design, size, duration: Endometrial biopsies were obtained from donors (n = 7) and ESC were isolated. In vitro decidualization was induced by a long protocol using P4/E2 (Estradiol) (8 days) and a short protocol using cyclic adenosine monophosphate/Medroxyprogesterone (cAMP/MPA) (4 days). To activate only membrane progesterone receptors we used a membrane-impermeable P4 (P4-BSA) that avoids P4 entry in the cell. Also, we employed AG205, a PGRMC1 antagonist that blocks PGRMC1 activation.

Participants/materials, setting, methods: We induced in vitro decidualization by long protocol with P4(1uM)/E2 (10 nM) or P4-BSA(1uM)/E2 in combination with AG205 (50uM) (PGRMC1 antagonist), and by short protocol with MPA/cAMP or P4-BSA/cAMP+AG205. Decidualization was checked by IGFBP1 and Prolactin (PRL) secretion by ELISA and F-actin staining with phalloidin in each condition (n = 7). Classic progesterone receptor (PRAB) and PGRMC1 localization was performed in non-decidualized ESC (ndESC) and decidualized ESC (dESC) of both protocols by immunofluorescence (n = 4).

Main results and the role of chance: PRL secretion significantly decreased in the presence of P4-BSA/E2 compared to dESC P4/E2 group ($p<0.05$). Nevertheless, PRL levels were significantly increased in the presence of P4-BSA/E2+AG205 compared to ndESC and dESC P4-BSA/E2 group ($p<0.05$). However, when we use the short protocol, all groups were able to secrete high levels of PRL included the P4-BSA/cAMP and P4-BSA/cAMP+AG205 groups. F-actin staining showed a lack of cytoskeleton reshaping in P4-BSA dESC group compared to the typical polygonal shape observed in decidualized controls. The F-actin filaments disposition of a dESC were restored in P4-BSA group in the presence of AG205. Again, dESC of all groups showed a correct F-actin structure when we induced decidualization with the short protocol, also in the P4-BSA/cAMP and P4-BSA/cAMP +AG205 groups. Immunocytofluorescence assay revealed a clear PGRMC1 perinuclear and nuclear sublocalization in P4/E2 and P4/E2+AG205 dESC groups, while it persisted at the membrane and cytosol in P4-BSA/E2 and P4-BSA/E2+AG205 dESC groups. Surprisingly, PRAB, continued translocating to the nucleus in P4-BSA/E2 dESC group as in the control (P4/E2). However, PRAB signal strongly decreased in P4-BSA/E2 +AG205 dESC group. Finally, immunocytofluorescence of PR receptors using the short protocol did not show localization differences between all the groups in both receptors.

Limitations, reasons for caution: Due to the variability between human endometrial samples and the low number of experiments performed, results should be taken with caution.

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Wider implications of the findings: According to our results, we demonstrated that in absence of the P4 entry into the stromal cell, the activation of P4 membrane receptors in concomitance with PGRMC1 inhibition, is sufficient to achieve in vitro decidualization. Further molecular experiments are needed to understand the underlying mechanisms.

Trial registration number: Not applicable.

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P-452 Sufficient vitamin D supplementation regulates maternal T-helper cytokine production in women suffering from infertility

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Study question: To determine the impact of vitamin D (VD) supplementation on maternal immune tolerance in vivo, as well as decidualisation of human endometrial stromal cells (HESCs) in vitro.

Summary answer: VD supplementation suppressed T-helper (Th)1 cell levels in vivo and secretion of interferon (IFN)-γ in decidual HESCs in vitro.

What is known already: Maternal immune tolerance during pregnancy has been associated with the balance between Th1 (IFN-γ production) and Th2 (IL-4 production) cells in favour of the latter. An imbalance in the Th1/Th2 cell ratio has been associated with reproductive failure, such as implantation failure and miscarriage. VD plays a significant role in regulating the immune system, including Th and other cells. However, the impact of VD on successful pregnancy and optimal preconception levels of VD have not been clearly understood.

Study design, size, duration: The present study was approved by the local ethics committee. This prospective intervention study was designed to determine the impact of VD supplementation on Th cell immune response. Various Th cell tests before and after VD supplementation (vitamin D3 1,000 IU/day) for 3 months were compared. In primary culture, HESCs were decidualised using 8-bromo-cAMP and progesterone with or without 1,25(OH)2VD for 4 days. Molecular analysis was then used to examine the cells and condition media.

Participants/materials, setting, methods: Out of 28 patients aged 40 or below suffering from infertility and low 25(OH)VD levels (<30 ng/ml), five were excluded (one was lost to follow-up and four had received immunosuppressive drugs for post-liver transplantation or collagen disease). Twenty-three women suffering from infertility underwent various Th cell tests before and after VD supplementation. We then examined the effects of VD on HESC decidualisation using real-time quantitative PCR, ELISA and immunohistochemical staining.

Main results and the role of chance: Among the 23 patients, 22 (95.7%) had increased 25(OH)VD ($p < 0.0001$). Moreover, VD supplementation significantly decreased the Th1/Th2 cell ratio (14.8% ± 4.0% to 13.1% ± 4.1%, $p = 0.015$). Sufficient VD levels [25(OH)VD level ≥30 ng/ml] had been found in 11 patients (47.8%), among whom significantly decreased Th1 cell levels and Th1/Th2 cell ratios had been observed ($p = 0.032$ and 0.010). However, no significant differences in Th cell levels had been observed in patients who did not reach sufficient VD levels after supplementation. No complications or adverse effects were identified in all patients.

Basic research has suggested that co-treatment of decidualising cultures with 1,25(OH)2VD increased the expression of vitamin D receptor (VDR) in decidual cells, leading to the upregulation of IGFBP1 expression, a marker of decidualisation. Moreover, 1,25(OH)2VD treatment of decidual cells in parallel cultures showed nuclear accumulation of VDR, unlike cells not treated with 1,25(OH)2VD. In a cytokine assay of decidualised HESCs in culture media with or without 1,25(OH)2VD, IFN-γ levels were significantly lower in cells treated with 1,25(OH)2VD compared to those without 1,25(OH)2VD ($p = 0.008$), but not IL-4.

Limitations, reasons for caution: Considering that VD levels can be affected by exposure to sunlight and diet, supplementation may not be the only factor affecting VD levels. We recognise that significant seasonal changes had not been observed in the present study.

Wider implications of the findings: Optimal Th1/Th2 cell balance is important during implantation and maintenance of pregnancy. Aberrantly high population of Th1 cells has been associated with embryonic rejection, leading to repeated reproductive failure and complications during pregnancy. Sufficient preconception VD levels may prevent maternal immune rejection of the embryo, leading to a successful pregnancy.

Trial registration number: None.

P-453 The effect of maternal BMI on the development of human fetal kidney

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Study question: How does maternal BMI affect development of the human fetal kidney?

Summary answer: High maternal BMI affects fetal kidney development and dysregulates development of the renin-angiotensin system.

What is known already: The obesity epidemic worldwide currently affects one-third of women in reproductive age, giving rise to higher prevalence of obesity in pregnancy. Children born to overweight or obese mothers (BMI ≥25) are at an increased risk of developing childhood obesity and type 2 diabetes, which in turn predisposes individuals to chronic kidney disease (CKD). Although clear associations have been demonstrated between maternal obesity and long-term consequences on offspring health, the mechanisms underlying the effects remain largely unknown.

Study design, size, duration: We aimed to characterize and identify phenotypic and molecular changes to the human fetal kidney in relation to maternal BMI status. A total of 112 human fetal kidneys were collected from elective terminations of normally-progressing pregnancies (7-20 weeks of gestation, Scottish Advanced Fetal Research Study, REC 15/NS/0123). 53 from mothers of BMI <25 and 59 from overweight or obese mothers of BMI ≥25.

Participants/materials, setting, methods: All 112 kidneys were weighed before sample processing. Whole kidney extracts were prepared from 58 fetuses. 23 transcripts of key renal developmental genes, renin-angiotensin system (RAS), and kidney injury markers were quantified by qPCR. 27 whole kidneys were processed for histomorphological analysis to assess gross phenotype and podocyte density. Statistical analysis was done by ANOVA, linear regression and non-parametric tests as appropriate.

Main results and the role of chance: High maternal BMI significantly increases the relative ratio of kidney weight to total fetal body weight in male fetuses ($p < 0.02$), but maternal BMI has no significant association with overall podocyte density in fetuses.

Expression of 11/23 transcripts (5 renal developmental genes, 4 components of RAS, 1 kidney injury marker and 1 hypoxic marker) significantly increased with fetal age, while the erythropoietin (*EPO*) transcript was undetectable in all groups. The renal renin encoding gene, *REN*, involved in blood pressure and fluid balance control was significantly increased in fetal male kidneys if maternal BMI was ≥25 ($p < 0.04$).

These findings are suggestive of altered kidney development, particularly the renin-angiotensin system in male fetuses carried by women with high maternal BMI. This study is currently being expanded by analysis of the RAS and kidney damage markers in fetal human plasma.

Limitations, reasons for caution: Human nephrogenesis begins from 4 weeks of gestation and continues into the late fetal period (gestation week 34-35). Our population sample includes fetuses up to 20 weeks of gestation, hence the final stages of podocyte development cannot be quantified in our study.

Wider implications of the findings: Disturbance of the fetal renal renin-angiotensin system is a major contributor to the development of hypertension and kidney diseases in later life. Insight into mechanisms underlying effects of maternal BMI on fetal kidney development may provide key data to prevent progression of kidney damage and reduce CKD complications in adulthood.

Trial registration number: Not applicable.

P-454 Clusterin, CXCL1 and MUC1 may play important roles in improving endometrial receptivity with local injury of endometrium in unexplained recurrent implantation failure patients

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Study question: How endometrial scratch improves endometrium receptivity in unexplained recurrent implantation failure (RIF) patients.

Summary answer: Endometrial scratch might be related to the up-regulation of the expression of CLU and MUC1 and the down-regulation of CXCL13 after the scratch.

What is known already: The effectiveness of endometrial scratch on improving endometrial receptivity in unexplained RIF patients has been reported in multiple clinical studies, but the molecular and genetic mechanism of this appearance remain unclear.

Study design, size, duration: It was a self-contrast designed study, 27 patients diagnosed as unexplained RIF were included. Patients diagnosed with intrauterine adhesions, poor ovarian response, autoimmune disease were excluded. Expression of 14 genes regulating the process of the response to wounding and 8 gene reported to be related to endometrial receptivity were analyzed in the endometrium sample before and after scratching. The immunohistochemistry tests were performed to further testify expression and distribution of deferential expression genes.

Participants/materials, setting, methods: Included RIF patients were monitoring by ultrasonic and endometrium samples were obtained on the fifth day after ovulation as control group. Scratches were performed on day 5 of following menstrual cycle with biopsy catheters and endometrium sample were taken on the fifth day after ovulation as test group. The expression levels of targeted genes were assessed by RT-qPCR tests, then immunohistochemistry (IHC) tests were performed to analyze the expression status of the significantly regulated genes.

Main results and the role of chance: We select 14 genes that have been reported as genes regulating the process of the response to wounding, including C4BPA, HPSE, FGB, SCYE1, CXCL14, THBD, CXCL13, BCL6, SERPING1, CLU, CFD, C3, PROS1 and AOX1. Meanwhile, 8 gene symbols reported to be related to endometrial receptivity were also tested, including TP53, COX2, CPLA2, MUC1, SGK1, LIF, ITGAV, and ITGB3. Our RT-PCR data demonstrate that there are no significant changes of the expression levels of most detected genes except for CXCL13 (fold change: 0.91), MUC1 (fold change: 1.42) and CLU (fold change: 2.19). Immunostaining endometrium were observed and analyzed with average positive staining method. The CXCL13 positive cells area percentage (PCA%) is 4.89% vs 2.78% ($p = 0.10$), showing reduction after the scratch but without statistical significance. On the other hand, MUC1 was specifically stained in the cytoplasm of endometrial glandular epithelial cells before and after the scratch, and the PCA% is significantly higher after the scratch (15.18% vs 33.20, $p = 0.01$). Unlike MUC1, CLU stained mostly in the cytoplasm of endometrial stromal cells, and the PCA% also elevated after the scratch but the difference is no statistical significance (15.51% vs 19.43%, $p = 0.37$).

Limitations, reasons for caution: This is an observation study and the regulating mechanism of MUC1 on human endometrium receptivity should be further evaluated by gene knock-out model.

Wider implications of the findings: Endometrial receptivity defect is a major factor that's hampering the embryo implanting and endometrial scratch is one of the most applied procedure in clinical practice on RIF. With the revealing of its detail, we are aiming for more targeted examination and therapy to improve endometrial receptivity for unexplained RIF patients.

Trial registration number: not applicable.

P-455 Pregnancy loss rates (PLR) in IVF patients as an overlooked topic

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Study question: Are IVF patients of increased risk for pregnancy loss compared to natural conception and is there a benefit of pre-implantation genetic screening (PGS)?

Summary answer: Interestingly, PLR were not increased in sub-fertile IVF-patients compared to spontaneous conception. No significant improve with PGS questions aneuploidy as major early PL factor.

What is known already: Human reproduction is characterized by a high embryo-wastage. The majority of embryos are already lost before implantation with chromosomal imbalance suggested as the most important cause for this phenomenon. Major concern in IVF was turned on implantation failure, including the implementation of PGS. Less interest was drawn on PL after successful implantation of the embryo. While several studies focused on PL in natural conception, e.g. the pioneering work of Wilcox *et al.*,1988, PL in IVF-patients and especially in PGS remains under-investigated.

Study design, size, duration: A retrospective study single center study was conducted including 1.383 vitrified/warmed blastocyst transfers between 01/2010 and 12/2016 to insure a standardized optimal endometrial preparation. Clinical data were extracted by using the DynaMed® software; statistics were performed using SPSS statistics.

Participants/materials, setting, methods: All patients with a positive pregnancy test after blastocyst transfer in the test period were included. Mean female age at the time-point of cryopreservation was 35 years. In PGS-cycles trophectoderm biopsy was performed with aCGH or NGS analysis. We compared outcomes of single or double blastocyst transfers with or without PGS by analyzing implantation rate (IR), pregnancy loss rate (PLR) and birth rate (BR). Obtained data were compared with data published on natural conceptions.

Main results and the role of chance: After positive hCG-test a PLR before detection of embryonic heart-beat of 22.8% was observed in IVF-cycles, no statistically significant difference between conventional and PGS cycles was seen (22.6% vs. 30.0%, respectively). Thirteen pregnancy losses (0.94%) occurred due to ectopic implantation. Distinctly higher IRs were found in single blastocyst transfer (SBT) compared to double BT (DBT) in both, conventional and PGS cycles (SBT 79.7% and 70.8% vs. DBT: 50.0% and 33.3%, respectively). SBTs resulted in a 1.5% monozygotic twin rate; DBTs in 28.8% twin pregnancies. PLR after implantation and detection of embryonic heart-beat was 13.7% after frozen/warmed BT in conventional IVF-cycles and 9.5% in PGS-cycles with not statistically significant difference. These observed PRL in sub-fertile IVF patients were close to natural embryo loss after detection of fetal heart beat (assumed 6-12%). No statistically significant difference was detected between singleton and twin pregnancies. In this line, similar BRs/BT were detected in both groups: 66.7% in conventional vs. 63.3 % in PGS cycles.

Limitations, reasons for caution: Only vitrified/warmed BT were included, thus results might be not applicable for fresh BT. Further limitations are the small data set of PGS BTs. Larger data analysis are needed to further confirm our data.

Wider implications of the findings: Our data show increased risk of non-implantation for embryos in DBT. However, PLR after embryonic heart-beat in both, conventional IVF and natural conception are similar, potentially due to the benefit of an optimal luteal phase support. The benefit of PGS in PRL remains to be proven.

Trial registration number: 'not applicable'

P-456 Human chorionic gonadotropin improves endometrial receptivity by increasing the expression and enhancing phosphorylation of HOXA10

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Study question: Does hCG affect endometrial receptivity by regulating the expression of homeobox A10 (HOXA10)?

Summary answer: hCG activates endometrial decidualization by reducing expression and concomitant DNA methylation of HOXA10 and enhancing phosphorylation of HOXA10.

What is known already: HOXA10 has emerged as an important molecular marker of endometrial receptivity. Recent evidence suggested that hCG may influence the endometrial milieu thereby enhancing receptivity and implantation success, but the exact underlying mechanism of this phenomenon remained elusive.

Study design, size, duration: Endometrial samples were collected from normal cycling women during the mid-secretory phase for endometrial stromal cells (ESCs) isolation used to investigate the molecular mechanisms in vitro. Blastocyst-like spheroid(BLS) implantation models were performed to examine the roles of hCG during embryo expansion on hESCs. This is an experiment study lasted nine months.

Participants/materials, setting, methods: Endometrial stromal cells (ESCs) and JAR cells were used as study models. Western blotting, real-time

RT-PCR and BLS expansion assay were used to determine the mechanism of endometrial receptivity changes induced by hCG. All experiments were conducted using at least three different cell culture preparations in triplicate.

Main results and the role of chance: Western Blot analysis revealed that addition of 0.2IU/mL hCG to ESCs induced the increase of HOXA10 and decrease of DNA methyltransferases 1 (DNMT1) at 48 h (both $p<0.01$ versus control). Increase of mammalian sterile20-like kinase1(MST1) in ESCs treated with 0.2IU/mL hCG at 20 min ($p = 0.043$ versus control). HOXA10 mRNA levels were significantly increased by hCG 0.2IU/mL treatment for 48 h ($p<0.01$) and DNMT1 mRNA levels were decreased by hCG 0.2IU/mL treatment for 48 h. hESCs treated with hCG 0.2IU/mL up to 48 h showed a good decidual response, mainly characterized by increased BLS expansion.

Limitations, reasons for caution: This is an in vitro study utilizing hESCs and JAR cells. The changes of DNA methylation of HOXA10 and phosphorylation of MST1 should be further evaluated. Furthermore, the results obtained should be confirmed in mice with hCG intrauterine perfusion treatment in vivo.

Wider implications of the findings: Our results suggest that hCG activates endometrial decidualization not only by reducing expression and concomitant methylation of HOXA10 also enhancing phosphorylation of HOXA10. These finding may subsequently provide novel potential therapeutic regimens for patients with poor decidualization.

Trial registration number: This work was supported by the National Natural Science Foundation of China (81571504).

P-457 Increased NK-cell subsets with inhibitory cytokines and surface receptors in patients with recurrent miscarriage

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Study question: How do the counter-regulating inhibitory mechanisms of NK-cell subsets differ in patients with recurrent miscarriage compared to healthy controls?

Summary answer: Patients with recurrent miscarriage show abnormally high NK-, NKT- and T- cells in the blood which express inhibitory cytokines and inhibitory surface receptors.

What is known already: NK-cells are in the focus of debate as potential immunologic markers and targets in recurrent miscarriage. There is increasing evidence for immunoregulatory NK cells that might mediate immune responses by secretion of IL10, IL4, and TGFß. Patients with recurrent miscarriage show upregulated cytotoxic NK cells that are suspected to play a causal role in the pathogenesis. Further elevated NK-cells are associated with decreased uterine blood flow in early pregnancy, possibly leading to hypertension, preeclampsia, and fetal growth restriction.

Study design, size, duration: Prospective cohort study, in total $n = 31$ patients with recurrent miscarriage and $n = 37$ healthy controls were recruited between 01/2016 and 09/2017.

Participants/materials, setting, methods: NK-, NKT- and T-cell subsets were analyzed in the peripheral blood of $n = 31$ patients with idiopathic recurrent miscarriage and $n = 37$ healthy controls using eight-color fluorescence flow cytometry. Patients had a history of 3 and more consecutive miscarriages and were screened for anatomical disorders (vaginal ultrasound and hysteroscopy), endocrine dysfunctions, autoimmune disorders, deficiencies in coagulation factors, inherited haemostatic changes and parental chromosomal abnormalities. All analyses were performed at least 3 months after the last pregnancy.

Main results and the role of chance: Compared with healthy controls, patients with recurrent miscarriage showed significantly higher absolute numbers of CD56+ NK-cells co-expressing the phenotype IFNyR+, IL4+, TGFβ+, IL4+HLADR+, TGFβ+HLADR+, IL4+TGFβ+, IL4+TGFβ-, IFNy+ and/or IL10- IFNy+ (all $p\leq0.01$), more IL17+CD56bright ($p = 0.028$) NK-cells, and more CD56dimCD16+ NK-cells co-expressing IFNyR, IFNy, IL4 and/or TGFβ (all $p\leq0.01$). Further, patients with recurrent miscarriage showed significantly higher absolute numbers of CD158a-CD158e+, CD158a+, CD158b+ (all $p<0.05$), NKG2D+NKG2A+, NKG2D+NKG2A-, NKG2D+ and/or NKG2A + (all $p\leq0.01$) CD56+ NK-cells and higher CD158a+, CD158b+ (all $p<0.05$), NKG2D+ and/or NKG2A+ (all $p<0.01$) CD56dim+CD16+ NK-cells than healthy controls.

Limitations, reasons for caution: This data needs to be confirmed in a larger cohort of patients.

Wider implications of the findings: Compared to healthy controls, patients with recurrent miscarriage have abnormal high circulating NK-cells expressing inhibitory cytokines and inhibitory surface receptors which might contribute to the pathogenesis. This immune profile might be used to identify patients that benefit from therapies that inhibit cytotoxic immune response in clinical trials.

Trial registration number: not applicable.

P-458 Recurrent implantation failure: should we refuse from scanning electron microscopy

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Study question: To evaluate clinical outcome of scanning electron microscopy (SEM) usage for Personalized Embryo Transfer (PET) algorithm in women with recurrent implantation failure (RIF).

Summary answer: Determination of the implantation window via SEM helps improve the strategy of PET, as well as pregnancy rate in women with RIF.

What is known already: Recurrent implantation failure is a challenge of modern reproductive medicine. The endometrium acquires the ability to implant a hatched blastocyst only within a specific time termed the receptive phase. Pinopodes have been suggested as the markers of uterine receptivity. Endometrial pinopodes formation can be detected by SEM. Controversial data are published on SEM as an efficient method of endometrial receptivity determination. Implementation of endometrial receptivity array (ERA) test for «Implantation Window» determination (IW) limited SEM usage. However, in recent years interest to SEM has revived, given its availability, reproducibility and informative value.

Study design, size, duration: A prospective study of 66 cycles in patients with recurrent implantation failure was conducted between 2012 and 2016. Patients with RIF who had euploid embryos according to PGD results were included in the study after Informed Consent was obtained. Hysteroscopy was performed for all patients to exclude intrauterine pathology. Patients were divided in two groups. 1.89 ± 0.05 embryos were transferred in the first group and 1.94 ± 0.06 - in the second, ($p> 0.05$).

Participants/materials, setting, methods: Patients were divided in two groups. 1 group - 36 patients - transfer of frozen-thawed euploid embryos on day (P+7) after initiation of progesterone therapy in hormone replacement cycle (HRC). 2 group - 30 patients - Pipelle endometrium sampling was performed on day (P+6) and day (P+8). SEM was performed for pinopodies imaging. Frozen-thawed euploid embryos in this group were transferred depending on the IW according to results of SEM.

Main results and the role of chance: There were no differences in age, BMI, reproductive and gynaecological history, as well as in the number of previous IVF cycles in the groups. Analysis of SEM results on day P+6: lack of pinopodes in 24 (80,00%) patients, sites of scant pinopodes around the uterine gland in 4 (13,33%), plenty of developing pinopodes – in 2 (6,67%). On Day P +8 we found lack of pinopodes in 5 (16,67 %) participants. Scant, moderate and abundant amounts of developing pinopodes - in 8 (26,67%); 6 (20,00%); 4

(13,33%) respectively. The abundance of developing and single developed ones in 4 (13.33%); and scant, moderate and abundant quantity of regressed pinopodes in 1 (3.33%), 1 (3.33%), 1 (3.33%) respectively. Transfer of frozen-thawed embryos was performed according to the IW: on day P+5–1 (3,33%); P+6–6 (20,00%); P+7–5 (16,67%); P+8–10 (33,33%); P+9–6 (20%). In 2 cases was performed double transfer. Endometrial thickness on the day of ET was 9,35 ± 0,31 mm and 9,34 ± 0,3 mm (P>0.05). Pregnancy rate was 4 (11,11 %) in the first group and 18 (60%) in second (P <0.05). Live birth incidence on ET was 3 (8.33%) and 16 (53.33%) (P <0.05).

Limitations, reasons for caution: For the effectiveness of the proposed approach it is necessary to know the exact day of administration of exogenous or secretion of endogenous progesterone. The IW formation is different in women in the natural cycle, in cycle with HRT, and in the cycle with controlled ovarian stimulation.

Wider implications of the findings: Knowledge of IW in patients with RIF helps to obtain pregnancy in this complex category of patients. Along with the modern transcriptomic genetic investigation of IW, SEM remains an informative, reproducible, accessible method.

Trial registration number: Not trial; this prospective study was approved by Ethics Committee.

P-459 Systematic review and meta-analysis of variants in cytokine genes emphasizes their clinical validity with respect to recurrent pregnancy loss

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Study question: Is existing evidence sufficient to conclude that testing variants in cytokine genes is not useful in the context of recurrent pregnancy loss (RPL)?

Summary answer: Systematic assessment of all association studies to date, followed by meta-analysis, reveals that 5 variants in 3 cytokine genes are significantly associated with RPL.

What is known already: Although immunological cytokines are involved in establishing and maintaining pregnancy, studies testing the association of variants in cytokine genes with RPL have yielded mixed results. Indeed, the ESHRE Early Pregnancy Guideline Development Group (2017) recommends against testing for cytokine polymorphisms because 'meta-analyses have not been able to find polymorphisms in relevant cytokine genes associated with RPL." However, only one of the studies cited to support this recommendation is a meta-analysis, covering 3 variants in 3 genes (Medica et al., 2009). Moreover, since the publication of this analysis, at least 26 studies of cytokine variants and RPL have been published.

Study design, size, duration: Natural language processing algorithms were used to identify articles in NCBI PubMed pertaining to genetics and RPL. Of the 1,599 articles identified, 684 were found to be true positives after manual evaluation. The evaluation also uncovered articles cited as references that were not identified algorithmically. Reported gene associations were ranked using an adaption of the Clinical Genome (ClinGen) Gene-Disease Clinical Validity Classification Framework. Data was extracted upstream of meta-analysis following PRISMA guidelines.

Participants/materials, setting, methods: Variants in genes that met ClinGen-adapted criteria for 'strong" evidence of clinical association with RPL were statistically validated using random-effects model meta-analyses. Variants were excluded from analysis if there were <2 published studies or evidence of overlapping cohorts, or if an article's presentation of information precluded the determination of the risk allele. p<0.05 was considered statistically significant, equivalent to a false discovery rate cutoff of approximately 0.10.

Main results and the role of chance: We found no genes demonstrating 100% penetrance with RPL over multiple independent studies, which is required for a "definitive" association ranking based on the adapted ClinGen framework. Our analysis revealed that 39 genes, including the cytokines *IFNG*, *IL1B*, *IL6*, *IL10*, *IL18*, and *TNF*, currently satisfy guidelines for strong evidence of association with RPL. This ranking requires at least 2 independent demonstrations of statistically higher prevalence of variants in affected individuals than in controls, as well as supporting experimental data (e.g., from mouse models or human gene expression studies). Of the variants within these genes, 62 had sufficient experimental evidence for inclusion in meta-analysis, including 19 in 6 cytokine genes. Five of these variants were found to be significantly associated with RPL after meta-analysis. Three of these 5 lie within *TNF* and include the c.-488G>A variant (OR = 1.43, 95%CI:1.09-1.76, p = 0.003), previously observed to have no association with RPL after meta-analysis. Notably, the *TNF* variants c.-1211T>C and c.-556G>A have an even higher effect size, with ORs of 2.64 (95%CI:1.77-3.50, p<0.001) and 2.29 (95%CI:1.07-3.51, p = 0.002), respectively, which emphasizes the importance of assessing different variants in the same gene. We observed a similarly high effect for the c.-887T>G variant in *IL18* (OR = 1.92, 95%CI:1.58-2.27, p<0.001).

Limitations, reasons for caution: We only included studies in which patients had experienced ≥2 losses in our analyses. Heterogeneity introduced by other factors, such as age, population stratification, stage of loss, whether or not losses were consecutive, and the wide variety of exclusion criteria, were not adjusted for.

Wider implications of the findings: The association of genetic biomarkers with RPL cannot be discounted without objective, systematic, and standardized analysis. While the significant variants identified here may not be causative by themselves, such markers may enable physicians to distinguish molecular subtypes of RPL, leading to treatment strategies that help mitigate inherent, patient-specific risk factors.

Trial registration number: N/A.

P-460 Which one is more effective on Recurrent Implantation Failure,innate or adaptive immunity?

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Study question: *Which one is more effective on Recurrent Implantation Failure, innate or adaptive immunity?*

Summary answer: adaptive immunity play more important role in recurrent implantation failure patient.

What is known already: The immune system is required for maternal immune tolerance, protecting the fetus, and regulating the placentation process. In RIF patients, Many factors may contribute to implantation failure as inappropriate immune responses at the time of embryo introduction is one of the reason for unsuccesful implantation. So, evaluation of innate and addaptive immune syatem in RIF patients seems valued.

Study design, size, duration: This study was a basic genomic analysis of human endometrial biopsies taken from 10 patients with repeated implantation failure and 6 healthy fertile women in the secretory phase. All participants are in reproductive age with normal menstrual cycles.

Participants/materials, setting, methods: Total mRNA were extracted from endometrial tissues of women with repeated implantation failure (on day 3-5 after ovulation, n = 12) and healthy fertile individuals (on day 3-5 after ovulation, n = 10) during luteal phase. The expression profile of 84 genes related to innate and adaptive immunity was investigated using qRT-PCR array. Informed consent was obtained from patients. All measurements were performed in triplicates on independent biological replicates.

Main results and the role of chance: Our data clearly showed that cytokines expressions like IL6, IL-2, IFN gamma, IL17, IL23 and IL13 are higher in RIF group than normal. The innate immunity pathways as, pattern recognition receptors and their pathways like TICAM1, TICAM2, IRAK1, TRAF, MYD88 expression are lower or not significant in RIF than normal group, also the expression of NFKBIA (IκBα, MAD3) as an inhibitor of NFKB1 are higher in RIF group. The adaptive immunity markers like T cell activation,Thelper1 and Thelper17 like CD86, ICAM1, CXCR3, TBX21 and FASLG (TNFSF6) expressions are higher in RIF than normal.

Limitations, reasons for caution: The results need to be confirmed in more cases and in protein level.

Wider implications of the findings: Results showed more proinflammatory environment than anti-inflammatory in RIF. Apparently, the source of inflammatory cytokines production are Tcells not PRR pathways. For example, MYD88, an important factor downstream of PRR was decreased in RIF patients. So, it seems adaptive is more contributing factor than innate immunity in pathophysiology of RIF.

Trial registration number: N/A.

P-461 Patient acceptability and perception of randomisation in a trial of Scratch in Recurrent Miscarriage (SiM trial)

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Study question: 1. Would women be prepared to be randomised to scratch vs sham examination?

2. Will patients be aware of endometrial biopsy compared to sham procedure?

Summary answer: Women were accepting to the randomisation process within the trial.

Women were not aware whether they had the endometrial biopsy or the sham procedure.

What is known already: The Cochrane meta-analysis of the effects of endometrial scratch in women undergoing assisted reproductive conceptions, suggested that obtaining endometrial biopsy in the luteal phase prior to the treatment improves the clinical pregnancy and live birth rates. This was particularly so in women with previous failed IVF's.

The trials in the review compared endometrial scratch vs endometrial injury by other methods such as hysteroscopy.

In the Endoscratch trial for women prior to their first IVF, women are randomised to scratch vs no examination.

The ESHRE RPL guideline acknowledges the gap in the evaluation of endometrial scratch in women with recurrent miscarriage.

Study design, size, duration: Evidence from trials in recurrent miscarriage (RM) patients suggests good outcomes in the placebo group. The presence of a sham arm was important methodologically, in the trial. However patient acceptability was unknown.

The SiM trial is a pilot randomised controlled trial of endometrial scratch vs sham procedure, in the luteal phase, on the pregnancy outcomes in RM.

109 women were recruited over a 2 year period (2015-2017). Ethics approval was obtained for trial, questionnaire collation.

Participants/materials, setting, methods: Women (age 18-42) were approached in the recurrent miscarriage clinic to participate in the SiM trial. There were given trial explanation and PIS by the research midwife and clinical fellow. The questionnaire was provided in a self addressed envelope at their randomisation visit, to be completed and returned within 4 weeks.

The questionnaire contained objective measures quantifying pain, bleeding, duration- to assess the difference between the groups and free text box for comments and feedback.

Main results and the role of chance: Of the 226 women screened, 150 women were eligible. 133 patients were consented and 109 women were randomised. The acceptance rate was 72.66 %.(10/24 women were excluded as pregnant before randomisation). 109 Questionnaires were given at randomisation. The response rate was 62.38 %(68/109), with similar numbers in both groups-(33 and 35), with most responses within 2 weeks from randomisation (76.5%, 52/68). There were no reported infection following the procedures.

More bleeding was reported in the biopsy group (28/33) compared to the control group (8/35), which was mild in 80% of the women. A significant proportion of women in both the groups experienced pain, but more women in the biopsy group (30/33) reported pain than the control group (20/35). However the pain score was reported as mild by the majority of women in both the groups. Interestingly 7of 20 women who reported pain in the control group described it as moderate pain. 1 in 10 patients experienced severe pain in the biopsy group.30/68 patients had prior analgesia intake. The duration was short lasting (< 10 minutes) in most women.

Women who experienced pain in the sham group thought they had the intervention and those painfree in the biopsy group vice-versa.

Limitations, reasons for caution: The numbers are small as this is a pilot trial. There is a possibility of recall bias especially when the response has been delayed. The influence of the procedure expectations and experience leading upto the randomisation may have contributed to the recall bias.

Wider implications of the findings:

- Women with recurrent miscarriage accepted to randomisation in the trial with a sham group.
- More women experienced bleeding in the biopsy group.
- As some women in both groups experienced pain and others did not during the procedure, they were not aware of the allocation group and so blinding occurred.

Trial registration number: The trial is registered on clinicaltrials.gov.

The trial registration number is NCT02681627

P-462 The effect of uterine cavity irrigation with office hysteroscopy during antagonist cycle 0varian stimulation on IVF outcome

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Study question: Dose uterine cavity irrigation with office hysteroscopy in the day 5-7 during antagonist cycle ovarian stimulation in the IVF cycle improve the endometrial receptivity and ivf outcome.

Summary answer: hysteroscopy could not improve the IVF outcome in the same cycle but could enhance the cumulative pregnancy rate.

What is known already: Although many studies were done about the effect of routine hysteroscopy prior to the first IVF cycle, there is still no agreement about the effect of hysteroscopy on IVF outcomes.

Study design, size, duration: This prospective randomized clinical trial was conducted in the shariati hospital infertility ward, Tehran university of medical sciences from June 2015 to June 2016.

250 women who were less than 40 years, with primary infertility and without the history of prior hysteroscopy examination in the first IVF cycle, and also with normal transvaginal sonography and hysterosalpingography were entered to the study.

Randomization was done with web-based concealed allocation and patients were randomly entered to each group.

Participants/materials, setting, methods: Controlled ovarian hyperstimulation was done by using antagonist regimen with r-FSH in two groups.

Hysteroscopy was performed in the early mid-follicular phase of a stimulation cycle (day 5-7) with a vaginoscopy approach in hysteroscopy group. Embryotransfer was done in the same cycle. The primary outcomes were clinical pregnancy rate and secondary outcome was live birth rate.

also we followed all patients without live births through the second freeze embryo transfer cycle for secondary outcome.

Main results and the role of chance: From June 2015 to June 2016 we randomly assigned 250 women in one of the two groups: hysteroscopy (case group) or without hysteroscopy (control group) and finally 228 patients finished the study. clinical pregnancy rate was 47% in the case group and 40% in the control group. (p-value = 0.396, RRR = 13.8% [-5.9% to 35.9%], NNT = 15 [-16 to 5])

Live birth rate was 41.28% in the hysteroscopic group and 31.9% in the control group (p-value = 0.18, RRR = 22.7% [-9.2% to 45.2%], NNT = 11 [-32 to 5]).

When the patients were followed for 2 months for an additional embryo transfer, LBR was 78% in the hysteroscopic group and 47.5% in the control group (p-value = 0.039, RRR = 26.9% [3-44.9%] and NNT = 8 [4-85]).

Limitations, reasons for caution: conduction in just one center and small sample size were two limitations in this study.

Wider implications of the findings: in order to decide about the generalisability to other populations, more robust RCTs must be coducted.
Trial registration number: IRCT2016011022795N2

P-463 The relationship between angiogenic markers in peri-implantation endometrium and hypertensive disorder in pregnancy in women with reproductive failure

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Study question: Do angiogenic markers in peri-implantation endometrium predict hypertensive disorder in pregnancy in women with reproductive failure?
Summary answer: Micro vessel density (MVD) in the peri-implantation endometrium may have a predictive value for hypertensive disorders in a subsequent pregnancy in women with reproductive failure.
What is known already: Vascular endothelial dysfunction in the placenta bed has an important role in the pathogenesis of gestational hypertension and preeclampsia. Barker hypothesis proposed that early intrauterine events might have a long-term effect on the later on pregnancy or even after birth. We aimed to examine the relationship between angiogenesis at the peri-implantation period and hypertensive disorders in the subsequent pregnancy in this study.
Study design, size, duration: This is a retrospective study including 110 women with a history of reproductive failure, including 58 women with recurrent miscarriage, 38 infertile women and 14 women with recurrent implantation failure.
Participants/materials, setting, methods: Endometrial biopsies were obtained precisely 7 days after luteinization hormone surge in natural cycles. Immunohistochemistry was used to determine expression of VEGF-A, VEGF-C and PLGF and micro blood vessels were identified by von Willebrand factor (vWF). A semi-quantitative analysis was performed by using *H*-score analysis of staining intensity for VEGF-A, VEGF-C and PLGF in the luminal epithelium, glandular epithelium and stroma, separately. The number of vWF-positive endometrial micro vessels were counted by Image J software.
Main results and the role of chance: Nineteen out of the 110 women were diagnosed as gestational hypertensive disorders in the subsequent pregnancy, including 16 women with gestational hypertension and 3 women with preeclampsia. The mean±SD number of micro blood vessels/mm^2 in women with gestational hypertensive disorders ($15.2 \pm 5.1/mm^2$) was found to be significantly ($P = 0.041$) higher than those with normal gestational blood pressure ($9.8 \pm 3.1/mm^2$). However, there was no significant difference in the expression intensity of VEGF-A, VEGF-C and PLGF in any endometrial compartments (luminal epithelium, glandular epithelium and stroma) between women with or without gestational hypertensive disorders.
Limitations, reasons for caution: Immunohistochemistry and H-score analysis for staining intensity are semi-quantitative methods to determine the amount of protein expression. The study could potentially be strengthened by other quantitative measurements.
Wider implications of the findings: Observations of relationship between MVD in peri-implantation endometrium and gestational hypertensive disorders may indicate the long-term effect of peri-implantation events on the later-on pregnancy. Further studies may be considered whether the same conclusion applies to other markers or other pregnancy complications.
Trial registration number: N/A.

P-464 Reduced adrenomedullin expression leads to aberrant macrophage activities in human oviduct: implications for the pathophysiology of tubal ectopic pregnancy

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Study question: Does adrenomedullin (ADM) play a role in the pathogenesis of tubal ectopic pregnancy (tEP) by modulating tubal macrophage activities?
Summary answer: ADM inhibits tubal implantation by suppressing the pro-inflammatory activities of tubal macrophages.
What is known already: tEP accounts for ~2% of all pregnancies in the western world and is the major cause of maternal morbidity and mortality in the first trimester of pregnancy. The most important predisposing condition of tEP is salingitis. ADM is an immune-modulatory molecule with anti-inflammatory activities. Its expression in human oviduct is steroid dependent and peaked in a steroid environment simulating the early luteal phase, a phase when embryo transport occurred. We have demonstrated lower plasma and oviductal ADM levels in patients with tEP than in normal pregnancy. Macrophages is the predominant leukocyte populations in the oviduct with tEP.
Study design, size, duration: Women undergoing salpingectomy due to tEP were recruited as tEP group (n = 19). Non-pregnant women undergoing hystero-salpingectomy for benign diseases were also recruited and divided into control (n = 11) or salpingitis group (n = 19) according to their clinicopathological diagnosis.
Participants/materials, setting, methods: Oviducts were collected from the patients. ADM and implantation-related molecules expressions were determined by RT-qPCR and immunohistochemistry. Tubal macrophages were isolated and cultured with or without ADM treatment. Their conditioned medium (CM) were collected for inflammatory cytokine level determination by cytokine array and ELISA. The effect of CM form macrophages on the implantation-related molecules expressions and implantation capacity of tubal epithelial cells was also studied by RT-qPCR and trophoblastic spheroid attachment model respectively.
Main results and the role of chance: There was a reduced oviductal ADM levels and an elevated implantation-related molecules levels in patients with salpingitis and tEP when compared to the normal patients. Interestingly, ADM treatment significantly suppressed the stimulatory effects of tubal macrophages on the implantation-related molecules expressions and implantation capacity of tubal epithelial cells. These observations were associated with a decreased pro-inflammatory cytokines secretion of tubal macrophages after ADM treatment. Since Implantation is widely accepted to be associated with local inflammatory event, the results indicated that reduced oviductal ADM level in tEP patients may contribute to exacerbating pro-inflammatory activities of tubal macrophages, leading to a permissive environment for the embryo-tubal ectopic implantation.
Limitations, reasons for caution: Our study only focused on the role of ADM on tubal macrophage-derived pro-inflammatory cytokines production. Further studies are required to illustrate whether there are other factors from tubal macrophages can be modulated by ADM and thereby affecting implantation process.
Wider implications of the findings: Our study provides a new insight on the microenvironment change leading to tEP, as well as promote our understanding on the immunopathological mechanism of tEP.
Trial registration number: Not applicable.

P-465 Personal frozen-thawed embryo transfer in unexplained RIF patients according to the blinded histological dating of endometrial biopsies

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Study question: To determine the clinical effect of blinded endometrial histological dating in RIF patients.

Summary answer: Personal frozen-thawed embryo transfer(pFET) according to more accurate endometrial histologic dating (ovulation monitoring by ultrasound) may improve clinical outcomes in unexplained RIF patients.

What is known already: Many endometrial markers, such as pinopods, immunohistochemical biomarkers, endometrial blood flow, have been used to determine the receptivity. They may interact transiently with the embryo at implantation but appear not to be reliable markers of receptivity as the precision clinical diagnostic tools. The morphological changes observed on histology for each specific day after ovulation were described by Noyes in 1950'. Endometrial biopsy that shows a difference of more than 2 days between the histologic dating and actual day after ovulation is considered to be "out of phase". But such pFET studies according to the Noyes criterion are relatively lacking.

Study design, size, duration: From July 2017 to December 2017, altogether 205 infertility patients were recruited in the prospective intervention study.

Participants/materials, setting, methods: A total of 50 patients in control group and 155 RIF patients underwent endometrial biopsy. In phase I, control group patients with PGD-CCS were recruited before their first FET cycles in luteal phase (postovulatory +3/+5/+7/+9/+11) (ovulation monitoring by ultrasound). Endometrial histological dating were evaluated in control patients who were ongoing pregnancy in next natural FET cycle. In phase II, according to the results of endometrial dating, pFET were performed in the RIF group.

Main results and the role of chance: In the phase I, criteria for endometrial dating according to the Noyes dating criteria was developed in different time (postovulatory +3, postovulatory +5, postovulatory +7, postovulatory +9, postovulatory +11). And all the blinded endometrium dating (n = 205) was evaluated by two experienced pathologists. The agreement between pathologist A and pathologist B was determined to be good (weighted kappa = 0.672; 95% CI 0.606–0.737; P < 0.001). In the phase II, the rate of out of phase in the day of postovulatory +7 was significantly higher (31.61% vs 8.0%, P = 0.015) in RIF group than control group.The ongoing pregnancy rate(OPR) was dramatically high in the pET RIF group (61.70%).

Limitations, reasons for caution: We didn't design randomized controlled study in RIF group which the endometrial dating was out of phase. Because the RIF patients who have experienced multiple IVF failure were mostly reluctant to try routine treatment protocol.

Wider implications of the findings: Provide a new insight for the unexplained RIF patients with individualized treatment.

Trial registration number: NCT03222830

P-466 Usefulness of hysteroscopic intrauterine perfusion in frozen-thawed embryo transfer (FET) cycles

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Study question: Patients who did not become pregnant after initial FET underwent hysteroscopic intrauterine perfusion. Its usefulness was examined by comparing pregnancy status.

Summary answer: Intrauterine perfusion with 500 ml of saline solution significantly increased the pregnancy rate even in women without macroscopic abnormalities in hysteroscopy.

What is known already: FET is now being performed more often in the world. FET is also what has mainly been performed at our hospital. Although the involvement of endometrial thickness in pregnancy is widely known, there are few reports on the other intrauterine environments.

Study design, size, duration: Between January 2013 and December 2017, the pregnancy rate in FET was 46.3% (1545/3337). Subjects comprised 710 women who did not become pregnant after initial FET. Conditions of embryo transfer limited materials strictly to an endometrial thickness of more than ≧8 mm and good-quality embryos (Veeck's classification for Day 3 embryos ≧6 cell G II, Gardner's classification for blastocysts ≧3BB) in this study.

Participants/materials, setting, methods: Subjects were classified into three groups: Group I (women who underwent FET without hysteroscopy), Group II (women who received treatment for polyps and inflammation detected by hysteroscopy and then underwent FET) and Group III (women who had no macroscopic abnormalities in hysteroscopy and underwent FET immediately after intrauterine perfusion). A comparison of the pregnancy rate was made between the three groups.

Main results and the role of chance: The pregnancy rate was 40.5% (85/210) in Group I, 57.9% (168/290) in Group II and 59.0% (124/210) in Group III, respectively. In terms of pregnancy rate, a more significant increase was recognized in Group II and Group III than in Group I (Group I vs Group II P<0.01, Group I vs Group III, P<0.01). Even in the women without any macroscopic abnormal hysteroscopic findings, the pregnancy rate increased significantly after intrauterine perfusion.

Limitations, reasons for caution: None.

Wider implications of the findings: It is suggested that intrauterine perfusion with saline solution during hysteroscopy might improve conditions for implantation. Further examination is underway to clarify differences in bacteria and endometrial microbiota before and after intrauterine perfusion.

Trial registration number: None.

P-467 Novel binding partners with serine protease A differentially expressed in recurrent pregnancy loss

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Study question: Could the role of serine protease A in regulating its matrix proteins be related to the pathophysiology of recurrent pregnancy loss (RPL)?

Summary answer: We identified novel binding partners of serine protease A and found that the regulation of this protease has an important role in women with RPL.

What is known already: In humans, serine proteases are responsible for various cellular processes including reproduction, immune response, and blood coagulation. In a previous study, a novel serine protease gene was identified to be more expressed in chorionic villi from the normal controls than in those from the RPL patients.

Study design, size, duration: The serine protease A was investigated to identify putative substrates using yeast two-hybrid screening. 22 different genes encoding proteins, which may interact with the novel serine protease A, were identified. The serine protease A and its putative binding proteins may play a role in the occurrence of RPL.

Participants/materials, setting, methods: The full-length serine protease A and human cDNA library were used for yeast two-hybrid Matchmaker GAL4 Two-Hybrid System 3. The novel serine protease A gene was transfected into HeLa cells, and cellular lysates from these cells were separated on SDS-PAGE, and proteins in these lysates were immunoblotted with diverse antibodies. In addition, bioinformatics tools were employed to identify putative substrates of the novel protease A.

Main results and the role of chance: In a previous study, we identified eight differentially expressed genes in chorionic villi between the normal control and RPL groups. Of these, a novel serine protease A gene was less expressed in chorionic villi from RPL patients than in those from controls. By performing yeast two hybrid screening, 22 different genes encoding proteins, which interacted with the novel serine protease A, were identified. In addition, we tried to identify the interaction between the novel serine protease A and known substrates through the bioinformatics tools. Immunoprecipitation assay revealed that putative substrates including immunoglobulin transporter (IGT) and inhibitor of apoptosis (IOA) interacted with the novel serine protease A. Exogenous and endogenous expression levels of substrates were decreased by the novel serine protease A in a dose-dependent manner.

Limitations, reasons for caution: The results of this study can only indicate the limitations of serine protease A function in vitro.

Wider implications of the findings: This is to explain the pathogenesis of RPL from a new perspective, and will be the cornerstone of the development for diagnostic reagents and the treatment of drugs.

Trial registration number: not applicable.

P-468 Effect of intrauterine perfusion of granulocyte colony stimulating factor on endometrial parameters, implantation and pregnancy rate in women undergoing IVF cycles: randomised controlled trial

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Study question: Whether there is any benefit of intrauterine perfusion of Granulocyte- Colony Stimulating factor (G-CSF) on Endometrial parameters, implantation and pregnancy rate in women undergoing IVF-ICSI cycles?

Summary answer: Intrauterine perfusion of G-CSF had no benefit in terms of clinical pregnancy rate and implantation rate in women undergoing fresh IVF-ICSI cycles.

What is known already: Implantation is the rate limiting step in ART cycles. The interaction between a good quality embryo and a receptive endometrium at the time of implantation determines the success of ART cycles. A lot of research has been directed towards studying the factors that influence endometrial receptivity, measures to assess and possibly improve endometrial receptivity. Role of G- CSF is being investigated in increasing endometrial thickness and pregnancy rate in women undergoing ART. Few studies have shown that it increases implantation in women with poor endometrial thickness. Its benefit in women undergoing IVF with normal endometrial thickness is unknown.

Study design, size, duration: A randomised controlled trial was conducted among 150 women undergoing IVF/ICSI treatment at ART Centre after taking approval from Institute Ethics Committee.

According to inclusion/exclusion criteria, 76 patients were randomised to the intervention group (I) and 74 patients were randomised to the control group (II). IVF protocol was decided according to age, BMI hormonal parameters and previous response. Primary outcome was clinical pregnancy rate. Secondary outcome were effect endometrial parameters, implantation rate and ongoing pregnancy rate.

Participants/materials, setting, methods: Patients in the intervention group received intrauterine perfusion of 300μgm (0.5 ml) of G-CSF on the day of ovulation trigger. Control group was administered intrauterine perfusion of 0.5 ml of normal saline. Endometrial thickness, volume and vascularity was assessed by TVS on trigger day and embryo transfer day. The IVF protocol was started according to age, BMI, previous IVF response, ovarian reserve, AMH and FSH levels. Clinician and participants both were blinded about the group allocation.

Main results and the role of chance: Baseline demographic parameters and IVF characteristics were comparable between the two groups. Endometrial thickness on day of ovulation trigger was 9.3 ± 1.6 mm in group I and 9.4 ± 1.6 mm in group II and was comparable (p value 0.660). Endometrial volume on day of trigger was 5.4 ± 1.7 ml in group I and 5.3 ± 1.8 ml in group II and was comparable.Endometrial thickness on day of embryo transfer (ET) was comparable in group I (11.3 ± 2.0 mm) and group II 11.2 ± 1.8 mm (p value 0.697). Endometrial volume was also comparable. Only parameter which showed significant improvement in intervention group was endometrial vascularity on day of embryo transfer (CI 1.67 (1.24, 2.24); p-0.001). Though there were more clinical pregnancies in group I (21/76; 27.6%) as compared to group II (14/74; 18.9%), but the difference was statically significant (CI-1.25 (0.9, 1.74) p value- 0.207). Implantation rate was also comparable between the two groups. Ongoing pregnancy rate (pregnancy beyond 12 weeks) was 20/76 (26.3%) in group I and 12/74 (16.2%) in group II. But the difference was not statically significant (p value 0.131).

Limitations, reasons for caution: Main limitation of this study was small sample size. Further large number data is required to document the role of intrauterine perfusion of G-CSF on clinical pregnancy rate and implantation rate in women undergoing IVF-ICSI cycles with normal endometrial thickness.

Wider implications of the findings: As published previously, our study also did not find any benefit of intrauterine perfusion of G-CSF on clinical pregnancy rate and implantation rate in women undergoing IVF cycles with normal endometrial thickness.

Trial registration number: CTRI/2017/10/010310

P-469 Mutations in the sperm-activating factor PLCZ1 causing fertilization failure after ICSI lead to partial hydatidiform moles

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Study question: Is the occurrence of partial hydatidiform moles (PHMs) related to a sperm-related activation deficiency, caused by mutations in the phospholipase C zeta (PLCZ1) gene?

Summary answer: Mutational screening of the PLCZ1 gene revealed three distinct point mutations in male partners of three women with a previous history of PHMs.

What is known already: PHMs are aberrant pregnancies characterized by trophoblastic proliferation and may contain embryonic tissues. PHMs mostly contain one maternal and two paternal chromosomes, in 90% of cases due to a dispermic fertilization. PLCZ1 is a sperm-related oocyte activating factor which is required to induce normal calcium oscillations after fertilization. Aberrant patterns of Ca^{2+}oscillations caused by sperm of infertile men have been associated with deficient oocyte activation and failed fertilization. Genetic predisposition for PHM in females have been described (e.g. NLRP7), but none are studied in males. We therefore investigated the possible.

new role of PLCZ1 mutations in males causing PHMs.

Study design, size, duration: Three couples with a history of PHMs and failed fertilization after ICSI were enrolled in this study. The mouse oocyte activation test (MOAT) was performed to reveal sperm-related activation deficiencies. Mouse and human oocyte calcium analysis were performed to evaluate the activation capacity of sperm. Male and female partners were screened for mutations in the PLCZ1 and NLRP7 genes respectively. Patients were counseled to undergo assisted oocyte activation (AOA) during ICSI to overcome fertilization failure.

Participants/materials, setting, methods: Mutation screening included all coding exons of the PLCZ1 gene. After PCR amplification, products were sequenced using Illumina MiSeq. Mouse oocyte assays were carried out with piezo-drilled microinjection. Human ICSI was performed following standard procedures. For Ca^{2+} imaging, oocytes were loaded with a Ca^{2+} sensitive dye before ICSI. For ICSI-AOA treatment, a sperm was injected into the oocyte together with 0.1 mol/l $CaCl_2$ followed by a double exposure to ionomycin (10 mM) 30 minutes after ICSI.

Main results and the role of chance: While two patients showed high activation rates after the MOAT, one patient showed a slightly reduced activation rate (78%). Remarkably, all sperm samples induced abnormal Ca^{2+} oscillatory patterns in mouse oocytes. This was even more pronounced when calcium analysis was performed using human oocytes, with most of the oocytes failing to show calcium rises. However, in accordance with the hypothesis of dispermic fertilization causing PHM, when two spermatozoa were injected into human oocytes, an increase in the number of oocytes showing normal calcium oscillations was observed. Sequencing of the three patients revealed three different heterozygous mutations in the PLCZ1 gene which include two missense mutations: D46N and H233L, and a splice mutation: c.136-1 G>C.RT-PCR on the cDNA of the patient sperm revealed that the c.136-1 G>C mutation leads to loss of exon 4, subsequently leading to a truncated protein. No mutations in NLRP7 were found in their female partners, thus excluding its role in these PHM. To overcome fertilization failure after routine ICSI, ICSI-AOA was

successfully employed for two couples, resulting in a total of 5 healthy children. The other couple conceived spontaneously while waiting to start an ICSI-AOA cycle, this pregnancy did develop normally, and a healthy child was born.

Limitations, reasons for caution: More studies confirming this new role of PLCZ1 in causing PHMs are needed. The functional effect of the mutations identified has to be studied by making recombinant protein with these mutations and look at their effect on activation rate, the pattern of Ca^{2+} signals, and embryonic developmental potential.

Wider implications of the findings: This study shows for the first time the involvement of mutations in the sperm-related oocyte activating factor causing PHMs in vivo, instead of an oocyte-related deficiency, as previously thought. PLCZ1 gene screening may contribute to better understanding of the pathophysiological mechanisms in PHM, thus improving clinical management of these cases.

Trial registration number: not applicable.

P-470 A comparison of miscarriage and live birth rates between hormone replacement treatment cycle versus natural cycle for frozen embryo transfer

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Study question: Primary objective is to compare miscarriage rates for frozen embryo transfer cycles, between the hormone replacement treatment (HRT) thaw cycle and the natural thaw cycle.

Summary answer: A significant increase in miscarriage rates in frozen embryo transfer(FET) cycles for patients who underwent the HRT thaw cycles compared to the natural thaw cycle.

What is known already: The FET protocol is easy to perform and can be accomplished in a shorter period of time than repeated in-vitro fertilization or intracytoplasmic sperm injection cycles. FET can result in extra work on weekends for both clinicians and laboratory staff. This is more likely to happen with natural thaw cycles, as the ovulation timing is not predictable.The recent Cochrane database stated that there is no difference in the outcome with the various methods of endometrial preparation used for FET.Thus we embarked on this study to evaluate if it will be worthwhile to continue performing natural thaw cycles at our centre.

Study design, size, duration: Design: Retrospective analysis of hospital records.

Setting: KK Women's and Children's Hospital, Singapore. An Academic Hospital with reproductive care.

Patient(s): Women undergoing frozen embryo transfer cycles either with HRT thaw cycle or the natural thaw cycle from 1st January 2011 till 31st December 2015.

Sample size: We would need 957 subjects in each arm to achieve a power of 90% to detect the difference at 5% statistical significance level in a two-sided chi-squared test after applying continuity correction.

Participants/materials, setting, methods: We had a large sample size of 2752 subjects.To exclude any pelvic pathology, all women included in our study had a recent endometrial cavity assessment within the last 6 months to exclude any pelvic pathology.Patients with regular menstrual cycles were mostly selected for true natural thaw cycle and irregular menstrual cycles were selected for HRT thaw cycle.Statistical analysis was carried out using R version 3.1.1. All tests were done at 5% statistical significance level.

Main results and the role of chance: A total of 2752 FET cycles were included in this retrospective study analysis.A total of 81 (6.6%) and 171 (11.2%) women suffered from miscarriage in the natural and HRT thaw group, respectively (p value 0.004). The live birth rate per transfer was significantly higher in the natural thaw group (22.8%) compared to the HRT thaw group (17.3%) with p value <0.001. Multivariable logistic regression models were used to control for factors that might be potentially associated with the risk of both the miscarriage and live birth rates as well as the choice of FET cycles. We adjusted for the patient's age at which the embryo was cryopreserved, race, BMI, main indication for IVF, number of embryo transferred, the type of embryo transferred and the type of protocol used for the FET cycle (either natural cycle or HRT cycle). The natural thaw group was considered as reference group to estimate the odds ratios. The protocol type used was independently associated with the risk of miscarriage (odds ratio 1.55; 95% confidence interval 1.15 to 2.08; p value 0.004) and live birth rates (odds ratio 0.69; 95% confidence interval 0.56 to 0.84; p value <0.001) after adjusting for potential covariates.

Limitations, reasons for caution: The limitation is that this is a retrospective study and the patients included were those with ovulatory and anovulatory menstrual cycles. Patient allocation to the different type of endometrial preparation based on their menstrual cycle characteristics can pose as a bias as patient characteristics differ in both the groups.

Wider implications of the findings: As the protocol type used for FET cycles was independently associated with miscarriage rates, patients with regular menstrual cycles should be offered natural FET cycles for better outcomes in live birth rates and reducing the miscarriage rates in the future.

Trial registration number: The Centralized Institutional Review Board at SingHealth Services approved this study (CIRB/2014/033/D).

P-471 The effect of low oxygen concentration on gene expression of potassium channels in human secretory endometrium

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Study question: Does changing in vitro partial oxygen pressure affect the molecular expression of potassium ion channels in the human endometrium?

Summary answer: A significant difference was observed on the expression of potassium channels in cells cultured in normal oxygen and low oxygen concentration.

What is known already: There is a known functional significance of intrauterine acute oxygen concentration changes in relation to both gamete viability, embryo implantation and development. Potassium channels have been shown shown to play an important role in implantation but the effect of oxygen concentration on potassium channels is not yet clear.

Study design, size, duration: This is a lab based study investigating the effect of oxygen concentration on potassium channel in normal human secretory endometrium (n = 6) and Recurrent implantation failure (RIF) human secretory endometrium (n = 4).

In each group we are comparing the potassium channels gene expression of KCNK9, KCNK10, KCNK17 and KCNMA1 under 2 different oxygen concentrations (atmospheric and 5% low oxygen concentrations). The experiment were carried out using qPCR and flow cytometry.

Participants/materials, setting, methods: Human endometrial samples are collected after consenting the patients. Samples are washed from blood in HBSS media and cultured in media supplemented with 10% fetal bovine serum (FBS) and incubated for 24 hours, half of sample in atmospheric oxygen incubators and other half in 5% low oxygen tri-gas incubator. After 24 hours, RNA is extracted, cDNA synthetized and qPCR carried out. Also same samples were prepared for flow cytometry.

Main results and the role of chance: Expression of all potassium channels (KCNK9, KCNK10, KCNK17 and KCNMA1) in all samples are tested. After the oxygen tension is modified, two of the channels showed significant altered expression (P<0.05). Translation to protein for the potassium channels was confirmed by flow cytometry.

Limitations, reasons for caution: Larger (n) number of samples is needed in order to apply two oxygen conditions on same patient sample.

The criteria of target group is so precise not allowing a high recruitment flow.

The heterogeneity of the tissue makes it difficult to identify which cell types showed the expression.

Wider implications of the findings: The findings from this study will provide new knowledge on the role of potassium channels contributing to normal and RIF endometrial function and may have future application in improving implantation rates and identifying novel targets for fertility regulation.

Trial registration number: 'not applicable'

P-472 Exosomal profile of the receptive endometrium: a source of non-invasive biomarkers for guiding of a successful embryo implantation

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Study question: Is endometrial fluid exosomal profile is a non-invasive source for biomarker for guiding a successful embryo implantation in order to reduce the time to pregnancy?

Summary answer: Exosomes playing key roles during embryo implantation, should be used to drive a single embryo transfer in the proper time with the highest endometrial receptivity.

What is known already: Accurate timing of embryo transfer is a critical clinical need in reproductive medicine. Several factors secreted by the endometrium in uterine fluid control implantation by directly affecting blastocyst development and/or by modulating the expression of key epithelial adhesion molecules and embryo-endometrial cross talk. Some of these molecules may be sorted from cytoplasmic endosomal compartments into secretory exosomes/Ev which selectively interact with specific target cells, delivering their biologically competent cargo of lipids, proteins and RNAs. Hence, Exosomes/Evs act at the same time as effectors of endometrium/embryo cross talk and as indicators of the specific molecular profile distinctive of the implantation window.

Study design, size, duration: Uterine fluid samples were collected from women undergoing hormonal stimulation for assisted reproductive techniques. The uterine flushing was collected at different times after the LH surge, from women volunteers with proven fertility (n = 14). All samples were taken with prior informed consent and exosome isolation and analysis were performed by researchers blinded to the study protocol. The study has been carried out during 12 months.

Participants/materials, setting, methods: Uterine washings, obtained by using a disposable catheter for histerosalpingography, were scheduled in LH +1/LH+3/LH+5/LH+7/LH+9, from women volunteers with proven fertility (n = 14) at the Centre for Couple Sterility, Siena University Hospital. EXs/MV were isolated by ultracentrifugation and precipitation and counted with NTA. Conventional technologies for RNA quantification and analysis have been used, including in-house optimized LNA PCR assays, and TaqMan miRNA arrays.

Main results and the role of chance: Two fractions of the uterine flushes, mucus (M) and supernatant (UF), were analyzed for overall vesicles content by NTA (Nanoparticle Tracking Analysis). Despite very low protein content UF appears rich in particles as measured by NTA and ELISA. Conversely, M, although rich in overall proteins, is very poor in vesicles. This drives us to conclusion that exosomes largely contribute to a proteome of UF while are not contained in its mucous fraction. By confronting the protein content and the particle number we can appreciate that protein equivalent of UF has about 16-fold more particles with respect to M. This is even more striking when we confront total proteins and exosome associated CD9 levels in ELISA, where the fold of exosomal protein enrichment of UF versus M is $>2*10^6$. To trace the portion of endometrial EVs in totals recovered from each sample we have assessed a small set of genes specific of the endometrium. Their assessment in EVs/exosomes recovered from healthy volunteer's raises the possibility that some of these genes, PAEP and PGR, are confined to, or increased in UF fraction, and their expression fluctuates along the phases of the cycle.

Limitations, reasons for caution: All findings have to be validated in a larger cohort. Moreover, Embryo implantation involves a complex crosstalk have to be established between the embryo and human endometrium. Therefore, our data regarding the maternal contribution must be interpreted with caution and integrated with data resulting from embryo derived exosomes.

Wider implications of the findings: Our study allows the identification of the informative biomarker pannel, with the potential to drive a single embryo/blastocyst transfer during the highest endometrial receptivity. This clinical approach would be widely applied not only in ART cycles, but also in treatment of uterine pathology negatively influencing the beginning of the pregnancy.

Trial registration number: None.

P-473 Effect of N-acetyl cysteine on expression of oxidation-reduction genes during implantation window in Recurrent Implantation Failure: a double blinded randomized placebo controlled trial, phase II

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Study question: Is N-acetyl cysteine (NAC) effective on the Expression of genes *NRF2*, *GPX3*, *GSTM1*, *GSTM2*, *GSTM5* and *PRDX6* in recurrent implantation failure (RIF)?

Summary answer: our preliminary results have demonstrated no significant differences in expression level of studied genes in comparing pre-treatment NAC-supplementation and placebo administered groups in RIF patients.

What is known already: Assisted reproductive techniques help infertile couples to conceive, however, the implantation rate is one of the limiting factors in assisted reproductive techniques. Endometrial receptivity plays an important role in embryo implantation. NAC as a nutritional supplement is a precursor of glutathione-biosynthesis, therefore, it has been greatly applied as an antioxidant. Since recent studies that showed high expression of genes which involved in oxidation-reduction pathway effect on successful implantation rate, thus we examined the expression of Oxidation-Reduction genes of *NRF2*, *GPX3*, *GSTM1*, *GSTM2*, *GSTM5*, *PRDX6* in NAC supplementation during implantation window in women with Recurrent Implantation Failure.

Study design, size, duration: A single center, double-blinded, placebo controlled, randomized trial was performed over one year with 40 women (age: 22-40 years) with at least two RIF history who were undergoing IVF cycle (long protocol for their ovarian stimulation). Subjects received either NAC or placebo with both effervescent tablets having similar color, size and appearance. Expression of genes *NRF2*, *GPX3*, *GSTM1*, *GSTM2*, *GSTM5*, *PRDX6* were evaluated on the day of Window of Implantation biopsies from the endometrium.

Participants/materials, setting, methods: forty RIF patients were randomized to receive NAC 1200 mg/day or placebo for at-least 6 weeks before starting ovarian stimulation. Their tissue took (Pipelled based biopsy from endometrium) on 19-21 day of their cycle, the day of WOI. Then patients were undergone ovarian stimulation (using NAC) ended to IVF. Total RNA-extraction and cDNA synthesis were performed from samples. Real Time PCR was conducted to evaluate Expression of genes *NRF2*, *GPX3*, *GSTM1*, *GSTM2*, *GSTM5*, *PRDX6*.

Main results and the role of chance: mean (SE) of the fold change of expression *NRF2*, *GPX3*, *GSTM1*, *GSTM2*, *GSTM5*, *PRDX6* were 2.62 (0.69), 14.83 (7.4), 19.82 (9.23), 9.73 (4.5), 7.40 (2.36), 1.74 (0.2) in drug group respectively and were 1.85 (0.3), 8.93 (5.4), 8.38 (3.7), 2.09 (0.7), 3.82(1.7), 1.75(0.3) in placebo group. No statistical significant difference between gene expression two arm were detected.

Limitations, reasons for caution: Owning the strict selection criteria in this late phase trial caused limitation to achieve enough patients participation in limited period of time (one year). Thus, to overcome this issue and also for the purpose of getting more experimental data, a larger number of RIF patients is needed.

Wider implications of the findings: There are no significant differences in expression of evaluated genes in comprising two NAC supplementation (as scavenging free radicals) and placebo groups during IVF protocols for RIF patients.

Trial registration number: Not Applicable.

P-474 Can we increase the Live-Birth predictive value of early beta-hCG level: A simple proposal for clinical practice

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Study question: Can the Live birth (LB) predictive value from serum concentrations of beta-hCG level (bHCG) in early pregnancy be enhanced in adding other simple predictors?

Summary answer: Early Single bHCG level remains a strong predictor of pregnancy outcome after embryo transfer. However, maternal age significantly increases the precision of prediction.

What is known already: bHCG rapidly increases during normal early pregnancy, however with a high inter-individual variation. bHCG blood level possibly dependent on the volume of distribution in the mother's body, and thus on maternal age and BMI, Eskild and al. (Fertil Steril. 2012) suggested that bHCG were negatively associated with maternal BMI, and Haavaldsen and al. (ACOG, 2014) found the same negative association with maternal age. In parallel, Age and BMI are predictors of live birth (Arvis et al 2012).

Study design, size, duration: In this non-interventional, retrospective, observational, single-centre cohort study, 3,077 live-births (LB) followed 16,105 embryo transfers after IVF or frozen-thawed embryo (FTE) cycles during 2000-2016 period. Intent to treat principle was used, no inclusion or exclusion criteria were applied. Luteal phase was supported by vaginal progesterone only. Serum bHCG were measured on day 14 after oocytes retrieval, or on day 14 embryo in FTE.

Participants/materials, setting, methods: The association between LB and maternal age, BMI and log-transformed bHCG was estimated by a linear mixed model, patient considered as random factor, Log-Transformed (or not) BHCG, BMI and age as fixed covariates. Main effects and Interaction with bHCG were tested. The final model was obtained through a backward strategy based on likelihood ratio.

Main results and the role of chance: The determination coefficient based on Log-transformed BHCG was higher (R2 nagelkerke=.67) than its untransformed value BHCG (R2=.56). The backward process identified a simple model where only log(bHCG) and its interaction with age remained the only significant predictors. Area under the ROC curves with this model reached a value of c = .91 95%CI [.86,.93] compared with the bHCG only (C=.82 95%CI [.79,.86] with a significant difference, p=.015).

Predicting values of this model are illustrated by a curve, providing LB probability according to bHCG level and age.

As a conclusion, although bHCG remains a strong predictor of LB, adding age adds important precision. Neither BMI, nor other predictors, influence significantly this probability. However bHCG exhibit a non-linear variation: the age effect is not constant with bHCG, the difference being the largest for intermediate values of bHCG. For example, for a bHCG level of 50 UI/L, probability of LB is 72% at the age of 20, 55% at 30 and 37% at 40. Above 200, the probability is closed to 100% for youngest patients.

Limitations, reasons for caution: This was a Retrospective uni-center study, needing external validation, and the assessment of other potential predictors of live birth.

Wider implications of the findings: Such result remains simple to used for clinical practice, while significantly increasing the precision of prediction.

Trial registration number: not applicable.

P-475 Mucin1 (MUC1) localization and quantitative study in luminal epithelium during implantation window

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Study question: Are MUC1 differentially expressed among different develop stage of pinopodes in fertile and recurrent implantation failure (RIF) patients?

Summary answer: MUC1 only expressed on the surface of ciliated cell, and showed no relationship with pinopode. Its expression level was significantly decreased in RIF patients.

What is known already: MUC1 has long been supposed as an anti-adhesion molecule during embryo implantation because of its molecule structure. But its expression level significantly increased in human endometrium during implantation window. The exact function of MUC1 in early development of pregnancy is still unknown. Endometrium is made up by ciliated cell and microvilli cell. During implantation window, microvilli cell lost its microvilli and developed as pinopodes. Animal and in vitro study have showed that pinopodes provided the implant site for embryo. However, the relationship between MUC1 expression concentration and different develop stage of pinopodes have not be studied.

Study design, size, duration: Twenty-five fertile control and 25 RIF patients were recruited in this study. All participants underwent daily urine test from day 9 of the cycle onwards to identify the LH surge, and endometrial biopsy was obtained at LH+7 day.

Participants/materials, setting, methods: Recurrent implantation failure was defined as failure to achieve a clinical pregnancy after at least 4 good-quality embryos have been transferred in 3 or more transfer cycles. Infertile patients who got ongoing pregnant result from a FET cycle were recruited as fertile control, and the endometrial biopsy was conducted just one cycle preceded embryo transfer. Scanning immunoelectron microscope and double immunofluorescence staining have been used to determine MUC1 expression on luminal epithelium.

Main results and the role of chance: MUC1 expressed mainly on the surface of ciliated cell. There was no MUC1 expression on developing, developed and regressing pinopodes. Double immunofluorescence staining showed that ciliated cell counting and MUC1 expression level were significantly decreased in RIF patients ($P < 0.05$).

Limitations, reasons for caution: The main limitation of this study is its only one-time point biopsy study, which cannot demonstrate MUC1 dynamical change as menstrual cycle proceeding. And this is only an observational study, research into the mechanism of MUC1 and pinopodes in embryo implantation is needed.

Wider implications of the findings: This investigation reveals that MUC1 is not expressed on the surface of potential implantation site. Its anti-adhesion function need to be further discussed. And ciliated cells maybe played a role in embryo implantation.

Trial registration number: NO.

P-476 Capturing the physiological characteristics of early pregnancy using wrist worn wearables

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Study question: Could wrist worn wearable sensors capture the physiological changes associated with early pregnancy?

Summary answer: We observed significant differences in heart rate variability, pulse rate, breathing rate, perspiration, temperature, and susceptance (of the wrist skin).

What is known already: The physiological changes associated with the reproductive hormones are well studied and widely reported (e.g., the increase of temperature associated with post-ovulatory progesterone rise). The onset of pregnancy is known to impart significant endocrinological changes. The

hormonal profile of the late luteal phase is therefore significantly different when comparing conceptive cycles to non-conceptive cycles. Consequently, it is plausible that these physiological changes could be captured by wrist worn wearables.

Study design, size, duration: This study is an interventional longitudinal observational study conducted at the University Hospital Zurich. The study is planned to run from the fourth quarter 2016 to the second quarter of 2018, with 430 participants. The participants are requested to measure the specified parameters for 6 months, and if they conceive, to term.

Participants/materials, setting, methods: We included healthy, eumenorrheic women, 20-40 years old, who are trying to conceive. Participants wore Ava bracelet which measures temperature, pulse rate, and heart-rate-variability ratio among other parameters. An LH home-urine test was used to estimate the ovulation day. The late luteal phase was defined as ovulation+7 to the end of the cycle for the non-conceptive cycles, ovulation+10 to ovulation +18 for conceptive cycles. Associations were evaluated using linear-mixed-effects models with random intercepts for each participant.

Main results and the role of chance: We included 49 conceptive cycles and 560 non-conceptive cycles from 253 women in the analysis. In comparison to the late luteal phase of non-conceptive cycles, conceptive cycles were characterized by: an increase in pulse rate^{+}, breathing rate***, perspiration^{+}. In addition, non-conceptive cycles were more likely to have lower wrist skin temperature***, heart rate variability ratio***, and wrist skin susceptance*. Overall, the physiological parameters trends in conceptive cycles had steeper rises and falls, and more pronounced peaks and troughs, compared to non-conceptive cycles. This suggests that the underlying hormonal dynamics (which are responsible for the physiological changes) in conceptive cycles are similarly steeper in rising and falling, and have higher peaks and lower troughs.

Note: $^{+}$p<.10, *p<.05, **p<.01, ***p<.001

Limitations, reasons for caution: The limited number of conceptive cycles limits the generalization of the results and the development for a pregnancy recognition classifier. Pregnancy tests are known to have limited sensitivity at the beginning of the pregnancy, hence, some conceptive cycles could be labeled as non-conceptive.

Wider implications of the findings: Wirst worn wearables are capable of capturing known and novel early-pregnancy associated physiological changes. Wearables represent a useful and non-invasive research tool to monitor women's health, and given sufficient data could allow for the continuous monitoring of early-pregnancy.

Trial registration number: NCT03161873.

P-477 What is the real length of the window of implantation (WOI) in humans?

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Study question: How long the human window of implantation (WOI) last?

Summary answer: Using endometrial receptivity analysis (ERA) in paired samples (same patient), the WOI last from 29-36 hours, exceptionally shorter WOI than 24 hours are found.

What is known already: The window of implantation (WOI) is a transient functional state when the endometrium became receptive for the implantation of the blastocyst. The endometrium is a hormonally regulated organ, progesterone (P) induces the acquisition of endometrial receptivity that theoretically last from LH+6 to LH+9 of the menstrual cycle or from P+4 to P+7 in a hormonal replacement therapy (HRT) cycle. Currently, it is possible to determine the molecular diagnosis of the WOI analyzing the transcriptomic signature known. But, the length of the WOI has never been previously determined.

Study design, size, duration: From the paired-biopsy dataset (n = 3,616) from patients who underwent ERA to test their endometrial receptive status, a selection of 2,874 endometrial samples from 1,437 patients were analyzed. The inclusion criteria was that the first biopsy was taken in the time interval of 120 +12 hours (P+5). Samples were collected from the same patient and HRT on P +5 and P+7 (n = 627 pairs) or on consecutives HRT cycles with the second biopsy made at P+6 toP+7 (n = 810 pairs).

Participants/materials, setting, methods: ERA results were reported as 1 day pre-receptive, 12 hours to Receptive, receptive (R), 12 hours late R and 24 hours or more post-receptive.

A cross-tabulation analysis of time between biopsies (10-hours intervals) was performed for each combination of paired-samples. A descriptive analysis of the obtained percentages for each time-interval was used for describing the length of WOI. Average time between paired-biopsies of main stages combinations was studied and a confidence interval was computed.

Main results and the role of chance: In general, we stablished the opening of the WOI at early-receptive stage and the closing at late-receptive stage. The average length of the WOI between these two stages was 32.8 hours (95% IC, [29.2, 36.3] (n = 47 patients).

The average length of the WOI between early-receptive to post-receptive was 48.2 hours (95% IC, [45, 51.4] (n = 94 patients), indicating the WOI was already closed. Although few patients passed from early-receptive to post-receptive stages in less than 24 hours (n = 4 patients), indicating that they have a narrow WOI (<24 hours).

When analysis was performed from previous non-receptive stages to our reference (late-receptive stage), the average time between 1 day pre-receptive and late-receptive was 41.7 hours (95% IC, [39.4, 44], n = 201 patients). However, some patients last less than 30 hours from 1 day pre-receptive to late-receptive (n = 46 patients) or less than 40 hours to post-receptive (n = 13 patients), showing narrow WOI.

Limitations, reasons for caution: This study has been performed using a retrospective descriptive approach. An increase sample size is needed.

Wider implications of the findings: This study suggests that using molecular diagnosis, the WOI length varies from 29 to 36 hours, although exceptionally few cases have shorter WOI.

Trial registration number: not-applicable

P-478 Changes in human chorion gonadotropin as a predictor of live birth outcome among Danish women with recurrent pregnancy loss

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Study question: Are changes in serum-human chorion gonadotropin (hCG) levels in early pregnancy a reliable predictor of live birth in women with recurrent pregnancy loss (RPL)?

Summary answer: A daily hCG increase of 20% and 40%, respectively, can predict a 65% and 70% chance of live birth among women with recurrent pregnancy loss.

What is known already: The increase of hCG in early pregnancy is used to clinically differentiate between viable and non-viable early pregnancies (ectopic/biochemical pregnancies and miscarriages). However, the evidence behind this practice is limited. A small study (n = 20) from 1990 showed a doubling time of 2 days for intrauterine pregnancies and this rate is often used in the clinic. Other studies have addressed the change in hCG according to the pregnancy outcome, but often only on symptomatic women (bleeding/pain) or IVF-patients – with inconsistent results.

No studies have evaluated the change in hCG in RPL patients, despite this being a crucial part of RPL care.

Study design, size, duration: This was a large retrospective cohort study at a national tertiary Recurrent Pregnancy Loss Unit.

In total 409 women were included between 2008 and 2016.

Inclusion criteria: ≥3 consecutive pregnancy losses; known outcome of the pregnancy; minimum two hCG samples in early pregnancy.

Exclusion criteria: Extra-uterine pregnancies (due to too small a number for the purpose of the statistics), pregnancies after donation of an ovum, and missing data on pregnancy outcome.

Participants/materials, setting, methods: The first pregnancy after referral to the RPL-Unit was included. Serum-hCG was measured in at least two blood samples few days apart during gestational week 4-7. Logistic regression analyses were used to investigate the change in hCG according to pregnancy outcome. Results were adjusted for maternal age, number of prior losses, and the between-sample interval.

When viability was uncertain, serial hCGs were performed until a final diagnose could be made.

P-values <0.05 was considered significant.

Main results and the role of chance: Pregnancy outcome was: 261 live births; 100 miscarriages; 38 biochemical pregnancies, not counting 10 excluded observations due to missing data/extreme outliers. In the final sample the mean age was 35.0 years (SD = 4.4), mean number of prior pregnancy losses was 3.8 (SD = 1.8), mean time of the first hCG sample was 4^{+4} weeks of gestation (SD = 5.7 days), and hCG samples were on average collected 3.1 days apart (SD = 2.0). The lowest initial hCG that subsequently led to a live birth was 12.

Main results: A 20% daily increase in hCG predicted a 65% chance of a live birth whereas a 40% increase predicted a live birth in 70% of the cases.

The association between change in hCG and live births was weaker for women with more than three losses, and was strongest for age <30years. The probability of a live birth decreased with increasing number of losses.

In summary, a daily increase in hCG between 20 and 40% is a strong indicator of live birth together with low maternal age and a lower number of losses. Increases above 40% did not add significantly to prognostic value.

All abovementioned results are significant at a 5% level. We therefore assess that the findings are not due to chance.

Limitations, reasons for caution: This study is limited by uncertainties regarding e.g. ovulation date, implantation date and the timing of both urine and blood testing depending on patient preferences. However, all measurements are related to the menstrual cycle and the date of last menstrual period was known for all except two women.

Wider implications of the findings: For women with RPL the uncertainty about whether a pregnancy is viable or not causes a lot of psychological distress. By this study clinicians can become better equipped to predict which pregnancies will lead to live births and thereby – hopefully – diminish the amount of distress for these women.

Trial registration number: not applicable.

P-479 Screening for pre-eclampsia: toward the development of a non-invasive repaid clinical mass spectrometry pregnancy urine test

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Study question: Can Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI ToF) Mass Spectrometry of early pregnancy urine be used to predict the likelihood of preeclampsa?

Summary answer: It is possible to use the analysis of data, following MALDI-ToF MS of maternal urine, to generate risk screening algorithms for subsequent development of pre-eclampsia.

What is known already: Identifying pregnant women who will develop pre-eclampsia is one of the last remaining grand challenges in obstetric medicine and pre-eclampsia occurs in around 1 in 50 pregnancies. The ability to do so non-invasively and at low cost in early pregnancy would dramatically impact maternal obstetric care worldwide. Recently proteomic and metabolomic analysis of maternal urine have been proposed as a possible route to an inexpensive and truly non-invasive screening test for Downs Syndrome. Using a similar approach it should be possible to achieve a risk score for preeclampsia.

Study design, size, duration: In this diagnostic test study to determine the analytical process and corrections needed to develop a clinical maternal urine screening test for risk of subsequent pre-eclampsia; we examined the spectral profiling of maternal urine at 15-17 weeks of gestation by MALDI ToF mass spectrometry from pregnant women being monitored for PE.

Participants/materials, setting, methods: A retrospective collection of 40 urine samples at 15-16 weeks of pregnancy 28 women (12 developed pre-eclampsia). Samples were examined by MALDI-ToF mass spectrometry in the mass/charge range between 1000 and 100,000 m/z. The spectral masses between 2000 and 11,000 m/z were specifically examined and a method was developed by mathematical algorithm to normalise spectral data in mass bins of 100 m/z and express as a percentage of the total mass spectra.

Main results and the role of chance: Of the ninety 100 m/z bins, forty-six were identified as m/z bins at which statistically significant spectral differences occurred between those who developed pre-eclampsia and control samples where pre-eclampsia was not subsequently diagnosed. Based on the differences and variance, for values at these bins, weighted scores of the probability of developing pre-eclampsia were assigned. Comparative algorithms consisting of various mass bins were tested for ability to distinguish those who developed pre-eclampsia as the pregnancy progressed. A change in pattern was evident from the raw and normalized spectra of pregnancy urine from women who developed pre-eclampsia and those who did not. Interestingly the changes seen were different to those found previously in screening for Downs syndrome by this same methodology. In this cohort decrease of signal intensity in the profile at 6000 and 7000 m/z were associated with those pregnancies that developed pre-eclampsia. An algorithms based on these changes had a sensitivity of 91.7% and specificity of 85.7%.

Limitations, reasons for caution: This study is limited by cohort size.

Wider implications of the findings: Using a simple, non-invasive screening tool such as MALDI ToF MS of early pregnancy urine could potentially improve pregnancy outcomes. Targeted maternal and fetal monitoring would lead to earlier detection of the clinical signs and, where necessary, intervention planned for those individuals at increased risk.

Trial registration number: not applicable.

P-480 Improved pregnancy rates following endometrial receptivity analysis and personalized embryo transfer in patients with previous failed implantation after euploid embryo transfer

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Study question: Does Endometrial Receptivity Analysis (ERA) with personalized embryo transfer (pET) of euploid embryos improve the reproductive outcome in patients with previous failed euploid embryo transfer?

Summary answer: Patients who experienced failed euploid embryo transfer had improved reproductive outcome combining pET of euploid embryos guided by ERA.

What is known already: Recurrent implantation failure (RIF) is a frustrating condition for patients and clinicians. The two most likely candidates implicated are embryo chromosomal abnormalities and/or alterations/desynchrony of endometrial receptivity. Preimplantation genetic testing for aneuploidy (PGT-A) identifies euploid embryos for selective ET improving reproductive outcomes in RIF due to embryo abnormalities (Rubio et al., 2013). The Endometrial

Receptivity Analysis (ERA) is based on the transcriptomic signature of 236 genes used to diagnose the receptive status of the endometrium guiding a pET. Increased reproductive outcome following pET guided by ERA has been demonstrated in patients with RIF of endometrial origin (Ruiz et al., 2013).

Study design, size, duration: In this prospective pilot study, fifteen patients with previous failed euploid embryo transfer after PGT-A in a hormone replacement therapy cycle (HRT) underwent ERA to assess the endometrial receptivity status. In a subsequent cycle, a pET guided by ERA with euploid embryo(s) was performed. This study was done in two independent reproductive centers from December 2016 to November 2017.

Participants/materials, setting, methods: Women of reproductive age (34.5+4), who have never achieved clinical pregnancy following at least one failed euploid ET in a HRT cycle were enrolled in this study. An endometrial biopsy for ERA was obtained in a HRT cycle identical to one carried out in the failed euploid ET cycle. Once a receptive result was obtained, the patient underwent a pET with the remaining euploid embryo(s). Pregnancy rates and follow-up data were collected from participating sites.

Main results and the role of chance: Thirteen of the fifteen patients with failed euploid ET were diagnosed with a non-receptive (NR) endometrium (86.7%), from those, four were early-receptive and nine were pre-receptive. The other two patients had receptive result (R). At this time, we have clinical follow-up data from all patients who have undergone pET of remaining euploid embryo(s). All fifteen patients (100%) achieved pregnancy (+hCG) when pET was performed based upon their personalized Window of Implantation (WOI) guided by ERA, one had a biochemical pregnancy (6.7%), three presented clinical miscarriages (20%) and the other eleven are ongoing or have delivered, corresponding to 73.3% ongoing pregnancy rate (OGP). The implantation rate was 87.5%.

Limitations, reasons for caution: The main limitation for this study is the number of patients included due to pilot nature. Further clinical validation in a Randomized Clinical Trial (RCT) is underway.

Wider implications of the findings: Our study demonstrates that patients with previous failed euploid embryo transfer could have WOI displacements in high proportion (86.7%) and it may benefit from ERA testing to guide pET when a displaced WOI is the cause of their failed implantation.

Trial registration number: Not applicable.

P-481 The inhibitory T cell co-receptor molecule B7-H4 (VTCN1) regulates major histocompatibility complex class I expression in a stem cell-derived model of primitive human trophoblast development

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Study question: Could the isolated expression of the inhibitory T cell co-receptor B7-H4 on human trophoblast cells during the first half of pregnancy be important in maternal-fetal immune interactions?

Summary answer: Downregulation of B7-H4 in an in vitro stem cell-derived model of primitive human trophoblast development dramatically increases HLA-A and –B, but not HLA-G protein levels.

What is known already: Downregulation of the classical MHC class I molecules, HLA-A and HLA-B on trophoblast cells in direct contact with maternal immune cells is thought to aid in the escape of the semiallogenic fetus from maternal immune rejection. B7-H4 is a co-receptor in MHC/T cell interactions and decreases T cell activities. Increases in B7-H4 expression correlate directly with cancer invasion and severity and **increases** in MHC class I expression. We have demonstrated that B7-H4 is expressed in first and second trimester placenta and in an in vitro human embryonic stem cell-derived model of early placental development ($hESC_{BAP}$), but not in term placenta.

Study design, size, duration: In vitro *B7-H4* knockdown study of human embryonic stem cell (hESC)-derived trophoblast cells.

Participants/materials, setting, methods: The human embryonic stem cell line, H1, was treated with BMP4, A83-01 and PD173074 (BAP treatment) to create an established in vitro model for peri-implantation trophoblast development. Cells were transfected with silencing *B7-H4 siRNA* or *control siRNA* on day 3 or 4 of differentiation. Proteins were collected on day 6 and expression of B7-H4 and MHC class I proteins compared to that of a housekeeping protein (GAPDH) using western immunoblotting.

Main results and the role of chance: In direct contrast to findings in cancer cell lines, downregulation of B7-H4 expression in cells representing the earliest stages of placental development dramatically increased the expression of HLA-A and HLA-B molecules at the protein level. Interestingly, knockdown of B7-H4 had little effect on the expression of HLA-G, a non-classical MHC class I molecule whose normal tissue expression is restricted to a subset of invasive placental cells called extravillous cytotrophoblast cells.

Experiments were repeated at least three times and after 3 days as well as after 4 days of differentiation. Endpoints were assayed at 48 and 72 hours after knockdown, all with consistent results. Comparison to experiments using a scrambled siRNA control limits the chance that the effects noted are the result of exposure to gene silencing methods. No differences in cell proliferation or cell viability were observed between the B7-H4 knockdown and control cells across the time period studied. This is consistent with equivalent protein expression of the control housekeeping gene, GAPDH.

Mechanism identification using comparisons of RNAseq datasets from knockdown and control cell lines is ongoing.

Limitations, reasons for caution: The cell system used is an in vitro system and may not directly mimic in vivo occurrences. Unfortunately, ethical and logistical factors limit access to human tissues directly post-implantation and before clinical detection of a pregnancy is possible. In vitro surrogates remain our best methods to study early post-implantation events.

Wider implications of the findings: Discrepancies between HLA responses to B7-H4 knockdown in cancer cells and in early trophoblast cells suggest the B7-H4 pathway regulates the unique transplantation antigen expression patterns found in the human placenta. This exposes opportunities to fill the pronounced knowledge gap in our understanding of HLA regulation in the human placenta.

Trial registration number: not applicable.

P-482 The uterine cavity in patients with recurrent pregnancy loss (RPL)

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Study question: What is the frequency of uterus pathology and histological analysis in a consecutive cohort of well characterized RPL patients, and is evaluation justified?

Summary answer: We report lower incidence of intrauterine adhesions and chronic endometritis compared to previous reports. However, 14.3% of the patients had intrauterine pathology justifying sensitive investigation.

What is known already: RPL patients have often had several uterine evacuations (medical (with Misoprostol) or D&C), which increases the risk of chronic endometritis (CE) and intrauterine adhesions (IUA). A meta-analysis from 2014 found that 19.7 % of women with one pregnancy loss had IUA, the incidence increases significantly with further pregnancy losses. Other studies evaluating biopsies from the uterine cavity suggests: that CE is as high as 10–30 % in patients with RPL.

We aimed to evaluate the uterine cavity in a well characterized group of RPL, and compare our incidences of CE and IUA to international findings.

Study design, size, duration: The study design is a macroscopic and histological description of the uterine cavity and endometrium in a cohort of women with RPL. We recruited a cohort of 70 women with RPL, over a time period of 19 month from May 2016 to December 2017. Inclusion criteria: three consecutive first- or second trimester miscarriages and no known uterine abnormalities or pathologies.

Participants/materials, setting, methods: All the included patients had a diagnostic office hysteroscopy (OH) with biopsies. The OH was performed by trained gynecologists; fixated biopsies were evaluated by pathologists. We compared the macroscopic description with the histologic diagnoses. The OH was performed during the proliferative phase of the menstruation cycle to standardize biopsies, as the numbers of immune cells change during the menstruation cycle. We confirmed the timing by measuring peripheral Oestrogen- and Progesterone levels at the examination day.

Main results and the role of chance: Seventy patients with RPL had an office hysteroscopy with biopsy during the study period. One patient was excluded due to the formalities. Histological diagnoses: 1/69 had signs of inflammation, with the presence of plasmacells, and was diagnosed with CE. Macroscopic diagnosis: 3/69 had IUA, 3/69 had placenta remnants, 1/69 had a polyp, 1/69 had a septum and 2/69 had intrauterine leiomyomas, macroscopic pathology in total 10/69.

These data diverse from earlier published data. We describe a much lower incidence of IUA (only 4% compared to 19.7 %) and only one patient were diagnosed with CE (1.4 % compared to 10 % - 30 %) in our clinical setting/cohort. Although we do not describe the same incidence of IUA and CE, we describe intrauterine pathology in 14.5 % of the women with RPL. All women were examined with TVUS, none were suspected to have the positive findings subsequently found at OH, despite a high incidence of earlier interventions (Misoprostol or D&C). Hereby we suggest that standard OH is a highly sensitive diagnostic tool for evaluating women with RPL.

Limitations, reasons for caution: This is a descriptive study on a large cohort of well characterized RPL patients. The study is not powered or designed to study the prognostic impact of uterus pathology or histology.

Wider implications of the findings: We describe low incidence of IUA and CE compared to historic data. We hope this confirm a trend towards higher quality in treatment of pregnancy loss. Further, we suggest OH as frontline examination elucidating the uterine cavity; reducing uterine pathology in women may increase the chance of a successful pregnancy.

Trial registration number: NCT02746588

P-483 Global Gene Expression Analysis of Blastocyst Implantation on 2D and 3D Implantation Models: Towards a Comprehensive and Inclusive Research

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Study question: Does the use of mesenchymal stem cells or endometrial cells on 2D and 3D implantation models enough for demonstrating large scale molecular regulation?

Summary answer: Endometrial and mesenchymal stem cells can be used for constructing implantation models with gel or polymer-based carriers to analyse differential expression of implantation related markers.

What is known already: Implantation is complex series of events; apposition, adhesion and invasion; that happens during a well-defined period with competent embryo and receptive endometrium. Since the process takes place in 3D environment, it is difficult to mimic implantation process in vitro. To examine invasion, human trophoblast cells and embryos have been cultured with human endometrial stromal cells. Endometrial cells cultured in collagen matrices to create a 3D model with different layers of trophoblast spheroids and embryos. The pregnancy is balanced with fetal decidual defense against pathogens therefore; immunomodulatory properties of MSCs could have positive effects on implantation and embryo development.

Study design, size, duration: Effects of 2D and 3D implantation models; designed with gel or polymer-based carriers with endometrial and mesenchymal stem cells; were analysed to reveal differential expression of 24 different implantation related markers; LIF, Hoxa10, Hoxa11, Wnt4, IHH, E-cadherin, Trophinin, Fibronectin, L-selectin, Laminin, Entactin, Integrin, MMP9,TIMP3, HBEGF,ErbB1, ErbB2, HBEGF, Progesteron and Estrogen Receptor and Collagen I,III,IV,V. Comparative analysis at 48 and 96 hours were performed by; IF, WB, qRTPCR and by multiphoton microscopy (for invasion depth) quantitavely.

Participants/materials, setting, methods: Mouse blastocysts were cultured in four groups as: i. routine IVF drop culture, ii.blastocysts on collagen coated biofilm, iii. blastocysts on gel matrices over biofilm decorated with endometrial cells, iv.blastocysts on collagen coated biofilm with human mesenchymal stem cells.Groups were compared in terms of structure, function and invasion by several signalling molecules, including cytokines, growth- transcription factors together with hormone receptors. Live/dead cell assay was also performed. Statistical analysis was performed by student's t-test.

Main results and the role of chance: Two very effective gel and polymer based 3D in-vitro implantation models, supported by either mesenchymal stem cells or endometrial cells,were constructed successfully and efficacy of new systems were compared with control group which is of routine-IVF culture. Both can be used to see the in-vitro development and survival of the blastocyst together with the newly differentiating trophoblasts. Comparative analysis was made between groups and also within groups from 48 to 96 hours. There is increase in LIF, trophinin, entactin, integrin expression of all groups. Additionally; LIF, MMP9 and PR showed significant increase with approximate p-value of 0,02. HOXA10 and HOXA 11 showed increase with tissue remodelling enzymes; MMP9 and TIMP3 in expression on 3D implantation models. E-cadherin and fibronectin expression decreased significantly ($p \leq 0,04$) in 3D models consistent with extracellular matrix remodeling process in vivo and suggest modulation of endometrial stroma during implantation. Among collagen types, collagen V was detected the most increasing type during implantation ($p \leq 0.05$). Endometrial co-cultures supported the blastocyst to invade horizontally under the implantation area as depicted by the increase of dead cells, and human MSC also supported the blastocysts for invasion, implicating that human MSC can be used for generation of 3D endometrial co-cultures.

Limitations, reasons for caution: This study is based on use of mouse endometrial epithelial and stromal cells and human MSCs.The limitation is the inter-species implantation differences between of mouse and human. Human blastocysts were intentionally not used for such trial, since there is a major ethical concern.

Wider implications of the findings: There is a clear need to develop 3D in vitro models to investigate embryo implantation.The methods proposed here are based on mouse blastocyst implantation, they provide information about different implantation models with the effects of human mesenchymal stem cells and endometrial cells to reveal extensive analysis of implatation markers.

Trial registration number: Not applicable.

P-484 Cripto is required for normal uterine remodeling, decidualization and development of placental vasculature

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Study question: What is the role of CRIPTO, a member of the TGFβ superfamily, in female reproduction?

Summary answer: Deletion of uterine *Cripto* causes defective uterine remodeling, decidualization and placental development resulting in subfertility, fetal intrauterine growth restriction (IUGR) and fetal loss in mouse.

What is known already: Cripto, a member of the TGFβ superfamily, encodes for a cell surface receptor whose role in embryonic development and stem cell maintenance has been studied. Cripto mRNA and protein have been detected in the human uterus at all stages of the menstrual cycle with moderately higher levels of expression during the secretory phase and its dysregulation has been found in *placenta creta* and *endometriosis* in humans. To date, there is not much known on Cripto's role in female reproduction.

Study design, size, duration: As Cripto null Knockout (KO) is embryonic lethal, we created a conditional KO (cKO) mouse model in which Cripto is deleted only in the reproductive tissues using a Cre-loxP system. We assessed

major reproductive events necessary for the establishment and maintenance of pregnancy (e.g. ovulation, fertilization, implantation, decidualization and placental development) in Cripto cKO versus control mice. For every studied stage, 8 to 10 mice were included in each of cKO and control groups.

Participants/materials, setting, methods: Cripto deletion in cKO mice was confirmed by PCR and insitu hybridization. Pregnancy rate and number of pups/litter were evaluated as general fertility indices. To determine the underlying causes of the observed subfertility, these approaches were used: embryo flushing and retrieval, dissections and histology of uterus and placenta at several time points during pregnancy, measuring blood Progesterone level, size and weight of decidua, placenta and fetus, artificial decidualization, quantitative PCR, immunofluorescence staining and western blot.

Main results and the role of chance: We observed a significant decrease in pregnancy rate (cKO 62% vs Control 100%) and litter size (cKO 7.68 vs Control 9.51 pup/litter) with loss of uterine Cripto. Even though no significant effects were detected in the pre-implantation period, assessment of the post-implantation period showed that 20% of cKO females fail to establish pregnancy and an additional 20% of females undergo full litter loss after implantation (between day 5.5 postcoitum and d8.5). By means of histology (d5.5, d7.5), quantitative PCR (d5.5) for assessing expression of important uterine remodeling and decidualization factors (Wnt4, Bmp2, Hoxa10, components of notch signaling pathway, etc.) and artificial induction of decidualization, we showed this is due to defects in uterine decidualization and remodeling/luminal closure.

During placental development, we observed that decidual area (d10.5) and fetal weight (d16.5) were significantly lowered, while the number of fetal deaths was significantly increased in cKOs. Histological examination of the placenta revealed abnormal labyrinth development; showing increased cellular density. The surface area of fetal vasculature in the labyrinth of the developing placenta on d12.5 was significantly reduced in the cKOs compared to controls. Interestingly, surface area of maternal blood sinuses, however, doesn't show any significant difference. (Where indicated significant: $P<0.05$ or $p<0.01$)

Limitations, reasons for caution: Limitations in *in vivo* research on uterine-embryo interactions in humans has led to relying primarily on animal models. Although there are considerable similarities between reproductive processes in mouse and humans, there are some differences which should be kept in mind when extrapolating data obtained from mouse model studies to humans.

Wider implications of the findings: While the major physiological events associated with female reproduction have been characterized, there are still many molecular pathways that have yet to be uncovered in order to open new windows toward overcoming and managing infertility and pregnancy associated complications which place both mother and fetus's health at risk.

Trial registration number: not applicable.

P-485 The roles of let-7 contained in extracellular vesicles (EV) in embryo implantation in human

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Study question: human uterine fluid contained plenty of EVs, the precise functions are still not clear.

Summary answer: let-7 contained in EVs decreased embryo implantation potential in human and mouse.

What is known already: embryonic diapause is an evolutionarily conserved phenomenon across mammals. The expression of Let-7 family members was significantly higher in dormant mouse embryos than estradiol-activated embryos. Human ULF contained EV and Let-7 was highly expressed in EV of human endometrial cell line ECC1. To investigate if EVs have regulatory functions in human blastocyst, we collected EV from non-receptive endometrial cell line, HEC-1B, which were transfected with pre-let-7a

Study design, size, duration: firstly, we detected the expression patterns of small RNA in EVs of dormant and activated uterine fluid in mice; secondly, we demonstrated the functions of EV-containing let-7 in embryo implantation and dormancy in mice; finally, we tested the potential roles of EV-containing let-7 in embryo attachment in human.

Participants/materials, setting, methods: morphology of EVs was observed by electron microscopy, identification of EVs was done by western blotting, small RNAs were detected by RNA-seq, targeted miRNAs were validated by Q-PCR

Main results and the role of chance: electron microscopy showed the presence of EV-like structures in the mouse uterine lumen. CD63 immnoreactivities were also localized to the endometrial epithelium during dormancy, on Day 4 of pregnancy and after E2-activation of delayed implanting mice. To determine if blastocysts internalized EV from ULF, scanning electron microscopy showed binding of EV-like particles to the trophectoderm of in vivo isolated blastocysts. RAN-seq and Q-PCR results showed that the expression of let-7 family was significantly increased in EVs of dormant uterine fluids compared to that of activated uterine fluids. EVs-containing let-7 induced blastocysts diapause-like state in mice. Let-7 of dormant embryos was mainly derived from uterine fluids. To investigate if EVs have similar functions in human blastocyst, we collected EV from non-receptive endometrial cell line, HEC-1B, which were transfected with pre-let-7a. EDU incorporation assay showed that DNA synthesis was significantly decreased in blastocysts co-cultured with let-7a-EV for 2 days. To further demonstrate if EVs contained let-7 regulate human blastocyst implantation potential, attachment rates of BAP-EB (surrogate of human embryos) were detected in the presence of let-7-EVs. After 48 h of treatment by let-7-EVs, attachment rates of BAP-EB were significantly decreased compared to Crtl-EV (65% vs 95%)

Limitations, reasons for caution: BAP-EB is not human embryos although spheroids expressed many markers which are similar human embryos and have cavity after 72 h of differentiation.

Wider implications of the findings: small RNAs are differentially expressed in uterine fluid EVs during menstrual cycles. EVs of uterine fluid are used as marker of receptive endometrium.

Trial registration number: embryos were collected from 8 IVF patients.

P-486 Odds and predictors of monozygotic twinning in a multi-centre cohort of 25,794 IVF cycles

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Study question: To estimate the incidence of monozygotic twinning (MZT) in In-Vitro Fertilisation (IVF) and embryo transfer (ET; Single–SET and double DET) cycles and to determine the predictive factors of MZT.

Summary answer: MZT following SET and DET were 0.47% and 0.16% respectively with its odds being highest with fresh, blastocyst SET and lowest with frozen, cleavage DET.

What is known already: While the single best intervention to decrease multiple pregnancy rate is elective single embryo transfer (SET), the risk is not completely eliminated. The incidence of MZT from SET is up to around 2%, the risks are reported to increase with ICSI insemination, assisted hatching or blastocyst transfers. Some smaller studies reported frozen embryo transfers and advanced age increase the incidence of MZT. The data on MZT with double embryo transfers (DET) are limited.

Study design, size, duration: This observational study involved analysis of prospectively collected data of all consecutive IVF treatment cycles of seven tertiary fertility units in the UK between 2013 and 2017. Out of 26,347 embryo transfer cycles, 25,794 cycles matched inclusion criteria and were suitable for analysis. 16,845 cycles were SET and 8,949 were DET.

Participants/materials, setting, methods: All fresh IVF and intracytoplasmic injection (ICSI) cycles and frozen embryo transfer (FET) resulting in SET and DET were included. As per the unit protocol, all women were advised not to have unprotected intercourse during the treatment cycles. MZT was confirmed if two foetal hearts were detected for SET at the 8 week post

embryo transfer scan and three or more foetal hearts for DET. Data analysed by Chi square tests and logistic regression analysis.

Main results and the role of chance: While the over all risk of MZT was 0.37% (96/25794), the risks following SET and DET were 0.47% (82/16845) and 0.16% (14/8949) respectively. The risk of MZT in all SET cycles was significantly higher with blastocyst transfer compared to cleavage stage embryos (OR; 95% CI: 3.2; 1.01-10.1). But on subgroup analysis the increased risk persisted only with fresh SET cycles (OR 8.4; 1.16-60.9), but not with frozen transfers (OR 0.65; 0.16-2.67). With DET, there was no difference in the risks of MZT between blastocyst transfer and cleavage stage transfer regardless of whether the cycles were fresh or frozen. The risk of MZT was higher with SET than with DET (OR 3.12; 1.77-5.42) with the same effect seen with blastocysts only (OR 3.15; 1.58-6.26), but not with CSE (OR 1.18; 0.28-4.93). Both fresh and frozen SET had significantly higher MZT rates compared with DET (OR 3.15; 1.6-6.2 and OR 2.88; 1.02-8.1 respectively). On regression analysis women's age was not a significant predictor for MZT. There was no difference in MZT risks between IVF and ICSI cycles whether the cycles were fresh, frozen, SET, DET, blastocyst or cleavage stage embryo transfer cycles.

Limitations, reasons for caution: MZT was diagnosed if the number of foetal hearts seen on ultrasound scan were higher than the number of embryos transferred. While the chances of concurrent natural conception from unprotected sexual intercourse during treatment leading to dizygotic and higher order pregnancies with SET and DET respectively, such risk is low.

Wider implications of the findings: The study provides data to counsel couples undergoing IVF/ICSI treatment about the risks of MZT following SET and DET. The study agrees with the previous reports of increased risk of MZT with blastocyst SETs. However, no relationship between age, IVF or ICSI and frozen ET with MZT risks were observed.

Trial registration number: not applicable.

P-487 Effect of embryo morphology on maternal serum β-hCG level in pregnancies resulting from a fresh single cleavage embryo and a fresh single blastocyst

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Study question: Is there any effect of early embryonic factors on serum β-hCG levels in biochemical pregnancy resulting after a single fresh cleavage embryo or blastocyst transfer?

Summary answer: The day 12 serum β-hCG levels has no association with early embryonic factors and similar between a single fresh cleavage embryo and blastocyst transfer.

What is known already: Results of studies comparing β-hCG levels after the transfer of cleavage stage or day blastocyst stage have been mixed, with some studies reporting higher β-hCG levels after the transfer of blastocysts and others reporting higher levels after the transfer of cleavage-stage embryos. A limited numbers of studies have evaluated relation β-hCG levels with embryo quality parameters resulted with biochemical pregnancy from single fresh cleavage ETs or blastocyst ETs.

Study design, size, duration: In this retrospective cohort study, we reviewed all fresh single ET (sET) cycles performed between September 2011 and December 2016 at the University affiliated Assisted Reproductive Technology Center. Positive serum β-hCG levels (>5mIU/ml 12 days after ET, biochemical pregnancy) resulting from transfer of a fresh cleavage-stage embryo (day 2 or 3 after oocyte retrieval) and those of a fresh blastocyst transfer (day 5 after oocyte retrieval) were compared.

Participants/materials, setting, methods: Four thousand fifty-five fresh single ETs were analyzed, 82 biochemical pregnancies from single blastocyst transfer and 60 biochemical pregnancies from a single cleavage ET were included.

Main results and the role of chance: The median β-hCG levels resulting from a single fresh blastocyst stage transfer was 173.4 (88.8-476.0) IU/L and similar with hCG levels resulting from a cleavage-stage transfer (140.8 [66.5-448.3] IU/L) (p=.70). In the simple correlation analysis, the median β-hCG levels resulting from a cleavage-stage transfer or blastocyst transfer and early embryonic factors had no associations. The threshold value predicting a clinical pregnancy for a cleavage stage-embryo was 127.1 IU/L, and for a blastocyst transfer was 173.5 IU/L. The proposed optimal thresholds predictive for live birth were 129.4 IU/L and 178.5 IU/L for cleavage-stage and blastocyst stage ETs, respectively.

Limitations, reasons for caution: The limitations of our study include its retrospective nature and the ability to include only resulted with biochemical pregnancy from single fresh cleavage ETs or blastocyst ETs for avoiding missinterpretion of early embryonic factors on serum β-hCG levels.

Wider implications of the findings: The threshold value predicting a clinical pregnancy and live birth for a cleavage-stage embryo and blastocyst transfer provides information regarding the outcome of pregnancy.

Trial registration number: This study is retrospective cohort study, not clinical trial.

P-488 RCT depicting favourable IVF outcomes following ATT (anti-tubercular treatment) either on abnormal endoscopy findings or history of contact of tuberculosis

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Study question: Genital Tuberculosis is a major cause of infertility in India. Does Anti-Tubercular Treatment on the sole basis of abnormal endoscopy findings OR history of contact yield better IVF-Result?

Summary answer: Prior treatment of abnormal Endoscopy findings OR history of contact of Tuberculous Mycobacterial infection cases with no other demonstrable cause improves the ART results.

What is known already: The morbidity and mortality due to tuberculosis (TB) is high worldwide, and the burden of disease among women is significant, especially in developing countries. Mycobacterium Tuberculosis bacilli reach the genitaltract primarily by haematogenous spread and dissemination from foci outside the genitalia with lungs as the common primary focus. Genital TB in females is a chronic disease with low-grade symptoms.

The fallopian tubes are affected in almost all cases of genital TB, and along with endometrial involvement, it causes infertility in patients.

More research is needed on the changing trends in the prevalence and on the appropriate methods for diagnosis and management.

Study design, size, duration: 423 infertile patients below 40 years were evaluated by laparoscopy and hysteroscopy between 2010 to 2017. Out of these, 108 patients having abnormal hysteroscopic findings or history of contacts only, were prospectively randomised into Anti-Tuberculosis treatment and Non-treatment groups by a computer generated list, before undergoing IVF.

History of contact of Tuberculosis and Screening for genital TB needs to be a part of evaluation of infertility and menstrual abnormalities.

Participants/materials, setting, methods: 423 infertile patients below 40 years were evaluated by laparoscopy and hysteroscopy between 2010 to 2017. Out of these, 108 patients having abnormal hysteroscopic findings or history of contact only, were prospectively randomised into Anti-Tuberculosis treatment and Non-treatment groups by a computer generated list, before undergoing IVF.

The primary outcome measured was cumulative pregnancy rate following IVF, and secondary outcome was the miscarriage rate.

Main results and the role of chance: There was a statistically significant difference between the two groups, the cumulative pregnancy rate being higher following IVF in the group who took prior anti tubercular treatment - 34% versus 16%. Although the miscarriage rate was lower in the treatment group, it was not statiscally significant (9% Vesrsus 13%).

Most of the patients present in advanced stage with scarring, severe fibrosis and adhesions and treatment outcomes, especially with regard to infertility, are

poor. Hence, early diagnosis and correct treatment is vital to avoid complications and to restore fertility.

Clinicians need to be aware of this important cause of infertility and menstrual dysfunction in women and major cause of poor IVF results.

Limitations, reasons for caution: Genital TB is a major cause of infertility inwomen, and prevalence is generally under stimated because of the asymptomatic nature of the infection and diagnostic challenges. Large multicentric studies are needed to estimate the magnitude of FGTB and to identify the most sensitive test for diagnosis.

Wider implications of the findings: In resource poor developing countries where IVF cycles are self-funded and difficult to afford, prior treatment of abnormal hysteroscopic findings or history of contact of tuberculous mycobacterial infection cases with no other demonstrable cause improves the ART results. Genital Tuberculosis is a major cause of infertility in developing countries.

Trial registration number: no trial number.

P-489 Bed rest after embryo transfer might affect embryo implantation, clinical pregnancy and spontaneous miscarriage rates: a prospective cohort analysis

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Study question: Bed rest after embryo transfer has been routinely advised without clear clinical evidence and benefit. We investigated whether abolishing the practice would negatively affect the clinical outcomes of ART.

Summary answer: Routine bed rest after embryo transfer appeared detrimental to the clinical outcomes including clinical pregnancy and embryo implantation rates, and spontaneous miscarriage rate after ART.

What is known already: There are several routine measures for embryo transfer to maximize its efficiency since inception of ART. Bed rest after embryo transfer has been historically practiced without clear medical benefit and evidence by meta-analysis (Cochrane Review, 2014: CD006567). Other recent publications suggested bed rest for long duration after embryo transfer might lower implantation as well as ongoing pregnancy rates. Even short 10 minutes' bed rest after embryo transfer using donor oocytes had negative impact on ART delivery rate (Gaikuwad et al. Fertil. Steril, 2013;100:729).

Study design, size, duration: A prospective non-randomized cohort study: clinical outcomes of frozen embryo transfers were compared before (86 cycles) and after (83 cycles) abandoning routine bed rest in July, 2015. Ethics committee approved the study protocol and consent was obtained from each participant.

Participants/materials, setting, methods: Females under age 43 receiving autologous frozen embryos were recruited for the study at a single private ART institution. The endometrium was prepared using transdermal estradiol and vaginal progesterone was administered until 8 weeks' gestation. The vitrified embryos were thawed at cleavage or blastocyst stage and transferred. Bed rest group spent 30 minutes' in supine or prone position after embryo transfer, and no bed rest group was ambulatory immediately after embryo transfer.

Main results and the role of chance: Between the two groups with and without bed rest, the average female age (38.3 vs. 38.3 y.o.) and the order of transfer (2.8 vs. 2.9) and the number of embryos transferred (1.4 vs. 1.5) were similar. There was tendency of higher clinical pregnancy rate (38.6% vs. 27.9%, OR 1.6 ($p = 0.14$)), implantation rate (27.6% vs. 22.8%, OR1.3 ($p = 0.38$)) and lower miscarriage rate (45.8% vs. 28.1%, OR 0.46 ($p = 0.17$)) for the group without bed rest. These trends were similarly observed despite the stage of thawed embryos (cleavage or blastocyst), and the uterine positions (anterior or posterior). Further analysis with fresh embryo transfer had the same findings though not statistically significant.

Limitations, reasons for caution: Lack of study power due to relatively small sample size, and non-randomized and non-blinded nature of the study design are the major limitations.

Wider implications of the findings: Abolishing routine bed rest after embryo transfer increased pregnancy and implantation rates, and lowered chance of miscarriage. The effects were unrelated to the stage of thawed embryos, or uterine positions. The uterine condition or higher sense of self efficiency with early ambulation after transfer might be related to the findings.

Trial registration number: Not applicable.

P-490 Relationship between gestational age and the observed protein patterns of first trimester maternal urine analysed by maldi-tof mass spectrometry

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Study question: Does mass spectral patterns of healthy pregnancy maternal urine analysed by MALDI-ToF MS represent the gradual and directional changes associated with increasing gestational age?

Summary answer: The observed difference in maternal urine of different gestational age show a consistent pattern at selected regions corresponding to protein peak areas.

What is known already: Urinalysis by MALDI-ToF MS has been shown to be a useful tool for identification of pregnancy related diseases including pre-eclampsia screening, downs syndrome screening and assessment of pregnancy viability in case of threatening miscarriage. Furthermore it is also recognised, by both biochemical testing and MALDI-ToF MS technique, that gestational age is a very important factor affecting maternal urine protein levels. Therefore, this is likely to have an effect on any methods which involve the study relative protein in the urine of women during pregnancy.

Study design, size, duration: Retrospective longitudinal study analysing a total of 633 urine samples at an average of 8 weeks of gestation from four USA IVF clinics collected between May 2014 and August 2016. Samples were categorised into five groups for gestational weeks from six to ten (n = 187, 127, 162, 103 and 54). Pregnancy losses were excluded and only MALDI ToF MS profiles from healthy pregnancies were assessed for changes relating to gestational age.

Participants/materials, setting, methods: Subsequent to the mass spectral analysis all data was subject to meticulous pre-processing to render data comparable and the region between 2000 to 12000 m/z was subject to further testing. There were 274 peaks identified to be representative throughout the data and were quantified by summing the ion count in the peak regions. Non-parametric test for trend was applied to medians of five groups for all 274 peaks. Analysis was performed using R.

Main results and the role of chance: Total of 274 peaks were tested for upward or downward trends, each one representing the upregulated or down-regulated proteins present during the ongoing pregnancy. Visual assessment of the 274 peaks by scatter plots and boxplots led to the conclusion that such trends both positive and negative are present in about quarter of the peaks analysed. Twenty (7.3%) of the observed peaks had a strong (>0.8) negative correlation and forty two (15.4%) peaks had a strong positive linear correlation for gestational weeks. These significant peaks were further examined for trends by removing the categories and analysing the raw data by day, giving more certainty to the results and to confirm the existing trends. Furthermore, the increase of human chorionic gonadotropin (hCG) during first weeks of gestation was represented by the increase of ion counts in the mass region of 9425 m/z to 9847 m/z which corresponds to the hCG β subunit core fragment. The consistent presence of this peak provides additional confidence in the reliability of the data. Additionally, this has confirmed the previously observed pattern (unpublished data) for maternal urine in Downs screening data.

Limitations, reasons for caution: Urine samples were not analysed fresh and were subject to at least one freeze – thaw cycle.

Wider implications of the findings: The study furthers the understanding of maternal pregnancy urine analysed by MALDI-ToF MS which has several potential applications for diagnostic testing including pre-eclampsia and aneuploidies screening. This new knowledge will allow improvements to the diagnostic algorithms by adjusting predictive models to particular gestational ages.

Trial registration number: n/a.

P-491 Towards the development of rapid, first trimester, maternal urine maldi-tof ms screening tests for Downs syndrome

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Study question: Question - Can the examination of mass spectral data from maternal urine obtained from 12 to 14 week high risk pregnancies predict a risk of Downs syndrome?

Summary answer: Mass spectral examination of maternal urine, in the range of 2,000 to 11,000 m/z, gives rise to gestational age specific testing algorithms for Downs syndrome screening.

What is known already: The established methods of antenatal screening test for Down's syndrome are based on a panel of maternal serum biomarkers measured by immunoassay together with ultrasound markers. Recently, a method based maternal plasma cell free (cf)DNA has begun to be used but it has a number of limitations including excessive turn-around time and cost. We aimed to develop an alternative method based on urinalysis which is simple, affordable and accurate.

Study design, size, duration: A retrospective collection of 20 Down Syndrome and 100 non-aneuploid pregnancy urines collected in 2007-2008 from high risk pregnancy cohorts.

Participants/materials, setting, methods: Samples were thawed and examined by MALDI-ToF mass spectrometry in the mass/charge range between 1000 and 100,000 m/z. The spectral masses between 2000 and 11,000 m/z were specifically examined and a method has been developed by which data was collected and analysed. Normalisation of spectral data was defined using mass bins of 100 m/z expressed as a percentage of the total mass spectra from 2,000 to 11,000 m/z.

Main results and the role of chance: Of the ninety 100 m/z bins, nineteen of them, covering the maximum amount of variation were selected, all demonstrating statistically significant difference between Downs and control/non-aneuploid samples as verified by Mann-Whitney test. Based on the differences and variance, for values at these spectral regions, weighted scores of the probability of being Downs were assigned. Comparative algorithms consisting of various mass peak regions were tested for ability to distinguish Downs syndrome from non-aneuploid pregnancy. Although various algorithms could distinguish Downs from non-aneuploid controls, it was found that gestational age was a confounding factor and that if separated into gestational age matched cohorts the ability to distinguish the groups improved dramatically e.g. whilst an algorithm consisting of nineteen measured bin spectral regions separated a 100% of Downs for non-Downs for 10% false positive in the mixed gestational ages cohort; a two mass peak algorithm distinguished 100% of Downs for 6% false positive rate for a cohort of 12 weeks gestation samples.

Limitations, reasons for caution: This study is limited by the cohort number and samples which have been stored for a considerable time.

Wider implications of the findings: Implementation of simple screening programmes based on rapid analysis of urine from women in early pregnancy would provide an economical alternative to complex screening programmes and costly genetic NIPT. This will be of particular use in countries with large and widespread populations.

Trial registration number: N/A.

P-492 Duration of medicated frozen embryo transfer cycles. Is length of estradiol supplementation having a role in outcomes? A retrospective study

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Study question: Is the length of estradiol supplementation affecting outcomes in frozen embryo transfer cycles?

Summary answer: Our data suggest that clinical pregnancies are significantly increased with duration of estradiol supplementation of twelve or more days.

What is known already: According to data presented in the recent ASRM[1], estradiol duration n does not have an impact on pregnancy rates. Other studies suggest that prolonging estradiol supplementation may have negative effects on pregnancy outcomes[2].

1. Sekhon L, Endometrial preparation for FET: Does the duration of estradiol supplementation matter? (2017) ASRM P675

2. Sunkara SK., The impact of the duration of estrogen supplementation on the outcome of medicated frozen-thawed embryo transfer (FET) cycles (2011) Fertil Steril, Vol96, Issue 3, S43

Study design, size, duration: Retrospective analysis of medicated frozen embryo transfer cycles in our unit from January 2016 until December 2017. We reviewed 378 consecutive medicated frozen embryo transfer cycles. Cycles were divided into 2 groups A. those with duration of estradiol supplementation of 11 days or less (duration varied between 5 and 11 days) and B. those with estradiol supplementation of 12 days or more (duration varied between 12 and 28 days)

Participants/materials, setting, methods: Group A consisted of 154 cycles frozen embryo transfer cycles and Group B of 224. Egg recipients were not included in the study. Five PGS cycles were included in the study (1 in Group A and 4 in Group B but their inclusion does not affect the significance of results). Main outcomes off the study were implantation (as defined by positive pregnancy test) and clinical pregnancy rates (presence of heartbeat in the 7th week scan).

Main results and the role of chance: Age and AMH were similar in the 2 groups (p: 0.22 and p: 0.385 respectively). BMI was significantly higher in Group A 23.9 vs 23.2 in Group B (p: 0.039). Endometrial thickness was significantly higher in Group A (8.7 mm vs 8.1 mm, p:0.002). Endometrial morphology was recorded as triple layer endometrial lining in the majority of cycles. Implantation rates were similar in both groups (40.25% vs 43.7%) but clinical pregnancy rates were significantly higher in Group B (30.8% vs 22.7%, p: 0.03). In the subgroup analysis, the increase involves mainly blastocyst transfers with cleavage stage transfers demonstrating similar results in both groups. More specific, for cleavage stage transfers implantation (Group A 35.5% vs Group B 33%) and clinical pregnancy rates (Group A 21% vs Group B 22.3%)were similar in both groups. In blastocyst transfers implantation was higher in Group B (52,9% vs 43.5%) but did not reach significance. Clinical pregnancies were significantly higher in Group B (38% vs 23.9%, p:0.012).

Limitations, reasons for caution: The major limitation of the study is its retrospective design. BMI and endometrial thickness were significantly different in both groups but it is difficult to quantify the effect of these features on the outcomes as in both groups BMI and endometrial thickness are within normal limits.

Wider implications of the findings: Improved endometrial maturity and receptivity are possible key factors implicated in lenght of estradiol supplementation of 12 or more days. Our findings are in contrast with current literature suggesting that length does not have a role or increased length may have a negative impact on outcomes.

Trial registration number: not aplicable.

P-493 Minimally-invasive RNA-Seq profiling of the endometrium during stimulated in vitro fertilization cycles to identify predictive markers of endometrial receptivity

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Study question: What genes are differentially expressed by the endometrium at the time of embryo transfer in stimulated cycles and how does this compare to natural cycles?

Summary answer: A distinct gene expression signature distinguishes the receptive phase endometrium in stimulated cycles, with 58 genes overlapping with the natural cycle.

What is known already: Several groups, including ours, have characterized the gene expression of the endometrium in natural cycles and identified

transcripts that are differentially expressed during the receptive phase (LH+7) versus the pre-receptive phase (LH+2). Most investigators have used invasive endometrial biopsy to sample the endometrium, which must be performed in a 'mock" cycle as the biopsy may disrupt implantation. Therefore, molecular characterization of the endometrium in stimulated IVF cycles and same-cycle correlation of gene expression to pregnancy outcome are lacking. We previously developed a minimally-invasive technique of uterine fluid aspiration (UFA) that allows for transcriptomic profiling during an active conception cycle.

Study design, size, duration: Healthy women ≤ 40 years of age with regular cycles and normal uterine cavities, undergoing their first or second IVF cycle, were recruited with informed consent. UFA sampling was performed at the time of oocyte retrieval (hCG+2), classified as 'pre-receptive" samples (N = 40). UFA sampling was also performed in the same women at the time of fresh day 5 embryo transfer (hCG+7), classified as 'receptive" samples (N = 26).

Participants/materials, setting, methods: Total RNA was extracted from samples and RNA-Seq analysis was performed. Reads from each sample were mapped to the human reference genome (UCSC hg19) using HISAT2. Transcript assembly and abundance estimation was performed using StringTie, and R statistical software with the Ballgown package was used to determine differentially expressed transcripts between timepoints (fold change ≥ 4, false discovery rate q<0.05). These transcripts were cross-referenced with our previously published data from natural cycles (LH+2 and LH+7).

Main results and the role of chance: Statistical analysis showed 11,152 genes were differentially regulated between hCG+2 and hCG+7 samples. Hierarchical clustering of the samples using these genes revealed a clear segregation of samples into two major sub-groups: the first consisting almost exclusively of hCG+2 samples and the second of hCG+7 samples. The differentially expressed genes between hCG+7 and hCG+2 were then compared to a list of 245 genes showing ≥ 4-fold change from our previous natural cycle data (LH+7 versus LH+2). Of those 245 genes, 58 overlapped with the stimulated cycle data (q<0.05). The RNA-seq data for these genes were then clustered using a divisive analysis clustering method (DIANA) and showed two major sub-groups that correspond to hCG+2 and hCG+7. Of the patients that underwent fresh embryo transfer, 7 conceived. No statistically significant differences were identified between the samples from pregnant vs. non-pregnant patients.

Limitations, reasons for caution: This translational study focused on discovery of candidate biomarkers of endometrial receptivity in stimulated cycles. The main limitation is a small sample size. The gene expression profile requires further validation through prospective clinical trials with more patients to determine the transcripts that may have predictive value for implantation.

Wider implications of the findings: This is the first whole-genome transcriptomic study on the stimulated endometrium, with direct correlation to embryo transfer outcomes. Prospective studies using this signature will allow identification of predictive markers of pregnancy. Overlapping transcripts between stimulated and natural cycles imply conserved pathways involved in endometrial receptivity that are of particular interest.

Trial registration number: Not applicable.

P-494 Clinical pregnancies achieved after embryo transfer date adjustment based on novel endometrium receptivity test

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Study question: Does the embryo transfer (ET) date adjustment based on the new endometrium receptivity test lead to increased clinical pregnancy rate?

Summary answer: We can report encouraging preliminary results in patients who had their embryo transfer date adjusted based on the results of the simple endometrium receptivity test.

What is known already: Embryo implantation is a crucial step in the in-vitro fertilization. The so called "window of implantation" is a 4-5 days usually between cycle day 20 and 24. This, however, can vary depending on the cycle type and on individual basis. Analysis of the reasons for implantation failure in cases of top quality embryos showed that a common cause is the lack of synchronization of embryonic development with endometrial maturation.

There are commercial and non-commercial tests available that assess endometrial receptivity based on expression levels of genes linked to the status of endometrial receptivity, using RNA sequencing.

Study design, size, duration: This is an ongoing study initiated in April 2017. Study will include 160 patients with 3 previous implantation failures after a transfer of top quality embryos (with a total number of transferred embryos being at least 4), who are planning a frozen embryo transfer (FET). Patients are divided equally into study group that undergoes endometrium receptivity testing and the control group of those patients who do not opt for the test.

Participants/materials, setting, methods: To date 68 patients were qualified and underwent endometrial biopsies. Endometrial biopsies on day 5 and 7 after ovulation or after start of progesterone supplementation, depending on the planned preparation for FET. Endometrial biopsy material was processed with immunohistochemical staining to evaluate presence of the following protein: estrogen receptor (ER), progesterone receptor (PR), BCL-6, interleukin 15 (IL-15), osteopontin, beta-3 integrins, HOXA10.

Main results and the role of chance: 58.2% of patients had a normal result for both biopsy samples - day 5 and day 7. 38.2% had at least one of the results showing endometrium as delayed in development, prematurely developed or showing mixed development. There were 2 non-diagnostic results (due to poor sample quality) where D5 result was Normal and D7 was non-diagnostic.

D5 samples were Normal in 88.1% cases (59 patients), Delayed - 6.0% (4), Premature - 3.0% (2) and Mixed - 3.0% (2). For the day 7 samples the results were as follows: Normal - 64.2% (43), Delayed - 9.0% (6), Premature - 11.9% (8), Mixed - 11.9% (8), Non-diagnostic - 3.0% (2).

It is worth noting that in 11.9% of cases D7 biopsy results showed mixed endometrium development, i.e., party delayed and party premature development. In those cases other mRNA based tests may show misleading results.

24 patients have already undergone an FET. 15 of those had a normal receptivity test result and 9 an abnormal one. In the normal result subgroup there are 4 clinical pregnancies (26.7%). For patients in the abnormal subgroup the FET date was adjusted based on the receptivity test result. In that group there are 5 clinical pregnancies (55.6%)

Limitations, reasons for caution: These are preliminary results and the group of patients that had already underwent the FET is relatively small.

Wider implications of the findings: This test may provide a simple and cost effective method of determining endometrial receptivity. The preliminary results are encouraging. It appears that when endometrial receptivity is determined the be the cause of implantation failure adjustment of transfer date helps in achieving clinical pregnancies.

Trial registration number: Not applicable.

P-495 Frozen Embryo Transfer Protocols: Which one is best?

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Study question: Is there a difference in live birth rates between frozen embryo transfer (FET) protocols?

Summary answer: In under 36 year olds having FET with single vitrified top quality blastocyst, live birth rates are significantly higher after natural cycle compared to medicated.

What is known already: Currently, there is no definitive evidence that there is a superior protocol for frozen embryo transfer. Several studies have compared natural cycle, modified natural cycle and medicated cycles including both with down regulation and with out. Whilst pregnancy rates have been shown to be higher in some groups in some studies, the live birth rates have not shown

any significant differences. Most of these studies have not be carefully selected with heterogeneity and confounding factors affecting reliability.

Study design, size, duration: This was a retrospective study, from July 2011 to Dec 2016. 1933 FET cycles were included.

Participants/materials, setting, methods: Inclusion criteria: ≤36 years, vitrified top quality blastocysts, elective single embryo transfers, own eggs, no assisted hatching, at least 75% recovery of embryos at FET, patients with over two previous failed embryo transfers.

Exclusion criteria: egg recipients, double embryo transfer, age over 36 yrs, embryos cryopreserved by slow freeze.

1933 FET cycles that complied with inclusion criteria during study period were categorised into 3 groups based on treatment protocol and live birth rates were compared.

Main results and the role of chance: 746 had down regulation protocol, 981 had non downregulation protocol and 206 had natural cycle. (There were no significant differences between three protocols (Down regulation protocol (DR), Non downregulation protocol (NonDR) and natural cycles) in positive pregnancy rates (58.7%, 56.7%, 60.2%, $p = 0.54$). The clinical pregnancy rates (44.1%, 43%, 53.9%, $p = 0.16$) and live birth rates (38.5%, 36.7%, 46.1%, $p = 0.008$) were however significantly higher in the NC group. This can be attributed to a significantly lower biochemical pregnancy loss and miscarriage rate in natural cycle group. The number of natural cycle protocols are less than the medicated and so perhaps there is the role of chance which can be confirmed by further prospective analysis.

Limitations, reasons for caution: The main draw back is the retrospective design.

Wider implications of the findings: Natural cycle FETs can be unpredictable and hence may increase workload over weekend. This can be particularly unsuitable for planning clinic timings.

Trial registration number: not applicable.

P-496 CCS-STR analysis as standard of care for loss of pregnancy has advantages over traditional culture and karyotype

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Study question: Does CCS-STR analysis of pregnancy loss products have advantages over traditional culture and karyotype analysis?

Summary answer: CCS-STR analysis has several advantages over culture and karyotyping as a method to determine cause of early pregnancy loss.

What is known already: Simpson (1990) summarized the results of culture and karyotyping studies of pregnancy loss products.

Study design, size, duration: 516 pregnancy loss products were analyzed over a 5 year period using CCS technology (array-CGH and NGS sequencing) with follow-up STR analysis when appropriate.

Participants/materials, setting, methods: SurePlex whole genome amplification products from dissected pregnancy loss products that were previously performed in a clinical setting were analyzed.

Main results and the role of chance: Unlike traditional karyotyping, CCS-STR analysis is able to confirm the presence of fetal tissue being analyzed due to unique STRs found in the purported fetal tissue compared to the maternal tissue. We found that the proportion of "normal" results in the karyotyping study (51%) was comparable to combining both the normal and maternal contamination results using the CCS-STR method (31.4% diagnosed normal, 23.3% diagnosed as maternal contamination, total 54.7%).

CCS-STR analysis is able to be accurately identify monosomic lines and abnormalities involving 2-3 or more chromosomes. Simpson identified no losses involving both a monosomy and a trisomy or three or more abnormalities, while CCS +STR analysis found 1.2% and 0.2% of losses in those categories, respectively.

In addition, karyotyping involves use of the subcategory "G", when the operator has difficulty differentiating chromosomes 21/22. CCS-STR analysis is able to confidently distinguish even small chromosomes accurately, including 21/22. While culture and karyotype and CCS+STR identified the same proportion of losses involving trisomy 21 (2.1% vs 2.3%), CCS+STR identified almost double the proportion of trisomy 22 losses (5.2% vs 2.3%).

Limitations, reasons for caution: CCS-STR is not able to identify certain forms of polyploidy, including high-order polyploidy and mitotically-derived polyploidy.

Wider implications of the findings: CCS+STR analysis is a highly accurate way to determine cause of pregnancy loss without many of the drawbacks involved in culture and karyotyping. Shifting pregnancy loss testing towards SNP array + STR analysis should maintain the advantages of CCS+STR in addition to being able to identify more types of polyploidy.

Trial registration number: Not applicable.

POSTER VIEWING

Male and female fertility preservation

P-497 Small or large ovarian tissue fragment. Which is the ideal size for fertility preservation?

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Study question: Does the size of the ovarian fragment affect stromal and follicular morphology and tissue physiology after vitrification?

Summary answer: Electron microscopy and Gomori´s staining show normal follicular morphology, collagen fibers and fibroblasts in small and large samples and similar lactate dehydrogenase production in culture.

What is known already: Reports describe the use of small and large fragments and slow freezing. Ovarian tissue vitrification is gaining attention, however, effects of the size of the fragment on stroma morphology and tissue physiology were not reported. Larger samples may reduce mechanical damage caused by dissection. Fibroblasts and extracellular matrix present in the stroma play a critical role in folliculogenesis and angiogenesis through microenvironment remodeling and growth factors delivery, two processes involved in successful grafting and prevention of necrosis. Gomori´s staining and electron microscopy reveal details of stromal components and lactate dehydrogenase (LDH) represents a signature of necrotic cells pos-cryopreservation and culture.

Study design, size, duration: Fresh small (S, 1 × 1 × 2 mm) and large (L, 1 × 1 × 5) fragments of bovine ovaries were fixed for light (LM) and electron microscopy (EM) observations, or vitrified. Fresh and rewarmed fragments were cultured for 48hrs. Next, tissues were fixed and processed for LM and EM. LM, sections were stained with Gomori´s trichrome for connective tissue and collagen fibers. Culture media was collected for LDH assay. Experiments were performed in triplicates.

Participants/materials, setting, methods: S and L fragments from 4-6 ovaries were fixed for LM and EM observations or vitrified using 15% DMSO, 15% ethylene glycol and 0,5 M sucrose in human tubal fluid. Fixed S and L tissues were prepared according to standard LM and EM protocols. Morphological analysis included: collagen fibers, fibroblasts, follicle and blood vessel structures. LDH activity was measured by enzymatic colorimetric assay. LDH measurements were compared by ANOVA. *P* value was considered significant when <0.05

Main results and the role of chance: Gomori´s trichome staining of S and L vitrified tissue sections showed that interstitial matrix, collagen fibers and fibroblasts maintain a similar structure to that found in fresh tissues. However, S fragments presented a looser matrix structure, than L samples. Follicles in S and L fragments show no major alteration in relation to findings observed in fresh tissues. These observations were confirmed by EM micrographs, where collagen fibers in vitrified samples appear as continuous structures, with no interstitial gaps between them. In addition, it is possible to observe the normal fusiform morphology of fibroblasts, intact blood vessel wall and endothelium. Ultrastructural

analysis of primordial follicles demonstrated well-preserved integrity of the oocyte and the surrounding follicular cells. No differences in LDH activity were found between fresh and vitrified, S and L fragments after 48 hours culture (P = 0,347).

Limitations, reasons for caution: The main limitation of our study refers to the fact that results were shown for ovarian tissue collected from one species, bovine. Further studies with human ovarian samples are necessary to confirm our findings. Also, EM micrographs show detailed structures within limited areas of tissue, not the whole fragment morphology.

Wider implications of the findings: The present study provides evidence that vitrification does not induce major morphological and physiological changes in the stroma and follicles of S and L ovarian tissue fragments. Thus, it is recommended to use L ovarian fragments for tissue banking.

Trial registration number: Basic animal research.

P-498 Luteal phase stimulation, the future of fertility preservation? Retrospective cohort study of luteal phase stimulation versus follicular phase stimulation

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Study question: Is luteal phase stimulation capable of improving fertility preservation?

Summary answer: Luteal phase stimulation appears to be a promising alternative to conventional protocols in view of the number of mature oocytes obtained.

What is known already: Ovarian stimulation with cryopreservation of oocytes / embryos is today the most widely used technique for fertility preservation in women. Usually, ovarian stimulation starts early in the follicular phase. However, this method requires, depending on the day of the cycle of the patient on the day of consultation, between 2 and 6 weeks of implementation. This can delay the beginning of cancer treatment. In this context, luteal phase stimulation appears as a solution. It would indeed reduce the time of completion so the cancer treatment can start quickly as possible.

Study design, size, duration: We performed a retrospective cohort study of consecutive ovarian stimulations, during January 2012 and October 2017 in the University Hospital of Strasbourg.

Participants/materials, setting, methods: Were included all patients under 40 who were referred to our centre for fertility preservation. All included patients had regular cycles and normal ovulation. Non-inclusion criteria are a low expected ovarian response defined by AMH<0.75 µg/l, polycystic ovarian syndrome, amenorrhea, history of infertility or hypofertilization treatment. The primary endpoint is the number of mature oocytes (metaphase II) obtained.

Main results and the role of chance: A total of 87 patients were included: 19 in luteal phase and 68 in follicular phase. A larger number of mature oocytes was obtained in luteal phase versus follicular phase (12.4+/-8.8 versus 7.9+/-5.6 with p = 0.046). A greater amount of total (mature and immature) oocytes was obtained in luteal phase versus follicular phase with a significant difference (15.5+/-10.0 versus 10.2+/-6.9 with p = 0.041). No difference was found for the initial and total doses of gonadotropin.

Limitations, reasons for caution: One of the issues of our study was to determine the progesterone level to define the beginning of the luteal phase. In our study, a progesterone level above 2 ng/ml arbitrarily defined the luteal phase, while many studies have used a higher rate.

Wider implications of the findings: Luteal phase stimulation has the advantage of a better flexibility with positives results as to the number of oocytes obtained. It is easy to imagine a future for this new protocol, particularly in the preservation of fertility, where it is essential to reduce the delay in achieving stimulation.

Trial registration number: Not applicable.

P-499 An existing tool in a new concept: Retrieval of immature oocytes during laparoscopy/hysteroscopy followed by in vitro maturation as a strategy for oocyte banking

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Study question: The feasibility of performing transvaginal retrieval of immature oocytes and subsequent in vitro maturation (IVM) in adjunct with gynaecological surgery for women themselves or donation.

Summary answer: IVM-surgery is simple, safe and associated with good results, which allows embryo or oocyte cryopreservation utilised by treated women or donation of spare oocytes.

What is known already: n contrast to conventional in vitro fertilization (IVF), IVM offers several advantages. It may be performed without ovarian stimulation with gonadotrophins, thus avoiding ovarian hyper stimulation syndrome (OHSS), and requires shorter treatment time. IVM also involves less complexity associated with planning and monitoring of the cycle, and incurs lower costs, which is expected to be more acceptable to the patients and to expand access to fertility treatments.

It's common that women undergo non- assisted reproduction technologies (ART) procedures, such as hysteroscopy or laparoscopy prior or during their fertility treatment.

Study design, size, duration: To assess the feasibility and the efficacy of IVM treatment when oocyte retrieval was performed in adjunct with laparoscopy/ hysteroscopy in infertile women. To evaluate the outcomes of the ART cycles with both fresh and vitrified-warmed fertilised IVM oocytes and the efficiency of this technique compared with conventional IVM in our center. In addition, we examined the safety of the technique including ovarian function.

Participants/materials, setting, methods: We included 158 consecutive women with PCOS who underwent laparoscopy or hysteroscopy had concomitant transvaginal oocyte retrieval followed by IVM between June 2014 and May 2016. Matured IVM oocytes were either fertilised (n = 46) or vitrified (n = 112) and then warmed and inseminated (n = 33). Women with PCOS who underwent conventional IVM and women who underwent gynaecological surgery without IVM served as controls. The analysis of outcomes included embryological data, clinical outcomes, and ovarian function 6 months post-procedure.

Main results and the role of chance: The number of oocytes retrieved per patient was 14.5 (9.0, 20.0), the average maturation rate was 47.4% ±16.5%. Fertilisation rate of fresh-inseminated oocytes was 64.6% and of vitrified-warmed oocytes was 50.0% with oocyte survival 83.0%. Vitrified blastocysts were available for 41.3% patients (2.4 ± 1.641). Vitrified-warmed oocytes resulted in high-quality cleavage embryos in 63.6% patients [32.5 (0.0, 50.0)]. Clinical pregnancy and live birth rates per single blastocyst transfer cycle in vitrified blastocyst group were 63.6% and 54.6%, respectively and per single or double day-3 cleavage transfer cycle in vitrified oocyte group were 31.8% and 22.7%, respectively. No adverse neonatal outcomes were recorded in either group.

IVM-surgery resulted in comparable oocyte yield and maturation rate as conventional IVM. The fertilisation rate and high-quality cleavage embryos from fresh-inseminated oocytes were also similar to those of conventional IVM.

IVM-surgery added 30 minutes to operative time, but was not associated with postoperative complications or longer hospital stay and did not seem to affect ovarian function compared with those of surgery alone group.

Limitations, reasons for caution: We included only women with PCOS because PCOS patients have larger follicle pool and were more likely to benefit from IVM cycle.

Sample size limited our ability for meaningful conclusions regarding perinatal outcomes.

The findings need to be confirmed in a larger population and in different phenotypic groups of women.

Wider implications of the findings: Combined IVM-surgery is a simple and safe procedure. It carries minimal risk of OHSS and is associated with acceptable live birth rate. Importantly, this approach provides additional avenue for social fertility preservation in treated women and allows utilising excess vitrified oocytes for oocyte donation program or for research.

Trial registration number: The study was retrospectively registered with the Chinese Clinical Trial Registry on 04.07.2017; clinical trial registration ID ChiCTR-ONC-17011861.

P-500 Effect of letrozole added to gonadotropins in controlled ovarian stimulation protocols for fertility preservation on the yield and maturity of retrieved oocytes

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Study question: Does the co-administration of letrozole and gonadotropins during ovarian stimulation influence oocyte yield and maturation in breast cancer patients prior to chemotherapy?

Summary answer: In apparently normal responders with breast cancer, the addition of letrozole to gonadotropins does not increase the number of retrieved oocytes or maturation rate.

What is known already: Ovarian stimulation with letrozole and gonadotropins is effective in patients with breast cancer and spares them exposure to high estradiol levels in fertility preservation cycles. Adjunctive letrozole administration in poor-responder patients decreased the rate of cycle cancelation with increased oocyte yield, perhaps due to increased endogenous gonadotropins secretion or increased follicular androgens concentration.

Study design, size, duration: A retrospective cohort design was used comparing oocyte cryopreservation cycles among patients with breast cancer patients with other oncological indications and women undergoing elective oocyte cryopreservation at a single tertiary medical center between 2007 and 2017. All patients were treated with GnRH antagonist protocol using GnRH agonist for final oocyte maturation. The breast cancer group was additionally treated with letrozole (5 mg/day) from the first day of treatment until the day of oocyte retrieval.

Participants/materials, setting, methods: The cohort included 418 patients: 145 breast cancer patients, 168 with other oncological indications, and 105 patients who chose to undergo elective oocyte cryopreservation.

Main results and the role of chance: There were no significant differences among the groups in number of retrieved oocytes (13.1 ± 11.9 vs. 14.4 ± 11.0 vs. 13.6 ± 10.8, respectively, p = 0.593) or proportion of mature oocytes (85 ± 16% vs. 81 ± 22% vs. 80 ± 20%, respectively, p = 0.125). On multivariate linear regression models, co-treatment with letrozole was not a significant factor for the number of retrieved oocytes (p = 0.408) or for oocyte maturation rate (p = 0.127) after controlling for age, body mass index, and FSH dose.

Limitations, reasons for caution: Our study has several limitations, including the fact that the groups were not evenly distributed, age differences, and the BRCA status was not available for all breast cancer patients.

Wider implications of the findings: The suspected biological effect of aromatase inhibitors and increased intrafollicular androgens level reported in poor responders is probably unique to that specific population and was not found in apparently normal responders.

Trial registration number: 0163-16-RMC

P-501 Oocyte vitrification for elective Fertility preservation. An excellent tool for fertility counseling

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Study question: Is the number of oocytes obtained for vitrification in an elective fertility program a good tool for fertility counseling?

Summary answer: Our results show that vitrification of oocytes for elective fertility preservation is an excellent tool to be use for fertility counseling.

What is known already: Vitrification has superior post-thaw survival and fertilization outcomes compared with oocytes frozen with the slow-freeze technique.Oocyte vitrification is being offered to healthy women to extend their reproductive options,providing similar results to fresh oocytes.Currently, delayed maternity is most motivated by career aspiration, relationship instability, breakdown and late marriage.Age is strongly related to the probability of having a child with better chances when they do younger than 35,and between 10-12 oocytes would be the ideal number to obtain good results in terms of live birth rates and cost effectiveness.Obteining less oocytes than expected make us provide an strict reproductive counseling.

Study design, size, duration: Retrospective study, September 2008 to December 2017

Participants/materials, setting, methods: A total of 280 patients (37.4 ± 2,8 years), in 327 cycles, vitrificated 1584 MII oocytes, All patients had normal values of FSH, AHM and E2. The length of stimulation was 11.2 (10.1 12.3), Mean FSH dose (IU) 1445.85(1408.5-1483.2), E2 on day of hcg (pg/ml) 1636 (1548.3-1725.4).

Main results and the role of chance: A total of 280 patients, vitrificated 1584 MII oocytes, the mean age was 37 ± 2,8, (<35 y: 45(16.07%), 35-37 y: 67 (23.92%), 38-40 y: 122 (43.57%), >40 y: 46(16.42%), E2 on day of hcg (pg/ml) 1636 (1548.3-1725.4) (<35 y 1404 ± 1244, 35-37 y: 1479 ± 1074, 37-40 y:1209 ± 911, >40 y: 905 ± 674, Number of oocyte obtained/patient age: < 3: 87 (< 35 y: 9, 35-37 y:19, 38-40 y: 37, >40: 22), 4-6: 87 (<35 y: 15, 35-37 y: 17, 38-40 y: 43, >40 y: 22), 7-10: 57(<35 y: 11, 35-37 y: 15, 38-40 y: 21, >40: 10), > 11: 49 (<35 y: 10, 35-37 y: 15, 38-40 y: 21, >40 y: 2).This retrospective analysis, shows that the 67.5% of patients were between 35 and 40 years old, even more the 43,57% were between 38 and 40 years old.The 62.14% of patient vitrified 6 oocytes or less, in patients younger than 35 years old 53,3% vitrified 6 or less oocytes (<3:9 patients,4-6:15 patients, 7-10:11 patients, >11:10 patients).The probability of having a baby according to N*of oocytes used is: oocytes/CLBR(95%CI) 5: 14.7 (6.2-23.1), 8: 30.9 (19.1-42.7), 10: 37.9 (24.9-51.0)

Limitations, reasons for caution: This is a retrospective study. Although the size of our sample is small, our data provides useful information regarding convenient reproductive counseling, even more when the amount of oocytes obtained is less than expected.(empty follicles,poor response) with normal values of serum estradiol at the day of HCG.

Wider implications of the findings: Oocyte vitrification is an efficient tool which can be helpful In women who delay child bearing,we should inform women about their specific probabilities according to their age and number of oocytes at vitrification,is important to counsel women that elective oocyte cryopreservation can increase future reproductive chances but cannot guarantee success.

Trial registration number: Not applicable.

P-503 Optimising ovarian stimulation protocol for oncology patients

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Study question: Do timing of start and dose of gonadotrophin stimulation effect oocyte yield in oncology patients.

Summary answer: The timing of the start of stimulation in menstrual cycles does not appear to affect oocyte yield but the starting dose does.

What is known already: Oocyte number is a good predictor of live birth when using cryo-preserved oocytes. Time is of essence in oncology patients as the ovarian stimulation cycle needs to be completed swiftly to enable oncology treatment to commence without delay. This may make conventional early follicular phase stimulations difficult or incur time delay. There is concern about oocyte yield in late follicular or luteal phase stimultion starts as it may affect quality and number of eggs. There is also concern about using high dose of gonadotrophin due to risk of OHSS.

Study design, size, duration: A retrospective analysis was carried out in 78 patients having 78 cycles having ovarian stimulation and oocyte collection for fertility preservation prior to oncological treatment.

The study period was 2014-2017

Participants/materials, setting, methods: An analysis of 78 cycles was performed.

Demographic characteristics like age and BMI were evaluated.

Ovarian reserve was assessed with AMH and AFC.

Oncology diagnosis was taken.

Start time of stimulation, dose pf gonadotrophin used and number of oocytes collefted were evaluated.

Any adverse events like OHSS or haemorrhage was noted.

Duration of treatment to oocyte collection was assessed.

All patients had antagonist cycles with agonist trigger.

The study was perfomed in a tertiary care reproductive medicine unit.

Main results and the role of chance: There were 8 late follicular and luteal phase starts and 70 early follicular starts. An average of 13.75 (9-17) oocytes were collected in the late follicular and luteal phase with an average of 10.25 metaphase 2 eggs. The average oocyte yield in the early follicular phase start was 12.45 (2-31).

AMH of <5pmol/l was associated with a lower egg yield as expected (7.25). AMH of <1 were noted in some of the pill users which did not affect oocyte yield.

An analysis of oocyte yield with AMH>15pmol/l revealed fewer than 10 eggs when start dose of gonadotrophin was 150units. Average oocyte yield was 15 (11-30) with starting dose of 225-300 units in the same group. In an analysis of 12 cases with an oocyte yield of <5neggs, 5 were in young women with AMH >20pmol/l started on 150 units of gonadotrophin. There was no significant difference in oocyte yield between different diagnosis and no significant difference in those patients who came to freeze oocytes after chemotherapy. There were no cases with OHSS. Patients did not have to delay oncological treatment in any case.

The late follicular/luteal phase start group was small and the absence of effect could be due to chance.

Limitations, reasons for caution: The retrospective nature of the study and low numbers are limitations.

Luteal phase starts need more numbers.

Wider implications of the findings: AMH is not lways reliabe as a measure of ovarian reserve prior to fertility preservation.

Conventional stimulation protocols are not always suitable for oncology patients.

Trial registration number: not applicable.

P-504 Long term cumulative rate of use of frozen sperm in cancer patients

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Study question: What is the long-term cumulative use rate of frozen sperm in men who storage the semen before cancer therapies?

Summary answer: The cumulative usage rate is 11%.

What is known already: Cancer therapies are frequently aggressive and unwanted side effects are common, including threats to fertility. Affected patients should be aware of the risk of permanent infertility and on the possible options for fertility preservation. Currently, the only way to efficiently preserve the reproductive potential in men remains sperm cryopreservation. However, the economical validity of this intervention has been questioned because of the low rate of use. A recent review reported a mean percentage of use of only 8%. Unfortunately, evidence on the long-term cumulative rate of use, which is far more significant than the crude rate, is scanty.

Study design, size, duration: The analysis includes all males cancer patients who cryopreserved semen from 1986 to 2009 at the Infertility Unit of the Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico of Milan. Information were obtained from patients' charts. Moreover, men with incomplete information were actively contacted by phone and interviewed using a standardized questionnaire.

Participants/materials, setting, methods: The analysis focussed on the following information: age at semen preservation, semen parameters at the time of preservation, oncological diagnosis, use of frozen samples and time of use, duration of follow-up, disposal of samples and reasons for their removal.

Main results and the role of chance: We included 1524 cancer patients cryopreserving at least one sperm sample. Most frequent classes of malignancies among patients were testicular cancers (n = 660, 43%) and lymphatic-haematological cancers (n = 651, 43%). Mean age at the time of sperm banking was 30.0 ± 7.5 years (range, 14–66 years) and the median time from storage to use or to the last follow-up was 12 years (range 0–28 years). Out of 1524 patients, 137 (9%) used their cryopreserved sperm sample to perform ART. The cumulative rate of use for the whole cohort at 10 and 20 years follow-up was 9% and 11%, respectively. We then evaluated predictive factors of use. The Cox regression analysis identified two factors that were significantly associated with the chances of use: 1) age at the time of cryostorage (B = 1.033, 95%CI: 1.011-1.056, p = 0.003), and 2) cancer diagnosis (haematological versus testicular tumours B = 1.808, 95%CI: 1.234-2.648, p = 0.002; other diagnoses versus testicular B = 1.675, 95%CI: 0.996-2.818, p = 0.052).

Limitations, reasons for caution: Duration of follow-up is insufficient to draw information regarding young adults. Distribution during the duration of the program is unlikely to be uniform because interest in fertility preservation has grown during the last two decades.

Wider implications of the findings: Using these data, the cumulative rate of men banking and subsequently using their semen is 11%. On this basis, one should claim in depth economical analyses to investigate the economical validity of sperm banking programs in cancer male patients.

Trial registration number: Not applicable.

P-505 Comparison of conventional and random start ovarian stimulation for fertility preservation in oncological patients

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Study question: To compare the number of retrieved oocytes in oncological patients, when ovarian stimulation started in early follicular, late follicular or luteal phase of menstrual cycle.

Summary answer: The number of retrieved oocytes was similar when the ovarian stimulation was initiated in early follicular, late follicular or luteal phase of the cycle.

What is known already: Chemotherapy and pelvic irradiation for malignant or autoimmune disease have improved survival of patients but may lead to premature ovarian failure and infertility. Therefore, young oncological patients should be referred to Reproductive medicine subspecialist for counselling and procedures to preserve their chances to be parents with their own genetic material. With the usage of short antagonist protocols the ovarian stimulation requires no important delay in systemic oncologic treatment.

Study design, size, duration: We included all women who were referred to the Department of Human Reproduction of the Division of Obstetrics and Gynecology of the University Medical Centre Ljubljana for fertility preservation before gonadotoxic treatment from January 2013 to the end of December 2017.

Participants/materials, setting, methods: A total of 127 female patients were referred to our Department for counselling in this period. Our Department is the only one offering genetic material preservation for oncological patients in Slovenia. If the time available for stimulation was > 1 month, the ovarian stimulation started in early follicular phase, otherwise it was initiated immediately or ovarian tissue cryopreservation was offered.

Main results and the role of chance: Out of 127 patients, 54 (42.5%) had breast cancer, 36 (28.3%) had lymphoma, 7 (5.5%) had leukaemia, 7 (5.5%) had borderline ovarian tumour, 6 (4.7%) had autoimmune disease, 5 (3.9%) had sarcomas, 3 (2.4%) had colon cancer and 9 (7%) had other rear malignant disease such as brain tumour, disgerminomas and vaginal cancer. The median age of included women was 29 ± 2.3 years (range 3 to 43 years). In 41 (31.5%) patients we did not preserve any material due to variety of reasons. In 67 (52.7%) patients oocytes were vitrified, in 14 (11%) patients embryos were vitrified, in 3 (2.4%) patients oocytes and

embryos were vitrified, in 6 (4.7%) patients ovarian tissue was cryopreserved. When the stimulation started in early follicular phase (N = 58), the mean number of retrieved oocytes was 10 (range 1-31 oocytes); 8.56 oocytes were mature at aspiration. With the start in late follicular phase (N = 11) the mean number of oocytes was 11.3 (range 1-35 oocytes); 10.2 were mature at aspiration. With the start in luteal phase (N = 14) the mean number of oocytes was 12.6 (range 1-30 oocytes); 9.6 oocytes were mature at aspiration.

Limitations, reasons for caution: The majority of patients had breast cancer and time interval to the initiation of systemic treatment was 6 to 8 weeks. Therefore, majority of patients had conventional start of stimulation in early follicular phase. The number of patients in other two groups is quite small, so further studies are needed.

Wider implications of the findings: It has already been published, that random start stimulation can be used in oncological patients with good results. Our study also showed that numbers of retrieved and numbers of mature oocytes were similar when the ovarian stimulation was started in early follicular, late follicular or luteal phase of menstrual cycle.

Trial registration number: not applicable.

P-506 Does oxygen tension influence in vitro maturation of human oocytes in a fertility preservation program? Preliminary results of a prospective auto-controlled study

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Study question: Are human oocyte (i) maturation rate (MR) and (ii) morphological score enhanced by low oxygen (O_2) tension applied during *in vitro* maturation (IVM) cycles?

Summary answer: Oocyte MR was similar whatever the O_2 tension applied during IVM, whereas morphologic Total Oocyte Score (TOS) was significantly higher under low oxygen tension.

What is known already: O_2 tension within the mammalian female genital tract is low, (2-8%), contrary to culture conditions applied in most of Assisted Reproductive Technology laboratories (20%O_2). Hypoxia might reduce oxidative stress commonly triggered by atmospheric O_2 tension, further allowing a more physiological process. Besides, the latest recommendations state that embryos should be cultured under hypoxia. However, several randomized studies in mammals have reported controversial findings concerning *in vitro* MR and subsequent embryo development when oocytes were matured under either 5% O_2 or atmospheric conditions. To date, no study has ever evaluated the impact of O_2 tension on human oocyte matured *in vitro*.

Study design, size, duration: prospective, observational, monocentric, auto-controlled study on sibling oocytes was performed from November 2016 to July 2017. Fifty-nine patients candidates for fertility preservation (FP) using vitrification of metaphase 2 (M2) oocytes from IVM cycles were included when at least 2 cumulus-oocyte complexes (COCs) were retrieved. Each COC cohort was randomly split into two equal groups: group 1=culture under 20% O_2; and group 2=culture under 5% O_2.

Participants/materials, setting, methods: COCs were incubated for 48 h using an appropriate media, in similar benchtop incubators (G-210, K-Systems®), either under 5 or 20%O_2. After 24 h and 48 h of culture, every oocyte was assessed for maturity and morphology, using 6 parameters (shape, size, ooplasm, perivitelline space, zona pellucida and polar body characteristics) each graded as -1, 0 or +1, giving a TOS ranging from -6 to +6 (Lazzaroni-Tealdi et al., 2015). MR and TOS were compared using paired-sample analysis.

Main results and the role of chance: We analyzed our preliminary data. Briefly, patients' mean age was 30.6 years. Their mean serum AMH levels and antral follicle count were 3.62 ng/mL and 33.2 follicles, respectively. On average, 13.13 COCs per cycle were retrieved, leading to 7.5 M2 oocytes vitrified (global MR = 58.01% (449 M2/774 COCs)). Respectively 383 and 391 COCs were included in groups 1 and 2. In group 1 (20% O_2), MR after 48 h of culture yielded 56.14% of M2 oocytes (215/383), which was comparable to MR achieved in group 2 (5% O_2) (59.85%; 234/391; p = 0.21). Considering M2 oocyte morphology, the mean TOS was significantly higher under low O_2 tension (3.44 per oocyte in group 2 *versus* 3.16 in group 1; p = 0.014). Finally, a subgroup analysis outlined a significantly higher number of high-grade oocytes (TOS ≥ 4) in group 2 than in group 1 (2.24 *versus* 1.64, respectively; p = 0.032).

Limitations, reasons for caution: This study, still in progress, needs to be continued to clarify the impact of O_2 tension on human IVM oocyte and their morphology. Furthermore, analysis of subsequent warming cycles would complete these data, in terms of survival, fertilization rates, embryo quality and clinical outcomes according to IVM culture conditions.

Wider implications of the findings: Although these preliminary results failed to highlight any increase in MR under hypoxia, oocyte morphology has been improved. Therefore, further analysis is needed to investigate whether oocyte maturation under 5% O_2 increases the chances of pregnancy after thawing.

Trial registration number: N° ID RCB: **2017-A02264-49**

P-507 Spermatogonial quantities in untreated prepubertal cancer patients are comparable to normative prepubertal spermatogonial values

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Study question: Does cancer affect spermatogonial quantity in the the testes of prepubertal boys?

Summary answer: Spermatogonial quantities in prepubertal boys with hematologic, solid cerebral, or solid extra-cerebral cancer were comparable to those in healthy prepubertal boys.

What is known already: The incidence of pretreatment azoospermia in adult men diagnosed with cancer is known to be ten-fold higher compared to the general population, with the highest rates among hematologic and testicular malignancies (9-13% and 10-15%, respectively). It has been hypothesized, that such fertility impairments are caused by disease-related effects on the body, including hormone and metabolic imbalances, and immune response. However, no studies have thus far investigated the effect of different types of cancers on the numbers of germ cells in prepubertal boys. If numbers are reduced, these cancer survivors may face sub-/infertility without the possibility to use fertility preservation treatments.

Study design, size, duration: In this study, we collected data on cancer diagnosis in 83 prepubertal boys, who underwent testicular biopsy for fertility preservation prior to cancer treatment between 2011 and 2017. We classified their diagnoses into three cancer categories (hematologic, solid cerebral and solid extra-cerebral) and examined histology of the testicular biopsies by immunohistochemistry. Patients with the history of testicular torsion or cryptorchidism, able to ejaculate spermatozoa, or with malignancies in the testes were excluded from the study.

Participants/materials, setting, methods: Patient data were identified through records of the Academic Medical Center in Amsterdam, the Netherlands, and included patient age at the time of testicular biopsy and cancer diagnosis. A small part of the testicular biopsies were fixed, paraffin embedded, and labelled for MAGE-A4, a biomarker for human spermatogonia. The amount of spermatogonia per tubular cross-section (S/T) was determined using bright-field microscopy and compared with the normative reference values. Polynomial regression was used for data analysis.

Main results and the role of chance: In the analyzed cohort of 83 prepubertal boys diagnosed with cancer, median age at diagnosis was 9 years (range 0.5-16 y). Altogether, 15.7%, 27.7%, and 56.6% of boys had hematologic, solid cerebral, and solid extra-cerebral malignancy, respectively. The average S/T in the total cohort was 4.4 (SD = 5.29), with 6.59 (SD = 7.34) in hematologic cancer, 3.10 (SD = 4.54) in solid cerebral cancer, and 4.43 (SD = 4.87) in solid extra-cerebral cancer patients. No significant differences or associations between S/T and cancer categories were found. The amount of spermatogonia per tubular cross-section in all three patient groups was within the range of the normative reference values for healthy prepubertal boys.

Limitations, reasons for caution: These results should be interpreted in the context of the small sample size per cancer category, as well as the high variance between patients' general health condition within each group.

Wider implications of the findings: Our data suggest, that in each cancer category the size of the spermatogonial pool in prepubertal boys was comparable to the normative values. Therefore, all pediatric cancer patients who might face high risk of spermatogonial depletion due to gonadotoxic treatment can be referred for fertility preservation.

Trial registration number: Not applicable.

P-508 New miRNAs to evaluate ovarian reserve in patients with breast cancer

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Study question: The aim of this study is to identify a new non invasive strategy to evaluate the ovarian reserve in patients with breast cancer.

Summary answer: We found 4 deregulated miRNAs as a potential target for fertility preservation in patients with breast cancer.

What is known already: Anti-Müllerian hormone (AMH) is a reliable quantitative biomarker of the ovarian reserve, but not after chemotherapy treatments. Indeed, young breast cancer patients have significantly lower AMH after chemotherapy than age-matched controls. In addition, AMH solely is not able to determine the quality and the fertilization capacity of oocytes containing in the resting follicles from patients who wish to conceive after breast cancer treatment. Indeed, the necessity to quantify the ovarian reserve in patients after chemotherapy treatment remains a major medical issue.

Study design, size, duration: 21 serum samples were obtained from three breast cancer patients aged between 35 and 37 years old (mean age±SD: 35.7 ± 1.1 years) with chemotherapy treatment. All patients were prospectively recruited before initiation of the chemotherapy.

Participants/materials, setting, methods: Serum miRNAs and AMH quantification were performed at baseline, 15 days after and before the first chemotherapy treatment and every 3 months after the end of chemotherapy until time + 18 months.

We used Serum/Plasma Focus PCR MicroRNA Panel (Exiqon) that allows to study a total of 178 miRNAs.

Main results and the role of chance: Our preliminary results revealed that four miRNAs (miR144-5p, miR186-5p, miR34-5p and miR21-5p) were overexpressed during chemotherapy treatment compared with both before and after the chemotherapy treatment although these differences were not significant. Meanwhile, serum AMH concentration dramatically decreased 6 months after chemotherapy treatment in breast cancer patients compared at baseline measure (3 ± 0.03 *vs* 10.3 ± 3.5 ng/ml), and thus, until 18 months post-treatment. One of these miRNAs, miR 21-5p was suggested to affect follicular development. These four miRNAs could be a potential target for fertility preservation in patients with breast cancer.

Limitations, reasons for caution: This result is issue from few samples and the study is in progress.

Wider implications of the findings: This finding opens new perspectives in the patient care management of patients with breast cancer.

Trial registration number: CHACRY-1501.

P-509 A follow-up survey on the reproductive intentions and experiences of women who underwent "social freezing" or elective oocyte cryopreservation

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Study question: What are the reproductive attitudes and experiences in women who previously underwent elective oocyte cryopreservation for anticipated gamete exhaustion (AGE)?

Summary answer: Women who previously underwent elective oocyte cryopreservation perceive fertility preservation as a useful procedure to enhance the probability of motherhood.

What is known already: Elective oocyte cryopreservation is available to women who prefer to or have to defer and delay motherhood until the right time or the right partner arrives. A previous survey has shown that women are highly satisfied with their decision to cryopreserve oocytes. However, follow-up data documenting their reproductive outcomes, and their beliefs and expectations regarding the utilisation of cryopreserved oocytes are limited.

Study design, size, duration: Cross-sectional Survey.

Participants/materials, setting, methods: Women who had stored oocytes in a tertiary university-based fertility clinic between January 2009 and September 2016 were invited to complete an anonymous questionnaire about their reproductive intentions and expériences.

Main results and the role of chance: Of the 342 potential respondents, 138 (40.3%) returned completed questionnaires. The average age of respondents was 40.2 y and the reported age at first cycle of cryopreservation was 35.8 y. Eighty-three % had been single when they underwent oocyte cryopreservation while 44% of respondents still were. Almost two-thirds (65%) anticipated to use their cryopreserved oocytes and were planning to do so before the age of 45 y (range 38-55 y). Sixty-six respondents (48%) had already tried to achieve a pregnancy, of which 69% had succeeded (79% spontaneously and 21% using their cryopreserved oocytes). Sixty-five percent would consider adoption, whereas only 31,4% would turn to oocyte donation if they failed to become pregnant with their vitrified oocytes. When asked if they would ever consider to remain childless 50.4% answered yes.

Limitations, reasons for caution: The results of this survey may have been biased by the limited response rate and potential misinterpretation of questions. National and international registries of larger numbers of women who undergo elective oocyte cryopreservation as a prevention for age-related fertility decline would offer the best prospect of generating wide-reaching qualitative data.

Wider implications of the findings: After a mean follow-up of four years, "past social freezers" report an overall high degree of satisfaction with their previous decision. Even if only a subset achieved a pregnancy using these vitrified oocytes so far, the majority of respondents still have the intention to use their oocytes to realise motherhood.

Trial registration number: Not applicable.

P-510 Professionals' barriers in female oncofertility care and strategies for improvement

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Study question: What are Dutch healthcare professionals' barriers and strategies for improvement in female oncofertility care?

Summary answer: Professionals particularly perceive barriers in knowledge, attitude and organization towards fertility preservation and are open to improve oncofertility care.

What is known already: One of the most important undesirable side effects of cancer treatment in young women is the potential loss of fertility. Guidelines recommend to discuss the potential loss of fertility with all female cancer patients and, if desired, offer a referral to and counseling by a fertility specialist. Unfortunately, studies have shown that not all patients receive information on their fertility risks and options to preserve fertility and only a small proportion of these women is referred. A study on barriers for discussing fertility preservation and appropriate referral in a country with structural reimbursement has not yet been performed.

Study design, size, duration: Semi-structured in depth interviews were conducted with a total of 24 healthcare professionals. Thereafter, a selection of improvement strategies was performed with an expert panel consisting of patients and healthcare professionals to overcome these barriers.

Participants/materials, setting, methods: Oncological professionals working in Dutch female oncofertility care were recruited out of the three Dutch expertise hospitals for female fertility preservation and their affiliated hospitals. In the Dutch setting financial aspects do not play a role in oncofertility care. Professionals were asked for their barriers and improvement suggestions in individual interviews. A meeting with an expert panel was held to select improvement strategies and reach consensus on an oncofertility program.

Main results and the role of chance: In total 31 barriers and 23 facilitators were identified. Barriers have been revealed distributed over three domains; patient, professional and organization. On the patients' domain a focus on surviving cancer and starting treatment and not a focus on their fertility have been identified as barriers. On the professionals' domain a lack of awareness and a lack of knowledge, and on the organization domain no written information available, no time and FP is not discussed at multidisciplinary meetings have been identified as main barriers. The expert panel selected different strategies to improve oncofertility care. These include provision of written and online information, education of professionals, reminders for professionals, fertility should be a standard discussion item on the agenda of multidisciplinary oncology meetings and specialized oncology nurses should play a role in informing patients in oncofertility care and patient navigators at the fertility department to facilitate referral and counseling.

Limitations, reasons for caution: Selection bias could have occurred because it is likely that only professionals who are interested in oncofertility care were willing to participate. However this means that we underestimated the barriers.

Wider implications of the findings: This study was a preview for a multifaceted oncofertility program which should be developed in order to increase adherence to the national clinical guideline.

Trial registration number: Not applicable.

P-511 IVM before vitrification induces alterations of maturation rates and deleterious effects on actin and tubulin architecture of human oocytes

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Study question: Is it better to vitrify oocytes before or after in vitro maturation?

Summary answer: Since we observed alterations of maturation and deleterious effects on actin and tubulin architecture in oocytes vitrified before IVM, IVM should be performed before vitrification.

What is known already: Human oocyte cryopreservation is routinely used for fertility preservation of women who will be exposed to gonadotoxic treatments. After ovarian stimulation, matured oocytes are vitrified. However, this strategy cannot always be used, particularly for hormone-sensitive cancer or when ovarian stimulation is not possible. An approach including immature oocytes and in vitro maturation (IVM) could be considered in these cases. While some qualitative analyses of oocytes vitrified before or after IVM suggest that vitrification should be performed after IVM, little is known about vitrification effects on actin and tubulin cytoskeleton and on maturation of human oocytes.

Study design, size, duration: To answer to this question, we performed quantitative analyses comparing matured oocytes from three different groups: (i) oocytes vitrified before IVM, (ii) oocytes vitrified after IVM, and (iii) fresh immature oocytes. Non-vitrified (fresh) in vitro matured oocytes were used as controls. Kinetic of maturation and subcellular structures were analysed during maturation and in matured oocytes. 160 immature oocytes were collected from women without ovulation troubles from March 2017 to January 2018.

Participants/materials, setting, methods: A total of 160 immature oocytes were collected from 63 patients (≤37 years old, without ovulatory diseases) who underwent ICSI. Oocytes were matured in vitro with IVM medium (Origio) for 36 hours and vitrified in a closed system, Rapidi with RapidVit Omni and RapidWarm Omni (Vitrolife). Kinetics of maturation were analysed by Primovision™ (Vitrolife). The actin and spindle organisation were studied by immunostaining techniques, confocal microscopy (Leica SP6), and quantitative analyses (Imaris® and Fiji).

Main results and the role of chance: While the kinetics of maturation were similar for oocytes vitrified before or after IVM (17:27 before IVM versus 17:53 after IVM; $p>0.05$; h:min), a lower rate of oocytes vitrified before IVM extruded polar body (78% after IVM versus 56% before IVM, $p = 0.003$). Then, the cytoskeletal organisation of matured oocytes vitrified before or after IVM was quantitatively analysed. Using biophysical methods, our analysis revealed that spindle length and shape were significantly altered in oocytes vitrified before IVM, while oocytes vitrified after IVM appeared normal compared with fresh oocytes. By high-resolution imaging, we characterised for the first time the actin organization in human matured oocytes. Actin exhibits a complex organisation with a dense cytoplasmic network and cortical actin. Cortical actin thickness and actin filament lengths were measured in both conditions. Our results showed that the cortical actin thickness was significantly higher in oocytes vitrified before IVM compared with fresh oocytes; while it was not affected in oocytes vitrified after IVM. Analysis of actin filaments indicated that the average of filament lengths diminished similarly in both conditions compared with fresh oocytes. All these alterations observed in oocytes vitrified before IVM suggests that IVM should be performed before vitrification.

Limitations, reasons for caution: Since, we used oocytes from women without cancer; our data should be completed with an analysis of the effect of vitrification combined with IVM in oocytes from women diagnosed with a cancer. Moreover, homologous chromosome segregation during the first meiotic division should be investigated in order to detect aneuploidy.

Wider implications of the findings: Our preliminary data shows clearly that IVM should be performed before vitrification. After chromosome segregation analysis and validation on immature ovocytes from women with cancer, the protocol that we provide here, will be applied, with immature oocytes for fertility preservation.

Trial registration number: Clinical trial registration: NCT03416400.

P-512 Assessment of a sensitive and specific method to detect Ewing sarcoma minimal residual disease in testicular and ovarian tissue by RT-qPCR

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Study question: The aim of this study was to assess the sensitivity and specificity method to detect Ewing sarcoma minimal residual disease in human germinal tissue.

Summary answer: The detection of *EWS-FLI1* (RT-qPCR) enables sensitive and specific detection of Ewing sarcoma cells which may be contained in frozen germinal tissues for fertility preservation.

What is known already: Ewing sarcoma is a frequent solid tumor in children with high metastatic potential and high risk of infertility due to the potential toxic treatments for reproductive functions. Since the high survival rate of patients and deleterious effects of intensive chemotherapy, the cryopreservation of ovarian tissue or testicular tissue is recommended. However the risk to reintroduce cancer cells should be evaluated to ensure safety of fertility restoration with these tissues. The assessment of this minimal residual disease could be performed with frozen tissues by slow freezing or immediately after tissue collections by snap freezing.

Study design, size, duration: Ovarian tissues (OT) and testicular tissues (TT) were frozen (slow and snap freezing). After thawing, each fragment was contaminated with increasing amount of human Ewing sarcoma cells (RD-ES). The expression of *EWS-FLI1* mRNA was measured. To evaluate the invasive potential of RD-ES cells, cocultures (OT or TT / RD-ES cells) were performed. OT and TT from metastatic patients were analyzed once the high sensitivity and specificity of the detection method by RT-qPCR was demonstrated.

Participants/materials, setting, methods: OT: women with ovarian cyst (n = 12). TT: Non-obstructive azoospermia men (n = 14).

Tissues were frozen (slow/snap freezing). After thawing, they were contaminated with RD-ES cells (10, 100, 1000 cells). The expression of *EWS-FLI1* was quantified by RT-qPCR. The invasive potential of RD-ES cells was evaluated by cocultures and RT-qPCR, FISH and immunochemistry. Minimal residual disease was assessed in OT and TT of children with Ewing sarcoma once the efficiency of our *in vitro* model was demonstrated.

Main results and the role of chance: Yields of RNA extraction from OT (median weight: 39.2 mg [15.2 – 62.0]) and TT (median weight: 28.8 mg [16.6 – 48.0]) did not differ between slow or snap freezing (p = 0.54) method. The expression of *EWS-FLI1* transcript was positive in OT and TT samples contaminated with 10, 100 and 1000 RD-ES cells and was negative in uncontaminated samples. There was a strong correlation between the amount of RD-ES cells and the expression of *EWS-FLI1 mRNA* (OT: r = 0.93 and TT: r = 0.96 with p < 0.001) whatever freezing method. The sensitivity and specificity to detect the expression of *EWS-FLI1* transcript by our method was respectively 97% IC 95% [0.92-1.00] for OT and 99% IC 95% [0.98-1.00] for TT. After cocultures, we observed in germinal tissues the presence of Ewing cells by standard histology (HES coloration) and by immunohistochemistry with a diffuse nuclear pattern of ERG staining. Moreover, the expression of *EWS-FLI1* mRNA (qPCR) and the expression of t(11;22)(FISH) were clearly shown in the germinal tissues after cocultures. In OT and TT from children with Ewing sarcoma, we did not detect the expression of *EWS-FLI1* mRNA and did not observe Ewing cells by the histology methods.

Limitations, reasons for caution: The assessment of the method was performed by an *in vitro* model. An *in vivo* model by xenografting could be necessary to ensure the accuracy.

Wider implications of the findings: By our study, we demonstrated the high sensitivity and specificity of RT-qPCR to detect Ewing sarcoma minimal residual disease in human testicular tissue (TT) and ovarian tissue frozen by snap or slow freezing for fertility preservation. This is the first study to assess this measurement in TT.

Trial registration number: Clinical trial: NCT 02400970.

P-513 Texture profile analysis reveals a stiffer ovarian cortex after testosterone therapy

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Study question: Does prolonged testosterone administration induce stiffness in the ovarian cortex?

Summary answer: An increased cortical stiffness in the most outer part of the ovarian cortex could be documented following prolonged testosterone administration in trans men.

What is known already: The importance of ovarian stroma in the development and maturation of follicles has recently gained attention. The ovarian stroma does not only contribute to the structural support of the growing follicle, but also to the bidirectional paracrine communication between the oocyte and surrounding somatic cells, thereby influencing folliculogenesis. An aberrant extracellular matrix has been described in ovaries of patients with polycystic ovary syndrome (PCOS) where a more rigid structural environment, possibly induced by endogenous testosterone, impairs normal folliculogenesis. In this context, the effect of prolonged administration of testosterone on the ovarian cortex in female to male transgender persons was studied.

Study design, size, duration: Texture profile analysis (TPA) was performed on ovarian cortex of oncological (N = 3) and transgender patients (N = 3). A total of 34 frozen-thawed cortical strips (5 × 5 mm,) were subjected to TPA in order to measure stiffness, hardness, cohesiveness and springiness of the ovarian cortex (LRXplus universal testing system). Statistical analysis was performed using repeated measurements mixed models and the Spearman's rank order correlation test (IBM SPSS Statistics 23).

Participants/materials, setting, methods: Ovarian tissue was obtained of 3 oncology patients (25.00 + 3.61 years) (11 strips) and 3 transgender men (22.67 + 2.08 years) (23 strips). Oncological indications for fertility preservation comprised acute lymphoblastic lymphoma, breast cancer and acute myeloid leukemia. All trans men received intramuscular testosterone undecanoate 1000 mg every 12 weeks during 80.72 + 32.24 weeks. Cortical strips were compressed over a distance of 50% of the original sample thickness during 2 cycles.

Main results and the role of chance: A typical TPA double compression curve of ovarian cortex is characterized by a slower upstroke in the toe region, where increasing compression resulted in a steeper upstroke prior to the peak. Release of the maximum compression causes an immediate drop in the TPA curve. Typically a bigger first curve was noted, when compared to the second curve (Peak 2 < Peak 1), reflecting an incomplete recovery of the tissue post compression, probably as a result from the breaking points seen in the first upstroke. There was no difference in fragment thickness comparing tissue fragments originating from transgender (1.43 + 0.46 mm) and oncology (1.38 + 0.38 mm) patients (p = 0.826). The most outer part of cortex fragments of ovaries originating from transgender persons (fragments < 1.4 mm; N = 10) were significantly stiffer compared to the superficial area of ovarian cortex derived from oncology patients (N = 7) (p = 0.036).

Limitations, reasons for caution: TPA is applied in biomaterial industry to determine textural properties of foods, gels and pharmaceutical matrices. This is the first application of TPA in ovarian tissue, requiring optimization of the compression protocol. Future experiences in TPA on human tissue samples might contribute to optimization and lead to a standardized setup.

Wider implications of the findings: If increased cortical stiffness impairs folliculogenesis, it is advisable to interrupt testosterone prior to fertility preservation. Also, if aiming for *in vitro* follicle activation through Hippo signaling disruption, an adapted technique for tissue preparation is necessary. Last, these TPA parameters enable to optimize biomaterial characteristics in artificial ovary engineering.

Trial registration number: UZ Ghent reference: 2012/780 – Belgian registration number: B670201 21 5468

P-514 Controlled ovarian stimulation applied before ovarian cortex cryopreservation does not impact tissue histological aspect

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Study question: Is there an impact of controlled ovarian stimulation (COS) on the histological aspect of ovarian tissue cryopreserved for fertility preservation (FP)?

Summary answer: COS applied before ovarian tissue cryopreservation (OTC) allows optimizing the number of metaphase 2 oocytes vitrified when compared with IVM, without detrimental histological effect.

What is known already: Vitrification of in vitro matured oocytes (IVM) has been proposed in combination with OTC as a method for urgent female fertility preservation. However, both IVM and ovarian tissue transplantation remains experimental contrary to oocytes vitrification following COS. When COS is feasible in contexts of highly gonadotoxic treatment, vitrification of in vivo matured oocytes may be considered before OTC. However, the impact of ovarian stimulation on histological aspects of the ovary has never been described.

Study design, size, duration: This observational retrospective study conducted from 2009 to 2016 aimed to compare the histological aspect of ovarian tissue removed on the day of oocyte retrieval performed on stimulated (group 1, n = 13) or unstimulated (group 2, n = 12) ovaries. The number of metaphase 2 oocytes vitrified was also analyzed in both groups.

Participants/materials, setting, methods: Twenty-five cancer patients referred in our center for OTC before highly gonadotoxic chemotherapy were prospectively studied. Ovarian tissue was removed laparoscopically immediately after oocyte retrieval. The latter was performed after COS when feasible or from unstimulated small antral follicles. A sample of ovarian tissue was systematically addressed for pathological analysis.

Main results and the role of chance: Patient's age was similar between groups 1 and 2 (24.0 ± 6.2 versus 27.4 ± 6.3 respectively; p = 0.18). Histological aspects of ovarian tissue as well as the number of primordial follicles per mm^2 were similar between both groups. The total number of cumulus-oocyte complexes (COC) obtained in group 1 was 155 (mean 11.9 ± 5.7) and 122 (mean 8.6 ± 7.8) were vitrified. In group 2, 103 COCs were recovered (mean 9.4 ± 4.4), leading to 66 vitrified oocytes (5.5 ± 6.2) after an IVM rate of 64%.

Limitations, reasons for caution: Although our preliminary results are reassuring, histological data after thawing and further outcome after transplantation are lacking.

Wider implications of the findings: The competence of frozen-thawed ovarian tissue removed following COS constitutes an important issue. If it remains intact, oocytes vitrification after ovarian stimulation will have to be systematically considered before OTC, since this method is dramatically superior to IVM in terms of chances of pregnancy.

Trial registration number: Not required.

P-515 Dual In Vitro Maturation (Dual-IVM): an innovative fertility preservation strategy to increase the total number of cryopreserved oocytes when ovarian stimulation is unfeasible

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Study question: Is it feasible to perform within a short time frame two successive immature oocyte retrievals for fertility preservation (FP), especially during the same menstrual cycle?

Summary answer: Dual-IVM can be performed within a few days of interval to increase the total number of mature oocytes cryopreserved for urgent FP.

What is known already: Oocyte vitrification after IVM may constitute an alternative option for FP when controlled ovarian stimulation (COS) is unfeasible. Recent data reported that 8 to 20 cryopreserved oocytes are required to achieve reasonable success in healthy women. Since the competence of IVM oocytes is unknown, it is likely that the number of oocytes needed to obtain a pregnancy would be higher than that after COS. Combination with OTC has been proposed as an option to improve FP effectiveness. However, in women rejecting OTC, the strategy to be adopted for increasing the total number of cryopreserved oocytes can be questioned.

Study design, size, duration: Eighteen breast cancer patients, candidates for FP and presenting contraindication to COS for oncologic reasons, were prospectively included. All were offered ovarian tissue cryopreservation (OTC) combined with oocyte vitrification following IVM, and chose IVM only. They were systematically advised on reconsidering OTC and on the possibility of a second IVM cycle (IVM2), if these procedures did not require chemotherapy postponement.

Participants/materials, setting, methods: We hereby report a case-series of 18 breast cancer patients having undergone 2 successive cycles of IVM before initiation of chemotherapy. Cumulus-oocyte complexes (COC) were recovered under ultrasound guidance, and incubated 24-48 hours for IVM. Metaphase II oocytes were vitrified. When OTC was associated with IVM2, transvaginal oocyte retrieval was performed prior to laparoscopy. Outcomes of first and second IVM cycles (IVM1 and IVM2) were compared using Wilcoxon matched-paired signed rank test.

Main results and the role of chance: Median time interval between both IVM cycles was 24.5 days (ranging from 3 to 92 days). The mean number of COCs retrieved did not differ significantly between IVM1 and IVM2 (6.7 ± 2.9 *vs.* 7.1 ± 5.4 oocytes, respectively, NS). Maturation rates were not different (62 ± 23 % *vs.* 51 ± 34%, NS), leading to a similar number of metaphase II oocytes cryopreserved during IVM1 and IVM2 cycles (3.8 ± 2.6 *vs.* 4.0 ± 4.2, respectively, NS). IVM2 outcomes did not differ when it was performed during the same (n = 11/18) or different (n = 7/18) menstrual cycle than IVM1. No complication was reported during IVM1 or IVM2. Finally, performing IVM1 + IVM2 significantly increased the total number of oocytes vitrified *per* patient when compared with IVM1 alone (7.7 ± 5.7 *vs.* 3.8 ± 2.6, respectively, p < 0.001).

Limitations, reasons for caution: This study was conducted in a limited number of patients and further analysis will be required to confirm our preliminary results. In addition, the outcome of *in vitro* matured oocyte after thawing remains unknown.

Wider implications of the findings: Threshold values of cryopreserved IVM oocytes to achieve a live birth should be defined in order to assess the relevance of iterative oocyte collections.

Trial registration number: N/A.

P-516 Are Adolescents and young adults lymphoma patients less susceptible to chemotherapy than patients over 25 years old? Results of systematic prospective AMH levels follow-up

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Study question: To prospectively evaluate the influence of age and chemotherapy regimen on the longitudinal blood AMH variations in young lymphoma patients to elaborate fertility preservation strategies.

Summary answer: Adolescents and young adults (AYA) (15-24 years old) are less susceptible to chemotherapy than non-AYA (25-35 years old) even in case of alkylating regimen.

What is known already: Anti-Müllerian hormone (AMH) is now well-recognized to be a real-time indicator of growing follicle depletion and recovery in women treated by chemotherapy. Its longitudinal variations may discriminate between strong and soft protocol regarding the ovarian toxicity. It has been shown, mainly in breast cancer patients, that age, type of chemotherapy regimen and pre-treatment AMH levels are the main predictor of the ovarian recovery. Data on lymphoma are scarce but gives the opportunity to investigate the longitudinal AMH variations under chemotherapy at a younger age than breast cancer patients.

Study design, size, duration: Prospective monocentric observational study in the Fertility Observatory of the Lille University Hospital. Data were collected between 2007 and 2014. Non-Hodgkin or Hodgkin lymphoma patients (n = 122) between 15 to 35 years old were prospectively recruited before commencing chemotherapy. Patients were treated either by a non-alkylating protocol (non-alkylating group, ABVD; n = 67) or by an alkylating one (alkylating group; n = 55)P.

Participants/materials, setting, methods: Serial AMH measurements were performed at baseline (AMH0), 15 days after the start of chemotherapy (AMH1), 15 days before the last cycle (AMH2) and every 3 months from the end of chemotherapy until time + 24 months.

AMH variations were studied by ANOVA in the overall population according to the chemotherapy protocol. Then the study population was divided into 2 groups according to age: 65 AYA and 57 non-AYA.

Main results and the role of chance: Ovarian recovery in the non-alkylating group was complete and fast whereas no patients in the alkylating group recovered their initial pre-treatment value. There was no difference between the AYA and non-AYA groups regarding AMH 0 and the pattern of follicular depletion.

During the recovery phase, AMH levels re-increased more rapidly in the AYA group than non-AYA-group.

In AYA-group, the recovery phase is very fast and complete in non-alkylating group but progressive and partial in alkylating group. Only AMH+12 and AMH +24 were statistically different from AMH2 and all the serum AMH levels after chemotherapy remained different from AMH0 ($p<0,0001$).

In the non-AYA-group, the recovery phase is more progressive in the non-alkylating group. In alkylating group, serum AMH levels after chemotherapy remain low and not statistically different from AMH2.

AYA and non-AYA patients who received an alkylating protocol never recovered pre-treatment AMH values unlike those treated by a non-alkylating one. AYA patients treated by alkylating protocol highlighted higher levels of AMH during the recovery phase than non-AYA patients.

Limitations, reasons for caution: Large disparity in chemotherapy protocols in the alkylating group. The average duration of chemotherapy for patients treated with alkylating protocol was longer than treated with non-alkylating protocol.

Wider implications of the findings: These results allow for the first time developing evidence-based strategies for fertility preservation according to age and type of protocol in a large series of young lymphoma patients.

Trial registration number: DEC2015-112 and DC-2008-642

P-517 Identification and efficiency assessment of potential miRNAs targets for developing new pharmacological drugs against chemotherapy induced ovarian damage using mice model

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Study question: Can miRNA replacement approaches be used to develop a new adjuvant protective therapy to reduce chemotherapy induced ovarian damage?

Summary answer: Amongst several identified miRNAs that rapidly response to chemotherapy exposure, replacement of the selected miRNA, let-7a is feasible and efficient to reduce chemotherapy-induced ovarian damage.

What is known already: Cancer treatments as cyclophosphamide and its active metabolite, 4- hydroxycyclophosphamide can cause primordial follicle activation, apoptosis and ovarian reserve depletion. As these therapies negatively influence the ovarian function, fertility preservation is recommended. Today, several miRNA-based therapeutics have reached clinical trials, in oncology landscape. Several studies also indicate that miRNAs have a key role in follicle development as they can regulate the follicular growth, atresia and steroidogenesis. Last, miRNAs are involved in DNA-damage response, apoptosis and they can be themselves modulated during chemotherapy exposure which contributes to minimize the off-target toxicity.

Study design, size, duration: Postnatal day 3 ovaries (PND3) from C57blxCBAF1 hybrid mice were cultured under control and treated conditions-exposed to 4-hydroxycyclophosphamide (4-HC, 20μM, 1 or 24 h). Six ovarian samples (3/condition) were used for the miRNA expression profiling while 20 and 24 samples were used for the validation of miRNA levels after 1 and 24 h exposure to 4-HC respectively. Forty ovarian samples were used in mimic transfection experiments (10/condition: control, control with mimic-miRNA-transfected, 4-HC alone, 4-HC with mimic-miRNA-transfected).

Participants/materials, setting, methods: TaqMan Low Density Arrays were used for miRNA expression profiling. Custom Cards and QPCR -assays validated the differently expressed miRNAs. A functional annotation clustering was performed on DAVID database. A liposome-based system was used to deliver selected mimic-let7a into ovaries. After 48 h of mimic-transfection (plus 1 h exposure to 4-HC in treated samples), the efficiency was confirmed by fluorescence microscopy. The expression of the target genes was evaluated by QPCR. The apoptosis was also assessed.

Main results and the role of chance: The expression profiling of 384 miRNas in PND3 ovaries, revealed that miRNAs are differently expressed after chemotherapy exposure. Amongst 245 expressed genes, 74 stayed stable, 81 were upregulated and 40 downregulated after 1 h/4-HC/20 μM exposure. A set of these miRNas was selected for further validation. We found that mir-10a-5p, mir-146a-5p, mir-34a-5p, miR-494 and let-7a were significantly expressed. Then, we chose to focus on miR-10a and let-7a because they presented a stable and profound downregulated profile in all miRNA profiling assays. Moreover, their target genes are involved in DNA damage response, apoptosis and cell proliferation, based on functional annotation clustering results. As the let-7a is downregulated, we used a liposome-based system to deliver a mimic-let7a in PND3 ovaries in vitro. We evaluated the safety and efficiency of this transfection system and we found that we can successfully deliver the mimic-miRNA without causing significant apoptosis in the ovaries. The overexpressed let-7a was confirmed after transfection and was able to prevent the upregulation of the selected mRNA targets in response to chemotherapy, as genes involved in apoptosis (FASL, Bax) and in cell growth (STAT3). The potential protective effect of mimic-let7a will be confirmed by follicular count-follicle activation, DDR and apoptosis analysis.

Limitations, reasons for caution: The mimic-miRNA was delivered in vitro using the Lipofectamine/RNAiMAX in whole ovaries, avoiding dissection which could trigger the spontaneous follicle activation. Efficiency of the transfection could vary from one experiment to the other. Results must be confirmed in vivo using appropriate delivery system.

Wider implications of the findings: We identified several miRNAs target molecules involved in crucial cellular pathways during chemotherapy damage. By reducing chemotherapy-induced apoptosis, the mimic-let7a could be used for ovarian protection during treatment. Let-7a, may also affect pathways involved in follicle activation like PI3K/AKT, opening a new research area in fertility preservation by pharmacological protection.

Trial registration number: Not applicable.

P-518 Factors associated with the reproductive potential of vitrified-warmed, autologous oocytes derived from elective oocyte cryopreservation cycles

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Study question: What clinical factors are associated with optimal cryopreserved oocyte survival, fertilization, embryo culture, and transfer outcomes?

Summary answer: The outcome of autologous oocyte vitrification-warming cycles is optimized in younger patients who undergo transfer of blastocyst-stage embryos.

What is known already: In order to protect against the age-related fertility decline, women electively undergo oocyte cryopreservation to enhance their ability to conceive when they are ready. While enhancements in cryopreservation methodology, including vitrification techniques, have demonstrated the efficacy of elective oocyte cryopreservation as a means of fertility preservation, a relatively small proportion of women have returned to utilize their vitrified oocytes. Current data related to efficacy is limited to extrapolations from hypothetical models and case series. Given the paucity of experience, physicians seek prognostic information to guide and counsel patients and manage expectations regarding the likelihood of success from vitrified-warmed oocytes.

Study design, size, duration: This retrospective cohort study includes 1197 patients who underwent 1396 oocyte vitrification cycles, from 2010 to 2017. One hundred and eleven patients (9.3%) returned for oocyte warming (n = 118) and treatment. Fresh (n = 52) and frozen (n = 39) transfers of cleavage (n = 37) or blastocyst stage (n = 54) embryos were observed. Over half of the patients (52.3%) who warmed their oocytes were successful in creating embryos eligible for preimplantation genetic testing (PGT), that were then vitrified for future use.

Participants/materials, setting, methods: Patients presented to a private IVF clinic for elective oocyte cryopreservation. Cycles in which autologous, vitrified oocytes were warmed (n = 118) were analyzed using multivariate linear and binary logistic regression models to assess patient demographics (age, BMI, and AMH); and treatment related factors (number of oocytes cryopreserved) associated with oocyte survival and clinical outcome. The influence of these factors and the treatment approach (i.e. PGS, frozen embryo transfer (FET)) on embryo transfer success was determined.

Main results and the role of chance: One thousand and fifteen oocytes were thawed and fertilized using ICSI. Of the fertilized oocytes, 63.3% reached the cleavage stage, and 39.0% reached the blastocyst stage. Chemical and clinical pregnancies were achieved in 52.7% (n = 48/91) and 41.8% (n = 38/91) of cycles, respectively. Increased oocyte age (b = -0.6, p = 0.0003) and decreased AMH (b = -1.4, p<0.0001) reduced the number of oocytes available for warming, but not the rate of survival. Controlling for age, each additional oocyte warmed was associated with an increase in the rate of oocyte survival (b = 0.007, p = 0.04), embryos reaching the cleavage (b = 0.008, p = 0.01) and blastocyst stage (b = 0.008, p<0.0001) and being amenable to PGT (OR 1.182 [95% CI 1.051-1.33], p = 0.005) and vitrification (OR 1.212 [95% CI 1.048-1.403], p = 0.009). Oocyte age (b=-0.02, p = 0.02) and AMH (b = 0.05, p = 0.03) modified the rate of blastulation. The odds of having an embryo available for fresh transfer was independent of age, AMH or the number of oocytes warmed. Controlling for confounders (age, AMH, BMI, oocytes warmed, embryo stage, PGT, FET, embryos transferred and endometrial thickness), clinical pregnancy rate was negatively impacted by increased oocyte age (OR 0.78 [95% CI 0.60-1.01], p = 0.059) and transfer of cleavage embryos (OR 0.10 [95% CI 0.01-1.05], p = 0.05).

Limitations, reasons for caution: This analysis is retrospective and subject to confounding bias. Prospective randomization of patients to various treatment approaches (ie. transfer of day 3 vs. day 5 and screened vs. unscreened embryos) would ultimately validate our findings and define the optimal strategy for patients utilizing vitrified-warmed oocytes.

Wider implications of the findings: Women desiring elective fertility preservation should be encouraged to vitrify their oocytes at a younger age to increase their probability of future pregnancy. When possible, zygotes from warmed oocytes should undergo extended culture and be transferred at the blastocyst stage to optimize embryo selection and improve clinical outcome.

Trial registration number: This study was approved by the Western Institutional Review Board (Study Number: 1167398).

P-519 Effects of natural honey, as a new cryoprotectant, on viability and genes expression of vitrified mouse blastocysts

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Study question: To investigate if natural honey can be used instead of sucrose to optimize the vitrification procedure of mouse blastocysts.

Summary answer: Natural honey with a wide range of monosaccharides, disaccharides and antioxidants can be proposed as a cryoprotectant to reduce the cellular stress caused by vitrification.

What is known already: Profound understanding of cryobiology has led to novel protocols for vitrification of blastocysts in terms of media composition. It has been demonstrated that a combined presence of permeating and non-prmeating cryoprotectants, both monosaccharides and disaccharides, can effectively increase the viability of vitrified embryos. Although these improvements resulted in high survival rates, other analyses on post warming blastocysts with normal morphology demonstrated a wide range of cellular and molecular impairments.

Study design, size, duration: The first part of the experiment was conducted to determine the optimum concentration of honey in vitrification (0.5, 1, 2 M) and warming solutions (thawing: 1, 2, 3 M; dilution: 0.5, 1, 1.5 M). The second part was designed to compare the effects of honey-based selected solutions and sucrose-based one on embryo quality.

Participants/materials, setting, methods: In vivo produced mouse blastocysts were vitrified as described by Kuwayama (Kuwayama et al., 2005) using mentioned solutions. After 12 h, vitrified blastocysts were compared with fresh blastocysts in terms of survival and hatching rate as well as the expression level of genes related to apoptosis (*Trp53*, *Bax*) and oxidative stress (*Sod2*).

Main results and the role of chance: The optimum concentrations of 1, 2 and 1 M were chosen for vitrification, thawing and dilution solutions, respectively. Our results showed a high survival rate for both treatment groups and control one. The hatching rate of vitrified blastocysts was significantly lower than fresh blastocysts (58.47%). However, no significant difference was found between sucrose-based and honey-based mediums (40.8% and 38.3%, respectively). Furthermore, real-time RT-PCR analysis data showed a reduction in the expression of *Trp53*, *Bax* and *Sod2* transcripts in the honey group (1.8-, 2.15- and 1.1-fold, respectively) compared to the sucrose group (6.15-, 2.83- and 4.05-fold, respectively).

Limitations, reasons for caution: The implantation and live birth rates are necessary to give a complete picture of natural honey effects during vitrification procedure.

Wider implications of the findings: Our study considered antioxidant properties of vitrification solutions as a new parameter for the success of the procedure.

Trial registration number: None.

P-520 Slow passive freezing for ovarian tissue shows comparable in vitro activation of primordial follicles with no difference in apoptosis compared to traditional controlled slow freezing

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Study question: Is slow passive freezing (PSF) an alternative to the standard controlled slow rate freezing (CSF) of human ovarian tissue?

Summary answer: The PSF showed no loss on primordial follicle count, activation, apoptosis and cell proliferation compared to the traditionally CSF.

What is known already: Fertility preservation is an important issue in regard to the long-term quality of life. Cryopreservation of OT fragments is a strategy for fertility preservation for cancer patients. For the moment, CSF is the established method used to freeze cortical fragments of ovarian tissue. CFS requires expensive computerized equipment and the process is time-consuming. Moreover, there is a risk for technical failures with the potential loss of precious material. Several publications reported passive slow freezing (PSF) to be successful for testicular tissue of different animal species including for human testicular tissue (Goossens et al., 2013).

Study design, size, duration: OT strips (n = 50) from transgender patients (n = 10) were divided in 3 groups: (1)Fresh tissue; (2)CSF and (3)PSF. DMSO

(1.5 M) was used as cryoprotectant in both freezing protocols. CSF was performed with a programmable freezer including a manual seeding. For PSF, cryovials were placed in an isopropyl alcohol container in the -80°C freezer overnight allowing a cooling rate »-1°C/min. Samples were stored in LN. Strips were thawed, cultured for 2 h and for 2days (2d).

Participants/materials, setting, methods: OT strips were cultured individually in 24-well plates containing supplemented McCoy's 5a medium for 2 h and 2d at 37°C (6%O_2, 5%CO_2). The strips were fixed in 10% paraformaldehyde and embedded in paraffin. Histological sections were made for follicle count, apoptosis and cell proliferation analysis. Light microscopic evaluation was performed to study apoptosis by TUNEL en capsase-3 and cell proliferation PCNA and Ki67-staining. Statistical analysis (Wilcoxon Signed Ranks Test) was performed (SPSS vs23).

Main results and the role of chance: Both freezing procedures, PSF versus CSF, showed no loss in number of primordial follicle after 2 h of warming (54.6 ± 24.0% versus 64.1 ± 16.4%). The PSF technique, showed a significant higher amount of primary follicles when comparing the amount after 2 h and 2d of culture (30.3 ± 24.2% versus 66.1 ± 11.2% (P = 0.013)), indicating a clear activation of the follicles. This was also observed in the SRF (18.1 ± 11.2% versus 60.2 ± 15.5% (P = 0.005)). This activation shift was not significantly different between both techniques (Fisher's Exact p = 0.24). The PSF and CSF technique did not affect the apoptosis level of the oocyte nor the granulosa cells after 2d culture. There was no statistical difference in caspase-3 negative follicles after 2d of culture between PSF and CSF respectively (36.1 ± 27.4% versus 51.7 ± 33.7% for primordial follicles and 73.5 ± 22.1% versus 77.7 ± 19.9% for primary follicles). Likewise for the late apoptosis PSF versus CSF (89.2 ± 13.2% versus 73.4 ± 21.1% (TUNEL negative primordial follicles) and 80.2 ± 16.6% versus 77.9 ± 20.6% (TUNEL negative primary follicles)). For the cell proliferation PSF and CSF method showed comparable results after 2d of culture (14.5 ± 23.6% versus 13.7 ± 19.9% (PCNA positive primordial follicles and 23.1 ± 30.8% versus 18.4 ± 17.4% PCNA positive primary follicles)) and the ki67 positive staining (34.4 ± 34.1% versus 33.5 ± 35.3% (primordial) and 41.9 ± 22.1% versus 45.3 ± 24.3% (primary).

Limitations, reasons for caution: Further studies should be conducted to evaluate the true activation and maturation potential by xenotransplantation. It is also important to note, that these experiments should be repeated using OT from oncological patients as tissue characteristics might be affected by long term testosterone treatment in the OT of trans persons.

Wider implications of the findings: PSF could be an easy and cost-effective alternative to CSF for fertility preservation of ovarian tissue.

Trial registration number: This research is conducted with the approval of the local ethics committee 2015/0124 – B670201523543).

P-521 Autologous oocyte vitrification: outcomes assessment

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Study question: Do maternal age and number of collected mature oocyte affect post-warming biological and clinical outcomes?

Summary answer: Oocyte survival and fertilization rates after vitrification and warming are not impaired in older patients and in patients with large number of mature oocytes collected.

What is known already: Autologous oocyte vitrification is widely proposed to women receiving ICSI treatment in our fertility centre in order to limit the number of embryos created. So far, few data have been published regarding post-warming outcomes of autologous oocytes as main studies on oocytes vitrification were conducted on donated eggs from young women. As both the number of oocytes collected at pick-up and patient age are known to affect oocyte quality, there is a need to evaluate the impact of these factors on biological and clinical outcomes after autologous oocyte vitrification and warming.

Study design, size, duration: This is a retrospective study conducted over a 6-year period. A total of 1217 autologous oocytes vitrification cycles were performed. This program allowed the cryopreservation of 8926 oocytes. To date, 1080 oocyte warming cycles were performed including 5153 oocytes.

Participants/materials, setting, methods: 970 women receiving ICSI treatment were proposed to vitrify a part of mature oocytes concomitantly to ICSI procedure on sibling oocytes. Oocytes were vitrified and warmed using Kitazato vitrification media and system. Post-warming oocyte survival rate (Number injected oocytes/Number of warmed oocytes), fertilization rate (Number of zygotes displaying 2PN/Number of injected oocytes) and early pregnancy rate per transfer (βHCG blood measurement>100UI/l) were assessed and compared between groups of age and number of collected mature oocytes.

Main results and the role of chance: The mean age of women included in the study is 32.8 years. They were divided into age groups and 3 groups based on the number of mature retrieved oocytes. Our data show that maternal age at vitrification doesn't affect post-warming survival rate (<30years: 84.3%, 30-35years:83.8%, >35years: 84.8%, p>0.05), nor fertilization rate (<30years:64.2%, 30-35years: 65.7%, >35years: 63.1%, p>0.05). However, as expected, women above 38 years have significantly lower chances to achieve a pregnancy than women under 30years (28.0% *vs* 16%, p = 0.003) but the difference observed between the other age group is not statistically significant (30-35years: 22.7%, >35years: 21.3%, p>0.05). The number of mature oocytes available after pick-up doesn't change survival rate (≤10: 83.9%, 11-15:84.6%, >15: 83.7%, p>0.05), fertilization rate (≤10: 62.8%, 11-15:64.5%, >15:63.8%, p>0.05), nor early pregnancy rate per transfer ((≤10: 22.4%, 11-15:20.8%, >15:19.6%, p>0.05). Finally, when looking at the different age groups described above, no correlation was found between survival/fertilization rates and number of mature oocyte at pick-up.

Limitations, reasons for caution: The major limitation of the study is potential confounding factors such as infertility factor, ovarian stimulation protocol and treatment used for endometrial preparation for oocyte warming cycle couldn't be taken into account.

Wider implications of the findings: Since we have shown that critical factors like maternal age and number of oocytes have no impact on post-warming biological outcomes, one can suggest that the cryopreserved oocytes that are not intended to be used could be allocated to oocyte donation program with the consent of the couple.

Trial registration number: Clinical Trials Registration number: 209 R02

P-522 UV irradiation of freeze-dried human sperm shows high DNA integrity as compared to conventionally frozen sperm

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Study question: Is freeze-dried human sperm more resistant to UV irradiation than conventionally frozen?

Summary answer: Freeze-dried human sperm maintained a high DNA integrity compared to conventionally frozen sperm after UV irradiation.

What is known already: Recently, it was shown that mice sperm preserved in the dry state for 9 months in a space station and exposed to cosmic irradiation showed only minor DNA damages which were repaired by oocytes cytoplasm and resulted in normal offspring.

Study design, size, duration: Basic human experimental research using human sperm allocated to four different groups: (1) Fresh (2) Freeze-dried and rehydrated cells (3) Freeze-dried and irradiated prior rehydration. (4) Conventionally frozen and irradiated prior thawing. Each group contained 4 vials with a minimum of 10 drops. Drying duration was 48 hours. Dried samples were stored in vials at 4°C prior rehydration, and frozen samples were stored in vials in liquid nitrogen prior thawing.

Participants/materials, setting, methods: Sperm diluted 1:1 in lyophilization solution (LyoS). Freezing done by pipetting 10 µL drops into sterile liquid air (CLair, Fertilesafe, Israel). Freeze-drying done using a sterile drying device (Darya, FertileSafe, Israel) having a shelf temperature of -35°C and vacuum of

10mtorr. Samples were irradiated 30 min under a UV lamp. Rehydration was done using 0.2 mL/vial LyoS at 37°C, thawing was done on warm (37°C) microscopic slides. DNA integrity was evaluated using Halosperm G2kit (Halotech, Spain).

Main results and the role of chance: Fresh sperm concentration was $10•10^6$ cells/ml, motility was > 50% and DNA integrity was 81.06%±9.2%. Post thaw motility (after conventional freezing) was 65-80% of the fresh (normalized) same specimens. Rehydrated freeze-dried irradiated human sperm showed no significant cell loss $5•10^6$ cells/ml and no decrease in DNA integrity 84%±8.1%. After freeze-drying and rehydration cells concentration was $5.375•10^6$ cells/ml and DNA integrity was 81.3%±3.5%. The DNA integrity of the irradiated but conventionally frozen sperm was significantly lower, 9%±15% ($P<0.05$) and concentration was slightly lower $4.5•10^6$ cells/ml. Morphological analysis of irradiated conventionally frozen sperm was much different than the irradiated freeze-dried sperm displaying loss of tails and membranes and DNA showing larger halos. These results show that there was no cell loss and no additional damage to the DNA integrity due to the drying process and irradiation while at the dry state. Human sperm freeze-drying is a revolutionary technology that will allow long-term storage of sperm at room temperature and protection from UV irradiation.

Table 1 Summarizes the results of human sperm following freeze-drying, freeze thawing and irradiation.

Group	Sperm Concentration (cells/ml)	DNA integrity
Fresh control (undiluted)	$10 • 10^6$	81.06% ± 9.2%
Freeze-dried rehydrated	$5.375 • 10^6$	81.3% ± 3.5%
Freeze-dried irradiated	$5 • 10^6$	84% ± 8.1%
Frozen thawed irradiated	$4.5 • 10^6$	9% ± 15%*

*($p<0.05$)

Limitations, reasons for caution: Small number of samples and lack of data on embryonic development following ICSI.

Wider implications of the findings: These findings suggest that freeze-dried human sperm is more resistant to UV irradiation than frozen sperm. This may be due to a lesser amount of reactive oxygen species created in the dry state compared to the frozen state. Thus, freeze-drying may be preferred when storage conditions are less than optimal.

Trial registration number: N/A.

P-523 Follow-up of elective oocyte cryopreservation for age-related reasons: utilisation of vitrified oocytes and reproductive outcomes of women who return

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Study question: What is the come-back rate and what are the reproductive outcomes of women who previously had their oocytes cryopreserved to anticipate age-related fertility decline?

Summary answer: Of all women who underwent elective oocyte cryopreservation, only 7.6% have so far returned to use these oocytes. Of those, 32.6% had an ongoing pregnancy.

What is known already: Oocyte vitrification has become an established and efficient technology, which has facilitated the practice of elective oocyte cryopreservation for women who defer motherhood and wish to anticipate age-related fertility decline, for various reasons. Little is known about the utilisation of the vitrified oocytes of these "social freezers" and their reproductive outcomes. Follow-up data of reproductive behavior and outcomes in these women are needed for a comprehensive appraisal of social freezing.

Study design, size, duration: We performed a retrospective observational single-centre follow-up study in a cohort of 563 women who underwent elective cryopreservation of oocytes to anticipate age-related fertility decline between January 2009 and November 2017.

Participants/materials, setting, methods: A total of 563 women underwent 902 ART cycles for oocyte vitrification. Data were collected from computerised clinical charts. We evaluated reproductive treatment choices in social freezers who returned, warmed oocyte survival rates, fertilisation rates, embryo quality, the use of donor sperm, pregnancy rates, and live birth rates.

Main results and the role of chance: Mean age at oocyte vitrification was 36.5 y [95% confidence interval (CI) 36.3–36.6]. A mean number of 8.5 [95% CI 8.1 –8.8] oocytes were vitrified per cycle. Seventy-two women (12.8%) returned to the clinic for reproductive treatment at the moment of writing. In 43% of them (31/72), donor sperm was used for artificial insemination or ICSI. 7.6% (43/563) of women requested to have their vitrified oocytes warmed for ART, at a mean age of 42.1 y [95% CI 41.5-42.7]. Their oocytes had been vitrified at a mean age of 38 y. They underwent 72 warming cycles in total; overall survival rate of warmed oocytes was 73.4 %. The fertilisation rate was 66.8% and 64 warming cycles resulted in a fresh embryo transfer. In total, 25.6% (11/43) patients had an ongoing pregnancy after oocyte warming and fresh embryo transfer; the ongoing pregnancy rate (OPR) was 32.6% (14/43). Of women who returned, 32 underwent ≥1 ART cycles with oocyte retrieval (69 cycles in total), at a mean age of 38.9 y [95% CI 37.9-39.9] and the OPR was 34.4%. Nineteen patients underwent artificial insemination (15 with donor sperm), of whom 26.3% (5/19) had a live birth. Three patients had a live birth after oocyte donation.

Limitations, reasons for caution: Due to the limited follow-up period, the return rate of social freezers is low and utilisation rates of cryopreserved oocytes are incomplete. Hence, the reproductive outcome of only a subset of social freezers is currently known. Of patients who returned for reproductive treatment, 40.3% have not used their vitrified oocytes.

Wider implications of the findings: The majority of social freezers who returned have found a suitable partner to pursue motherhood. Whether their previous decision to undergo oocyte cryopreservation has enhanced the probability of a live birth will require confirmation in larger follow-up studies.

Trial registration number: Non applicable.

P-524 Serum AMH as predictor of ovarian stimulation outcome among women undergoing oocyte cryopreservation prior to breast cancer treatment

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Study question: Can AMH determination before hormonal stimulation in emergency fertility preservation for breast cancer help to evaluate the ovarian reserve and optimize the chance of a successful response?

Summary answer: AMH might not correlate with the ovarian reserve in patients with breast cancer prior to chemotherapy treatment, and novel biomarkers should be investigated.

What is known already: AMH is associated with the size of the follicle pool. In some studies AMH has shown to be predictive of the woman's ovarian reserve and the number of oocytes collected after hormonal stimulation. A successful fertility rescue treatment can be crucial for the woman´s future possibility of having biologically related children.

Study design, size, duration: Prospective cohort study between January 2012 and May 2016 of women with breast cancer that underwent a fertility rescue hormonal stimulation cycle in an antagonist protocol, with or without letrozole supplement, depending on their tumor estrogen receptor status, prior to chemotherapy. Stimulation started any day of their menstruation cycle, serum AMH was sampled before cycle start.

Participants/materials, setting, methods: A total of 124 women diagnosed with breast cancer were included, mean age 32,8 (range 21-42), mean BMI 23,0 (range 18-35). The mean AMH concentration was within normal ranges for age (mean 2,32 microgram/L, range 0,16-9,6). The majority of women were nulliparous. More than half (70 out of the 124 patients,56%) were on contraceptive pills at time of diagnosis.

Main results and the role of chance: There were only modest, non-significant correlations between age and AMH levels or between AMH levels and oocyte yield in the whole group. Possible explanatory variables were thus investigated. In the subset of 70 patients who had complete data on use of contraceptive pills at time of breast cancer diagnosis, sub-analysis indicated that patients who were on contraceptive pills and discontinued them at time of cancer diagnosis presented with significantly lower AMH levels than women who had not had contraceptives previously. However, women who were previously on contraceptive pills had a similar oocyte yield as women who were not on contraceptives.

Limitations, reasons for caution: Several clinicians planned and performed the stimulation of the patients and the retrieval of the oocytes. The time of the stimulation treatments varied between the patients and in some cases could not be prolonged due to planned chemotherapy initiation.

Wider implications of the findings: The predictive value of AMH determination before hormonal stimulation in emergency fertility preservation for women in breast cancer is still debatable. Measurements of serum AMH concentrations to evaluate ovarian reserve previously to fertility preservation might not correlate with the outcome in all patients with breast cancer.

Trial registration number: non-applicable

P-525 Serial serum cell- free DNA (cfDNA) quantification in lymphoma and breast cancer patients before and after chemotherapy: is there a link with the ovarian function?

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Study question: Can longitudinal study of serum cf-DNA levels serve to predict the recovery of the ovarian function after chemotherapy in different type of cancer?

Summary answer: Serum cf-DNA level can be used as a non-invasive promising prognostic biomarker to evaluate and predict ovarian reserve in hodgkin lymphoma and breast cancer patients.

What is known already: cf-DNA can be quantified in small amounts in serum resulting from the release of genetic materials from apoptotic/necrotic cells. Serum cf-DNA can serve to cancer diagnosis but also in predicting response to treatment. It still unclear the oocyte quality during fertility preservation procedures in the cancer area. Recently, cf-DNA has been shown to be significantly correlated to the oocyte as well as the embryo quality, suggesting that it may serve as an innovative and a non-invasive objective biomarker of the microfollicular environment and oocyte quality.

Study design, size, duration: 46 and 37 serum samples were obtained from five hodgkin lymphoma patients (mean age: 24.3 ± 1.8 years) and five breast cancer patients (mean age: 36.5 ± 1.8 years) respectively. All patients were prospectively recruited before initiation of the chemotherapy. Serum cf-DNA quantification and AMH measurements were performed at baseline, 15 days after the first cycle, 15 days before the last cycle and every 3 months after the end of chemotherapy until time 24 months.

Participants/materials, setting, methods: We performed cf-DNA qRT-PCR using Alu-115 (detecting both necrotic and apoptotic DNA) primers to evaluate serum cf-DNA level.

Main results and the role of chance: Our preliminary results revealed that the cf-DNA concentration was significantly higher in serum from breast cancer compared with Hodgkin lymphoma patients (Alu-115 mean ± SEM: 788.9 ± 129.5 vs 383.7 ± 99.6 $fg.\mu l^{-1}$, t-test $p = 0.08$). At baseline, serum AMH concentrations were 33.1 ± 9.8 and 24 ± 13.9 ng/ml (mean ± SEM) in hodgkin lymphoma and breast cancer patients, respectively. AMH concentration decreased significantly 15 days and 6 months after the first chemotherapy cycle in hodgkin lymphoma (6.7 ± 2.1 ng/ml, $p = 0.03$) and breast cancer patients (3 ± 0.03 ng/ml, $p = 0.05$), respectively. 18 months pos-chemotherapy treatment, AMH concentration remained stable in breast cancer patients (2.3 ± 0.9 ng/ml) while a tendency toward an increase of AMH concentration was observed (13.1 ± 5 ng/ml) in hodgkin lymphoma patients. Meanwhile, Cf-DNA concentration dramatically decreased 15 days after chemotherapy treatment in hodgkin lymphoma patients compared at baseline measure (441.2 ± 141.6 vs 1031.9 ± 719.7 $fg.\mu l^{-}$), and thus, until 18 months post-treatment (295 ± 141.6 $fg.\mu l^{-}$) although the tendency was not significant. In the breast cancer patients, there is no significant or tendency before, during and after chemotherapy treatment. As cf-DNA concentration has been reported to be negatively correlated to follicular size and embryo quality, our cf-DNA results question about cryopreserved oocytes quality in breast cancer and hodgkin lymphoma patients.

Limitations, reasons for caution: The few number of samples and patients and the heterogeneity of chemotherapy regimen in lymphoma patients.

Wider implications of the findings: This finding opens new perspectives in cancer patient care especially for fertility preservation program and counselling. cf-DNA may serve as a non-invasive objective biomarker of cryopreserved oocytes quality.

Trial registration number: CHACRY-1501.

P-526 Preliminary human application of optical coherence tomography for quantification and localization of primordial follicles aimed at effective ovarian tissue transplantation

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Study question: Is it possible to apply optical coherence tomography (OCT) intend for effective ovarian tissue transplantation with assessment numbers and localization of follicles on ovarian tissue?

Summary answer: OCT may detect primordial follicles non-invasively on fresh human and mice ovary coincident with ovarian reserve test.

What is known already: Primordial follicles in fixed and embedded human and bovine ovarian tissue could be detected using the current full-field OCT (FF-OCT) system. Although, in terms of effect for viability, non-invasiveness of near-infrared ray (NIR) used for OCT was indicated by glucose uptake assay and neutral red staining with bovine ovarian tissue, there are no reports about affect of OCT for oocyte to prove the reproductive ability including outcome of in vitro fertilization (IVF).

Study design, size, duration: To assess of NIR irradiation effect for oocytes, day3 mice were assigned to 4 groups: control (n = 3), 60 mW (n = 3), 110 mW (n = 4), 250 mW (n = 5). Also, to assess the affect of OCT for IVF, day 3 mice ovaries were assigned to with/without OCT group (n = 15, n = 10 respectively). The clinical study was conducted in four patients who received ovarian tissue cryopreservation from May 2016 to February 2017. Present studies were performed with informed consent and IRB approval.

Participants/materials, setting, methods: Fresh ovaries from each life stage mice were examined. And to confirm non-invasiveness of OCT, outcomes of IVF were compared among follicles from mice ovaries irradiated stepwise NIR. Also, day3 mice ovaries with/without OCT were transplanted under kidney capsule of host mice. Two weeks later, outcome of IVF with transplanted ovaries were examined. Additionally, newborns were bred and mated. Finally, OCT examination was performed using human ovary tissues. And follicle count using OCT was performed.

Main results and the role of chance: The standard OCT images were established including primordial follicle. And stepwise NIR irradiation (60-250 mW/cm^2) did not affect for IVF outcome (fertilization rate $p = 0.86$-1.00,

Blast cyst rate p = 0.86-1.00). Also, OCT examination did not affect for IVF outcome of transplanted ovaries (number of extracted oocytes p = 0.29, fertilization rate p = 0.35). Furthermore, all of newborns were shown normal appearances including reproductive ability. In the clinical study, three patients were received ovarian tissue cryopreservation for hematological disease as fertility preservation, and a patient received ovary extraction for in vitro activation intend for childbearing. Finally, OCT images of human ovary were well-accorded with histological images and ovarian reserve test on adult patients. While, OCT imaging detected primordial follicles fresh ovarian tissue from child patients (15 and 11 years-old) with unevaluable ovarian reserve by anti-Müllerian hormone and follicle stimulating hormone levels. As the result of follicle count, there were no significant differences between histology and OCT (p = 0.98).

Limitations, reasons for caution: The scanning area of commercial optical coherence tomography equipment is too small, and the scanning depth is insufficient. Therefore, it is presently difficult to assess actual ovarian reserve and follicle distribution using existing instrumentation. For the application for ovarian tissue transplantation, optimization of the OCT system is needed.

Wider implications of the findings: OCT holds the possibility for assessment of actual ovarian reserve and follicle distribution on each ovarian tissue. The selection of most suitable ovarian tissue using OCT may promote the development of effective ovarian tissue transplantation.

Trial registration number: The registraion numbers of present studies were No. 470 and 471(animal study) and UMIN000023141- No.3311 (clinical study) under IRB of St. Marianna University.

P-527 Towards fertility preservation for prepubertal boys – The role of gonadotrophins on functional maturation of Leydig and Sertoli cells in immature human testis xenografts

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Study question: We investigated whether gonadotrophin (hCG + FSH) administration promotes germ, Sertoli, and Leydig cell development in xenotransplanted immature human testis tissue for purposes of fertility preservation.

Summary answer: Sertoli cell maturation was induced or maintained in xenografts, whilst induction of steroidogenesis required gonadotrophin administration. Germ cell differentiation did not occur in xenografted tissues.

What is known already: For prepubertal boys facing gonadotoxic (e.g. chemotherapy, radiotherapy) treatments that are likely to result in sub/infertility, there are no established options to preserve their fertility. Whilst testicular tissue may be obtained and cryopreserved prior to treatment, no clinical applications have been developed to use this tissue to restore fertility. Transplantation of immature testis tissue into adults of several species has provided a proof of principle that grafting of tissue can generate gametes capable of producing progeny. However, the factors required to functionally mature immature human testis tissue to produce gametes have not yet been determined.

Study design, size, duration: Prospective laboratory study using immature human testis tissue obtained from boys (n = 3) undergoing testicular biopsy prior to gonadotoxic treatment for fertility preservation. Tissue was obtained with informed consent for clinical cryostorage and research purposes. Ethical approval was obtained from the Local Research Ethics Committee. Host mice xenografted with testicular tissue pieces were exposed to vehicle or treatment for 12wks prior to analysis by immunohistochemistry with quantification of cellular development and function.

Participants/materials, setting, methods: Testicular tissues from three boys (8, 13 and 14 yrs) were xenografted intratesticularly into immunocompromised castrate mice (n = 12). Hosts received subcutaneous injections of vehicle or gonadotrophins (hCG, 20IU, FSH, 12.5IU), 3× weekly for 12wks. Retrieved grafts were double immunostained for VASA, MAGEA4 (germ cell), SOX9 (Sertoli cell), Androgen Receptor (AR, mature Sertoli cell), Anti-Mullerian Hormone (AMH, immature Sertoli cell), CYP11a1 (Leydig cell - steroidogenic marker) and Ki67 (proliferation marker). Results analyzed by two-factor ANOVA.

Main results and the role of chance: Testicular tissue structure and histological appearance was maintained in xenografts. Administration of hCG/FSH was required to induce steroidogenesis (CYP11A1) in Leydig cells of xenografted tissue from one patient (8 yrs), whilst for the two remaining patients (13 and 14 yrs), steroidogenesis was maintained only in the hCG/FSH-exposed xenografts. Maturation of Sertoli cells (as indicated by AR expression) was induced (8 yrs) or maintained (13 and 14 yrs) in the xenografts, however, there was no difference in the number of Sertoli cells expressing AR between vehicle and hCG/FSH-exposed xenografts (2247.48/mm^2 vs 2411.13/mm^2, p>0.05). Sertoli cell number was significantly reduced in hCG/FSH-exposed xenografts, compared to vehicle control (3015.56/mm^2 vs 2548.92/mm^2, p = 0.01), and Sertoli cell proliferation was reduced although not significantly (7.65/mm^2 vs 1.35/mm^2, p>0.05). Germ cells were maintained in xenografts and germ cell numbers were lower in vehicle-, compared to gonadotrophin-exposed xenografts, although this was not significant (115.46/mm^2 vs 517.68/mm^2, p>0.05). Interestingly, for the youngest patient (8 yrs), whilst germ cell numbers were maintained in vehicle-exposed xenografts, there was a complete loss of germ cells in those exposed to hCG/FSH. Taken together, these results indicate xenografting immature testis tissue induces and maintains Sertoli cell maturation, whilst exogenous hCG/FSH promotes steroidogenesis in xenografted tissues.

Limitations, reasons for caution: Prepubertal human testis tissue is a finite resource limiting the number of samples in these studies. Inter-individual variation between patients is also a factor. Experimental design involving direct comparison of vehicle versus treatment for each sample and statistical analysis using two-factor analysis can account for some of these limitations.

Wider implications of the findings: These findings suggest that endogenous or exogenous gonadotrophins stimulate development and function of somatic cells in transplanted immature human testicular tissues. However, further work is required to determine the factors required to generate functional gametes from this tissue in order to develop viable options for fertility preservation in prepubertal boys.

Trial registration number: Not applicable.

P-528 Impact of breast cancer prognostic factors on the response to controlled ovarian stimulation in patients undergoing fertility preservation

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Study question: Do breast cancer (BC) prognostic factors influence ovarian function and response to controlled ovarian stimulation (COS) in patients seeking fertility preservation (FP)?

Summary answer: Neither SBRIII grade, high Ki67 expression, HER2 overexpression nor triple negative status influence COS outcomes in pre-chemotherapy BC patients undergoing FP.

What is known already: Since the development of FP, concerns about the potential impact of cancer status on ovarian reserve, function and response to COS have been raised. Studies evaluating COS outcomes before chemotherapy in FP context have reported conflicting results. On the other hand, BC tumor markers such as Scarff-Bloom-Richardson (SBR) grade, high Ki67, HER2 overexpression or triple negative status represent well established prognostic factors which might influence the treatment strategy. However, the potential impact of these factors on ovarian reserve or COS outcomes in BC patients undergoing FP has never been investigated.

Study design, size, duration: A total of 151 BC patients undergoing COS for FP were prospectively included between November 2013 and December 2016. COS was initiated regardless of the phase of the cycle, with or without letrozole supplementation. Matured oocytes and/or embryos obtained were vitrified. COS characteristics and outcomes were analyzed in all women.

Participants/materials, setting, methods: BC prognostic factors considered in the present study were SBRIII grade, Ki 67> 20%, HER2

overexpression and "triple negative" tumor. Univariate and multivariate analysis were performed to determine their impact on ovarian reserve markers (serum Anti-Müllerian Hormone (AMH) and antral follicle count (AFC)) as well as on ovarian response to exogenous FSH. Less than 8 mature oocytes vitrified, maturation rate under 70% or Follicle Output RaTe (FORT) under 35% were considered as poor COS outcomes.

Main results and the role of chance: A total of 154 COS cycles were performed and analyzed in BC patients 33.4 ± 4.1 years of age were analyzed. Mean AMH and AFC were 3.2 ± 4.5 ng/mL and 20.2 ± 14.3 follicles, respectively. HER2 overexpression was observed in 18.9% and 34.7% of tumors expressed estrogen or progesterone receptors. Triple negative status characterized 25% of tumors. BRCA1/2 mutation was found in 22.4% of patients. A mean of 9.2 ± 7.4 mature oocytes were cryopreserved per cycle.

After multivariate analysis, only serum AMH levels, AFC and smoking status were significantly associated with the number of mature oocytes obtained following COS. BC prognostic factors did not appear to have a significant influence on ovarian reserve markers, number of retrieved oocytes, maturity rate or on ovarian response to exogenous FSH assessed by FORT index, defined as the ratio of the number of pre-ovulatory follicle (16-22 mm) count on the triggering day X100 to the antral follicle (3-8 mm) count at baseline.

Limitations, reasons for caution: The main limitation is related to the patients themselves referred for FP, who usually have early stage disease. Therefore, these results cannot be generalized to patients with advanced tumoral stage. Since oocytes were not thawed, the actual impact of BC prognostic factors on live birth rate remains unknown.

Wider implications of the findings: BC prognostic factors probably have no or low impact on ovarian function in terms of ovarian reserve and response to COS. Further analysis, in particular after oocyte thawing will be needed to clarify a possible impact egg quality.

Trial registration number: Not applicable.

P-529 Effects of cancer on ovarian reserve and ovarian response to stimulation

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Study question: This study investigates differences in ovarian reserve and stimulation response in a group of cancer patients and in a control group.

Summary answer: Despite their reduced ovarian reserve, cancer patients did not show lower number of oocytes retrieved and oocyte cryopreserved when compared to healthy control group.

What is known already: Premature ovarian failure is a well recognized consequence of chemotherapy. Therefore counseling plays a critical role for these patients. A proper counsel should include fertility preservation options. Oocytes cryopreservation is a procedure accepted as non-experimental technique. The debate on the negative influence of cancer on ovarian stimulation is still open. Many studies compared oncological patients with a control group but reports are still in conflict. It is assumed that cancer can damage ovarian granulosa cells affecting their sensibility to exogenous FSH. Another theory recognizes cancer as responsible for generating a catabolic state that can increase oxidative stress.

Study design, size, duration: Retrospective study: 212 oncological patients aged 30.5 ± 6.1 years (16 to 40 years old) undergoing a fertility preservation treatment at the University of Bologna IVF Center were considered from 1997 to 2017. These patients have been compared with a control group of 733 women aged 30.6 ± 2.3 years, undergoing ovarian stimulation because of tubal or male infertility.

General characteristic and ovarian stimulation aspects were analyzed.

Participants/materials, setting, methods: Out of the 212 oncological patients, 96 had hematologic cancer, 61 had breast cancer, 35 had gynecological neoplasms and 20 had other tumors (brain, thymic, melanoma and colon). All patients were stimulated with FSH-r and GnRH antagonist or antagonist. Aromatase inhibitors were used in hormone-sensitive neoplasm. In the control group 733 women were considered. Abnormal Ovarian reserve was an exclusion criteria. Only mature oocytes were cryopreserved. T-student test was used to analyze the data.

Main results and the role of chance: Both groups presented comparable age (30.5 ± 6.1 versus 30.6 ± 2.3 P=NS) and FSH levels (6.7 ± 4.3 versus 6.6 ± 2.0 P=NS). However oncological patients showed lower AMH levels (2.18 ± 2.65 versus 3.5 ± 2.6 P<0.0001) and AFC (8.42 ± 3.9 versus 10.5 ± 3.3 P<0.0001).

No differencies in duration of ovarian stimulation in oncological patients and in healthy patients were detected (11 ± 3.4 versus 11.2 ± 2.4 P=NS).

Cancer patients needed significantly higher doses of FSH-r compared with controls (2182.5 ± 1235.3 UI versus 2025 ± 783 UI, P<0.05)

Oncological patients and controls produced a similar number of total follicles. (12.13 ± 6.7 versus 12 ± 5.2 P=NS)

On the other hand, cancer patients presented lower estradiol peak than controls (1148.65 ± 1235.38 versus 1913 ± 1490 P<0.0001).

There was no statistical difference in the number of oocytes retrieved (8.92 ± 6.24 vs 8.3 ± 4.8 P=NS) and mature oocytes cryopreserved (7.24 ± 4.75 vs 6.8 ± 4.4 P=NS) between cancer patients and control.

Limitations, reasons for caution: The estradiol peak reached during the ovarian stimulation is influenced by the use of the Aromatase inhibitors in breast cancer hormone-sensitive tumor patients.

Wider implications of the findings: Present study shows a reduced ovarian reserve in cancer patients. However, despite low AMH and AFC values, oncological women can produce the same number of oocytes suitable for cryopreservation as observed in non cancer patients.

Trial registration number: \

P-530 Protective effect of visnagin on doxorubicin-induced follicular apoptotic activation in ovarian tissue of rat

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Study question: Is there any efficacy of viscagin treatment to prevent tissue damage at cellular level in doxorubicin-induced ovarian toxicity?

Summary answer: Visnagin reduces the loss of follicles and protects the ovarian reserve against the gonadotoxic effect of doxorubicin.

What is known already: Chemotherapy causes loss of primordial follicles and impaired follicular maturation. Invitro studies suggest that apoptotic changes in pregranulosa cells lead to follicular damage. As a result of doxorubicin-induced toxicity, oocytes exhibit chromosome condensation, altered gene expression and oxidative stress. Oxidative stress at the cellular level causes DNA damage. There is an increase in apoptotic activation and an increase in follicular atresia and a decrease in ovarian reserve. Visnagin is an organic chemical compound and a furanochromone. It was reported that visnagin protects against doxorubicin-induced apoptosis in cultured cardiomyocytes.

Study design, size, duration: Experimental study conducted at Uludag University School of Medicine, the department of Histology and Embryology between 2016-2017 in master's thesis and licensed by the local ethics committe. A total of 24 Wistar albino adult female rats were used in the study. Ovarian toxicity was established with doxorubicin treatment in rats. Therapeutic effect of visnagin on ovarian toxicity was evaluated light microscopically and immunohistochemically.

Participants/materials, setting, methods: Female Wistar Albino rats that were six months of age (n = 24) divided 4 groups: GroupI: intraperitoneal (ip) saline, GroupII: ip doxorubicin (21 mg/kg), GroupIII: ip visnagin (30 mg/kg), GroupIV: ip doxorubicin+visnagin injected groups for 7 days. Vaginal smear was performed on all rats before application for drug administration and ovarian dissection. Paraffin tissue sections were stained with hematoxylin&eosin to morphological analysis. Apoptotic activation of ovarian follicles at different stages of development was assessed by the TUNEL assay.

Main results and the role of chance: In the doxorubicin group, there was a decrease in the numbers of primordial (p = 0,001), secondary (p = 0,002) and graafian follicles (p = 0,001) compared to the control, visnagin and doxorubicin + visnagin groups, and a significant increase in atretic follicle counts (p = 0,001), but there was no statistically significant difference unilaminar and multilaminar

primary follicles between groups. In the doxorubicin+visnagin group, the numbers of the secondary and graafian follicles were similar to those of the control and visnagin group, and the atretic follicle numbers decreased significantly compared to the doxorubicin group (p = 0,002). In vivo administration of doxorubicin resulted in an increase in the apoptotic granulosa cells poured into the antral fluid as a morphological indicator of atretic follicles. It was observed that the connections between the granulosa cells and the oocyte were deteriorated and inclusions were formed in the follicular cells. In granulosa cells of the secondary and graafian follicles, degeneration findings were observed in all follicles, with the pyknotic nuclei more prominent in the secondary follicles. The treatment of visnagin following the doxorubicin has a protective effect against the gonadotoxicity caused by doxorubicin. This effect showed protection in the number of primordial and developing follicles and decrease in the number of atretic follicles.

Limitations, reasons for caution: The limitations of the study is an experimental study and apoptotic activation could not be confirmed with different method.

Wider implications of the findings: This is the first study to show the protective effect of visnagin against the toxic effect of doxorubicin on the ovary. Visnagin therapy following chemotherapy is effective in protecting both of the primordial follicle pool and the developing ovarian follicles.

Trial registration number: The study is not clinical trials.

P-531 Social egg freezing in Israel – Do women take advantage of their opportunities?

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Study question: Do women utilize their legal privilege to perform up to 4 elective retrievals in order to maximize their chances for a future live birth?

Summary answer: Most women will undergo only one oocyte retrieval, even though the number of cryopreserved oocytes is less than optimal to ensure a live birth.

What is known already: In Israel, oocyte freezing has been approved in 2011, allowing women between the ages of 30-40 years to self-finance up to 4 retrievals or until 20 eggs are obtained (whichever comes first).

Previous studies indicate that 15 metaphase II oocytes will result in 90% cumulative live birth rate in women ≤35 years old, compared to 35% for women >35 years old.

Limited data has been published regarding this intervention results in Israel.

Study design, size, duration: A retrospective cohort study performed at a single academic center between January 2011 and December 2017. Computerized medical records of all fresh IVF/ICSI cycles during the study period were screened to identify patients undergoing social egg freezing.

Participants/materials, setting, methods: Patient's and cycle's data were collected and analyzed including age at first cycle, marital status, superovulation protocol, ovarian reserve markers, number of treatment cycles, number of retrieved oocytes, and number of oocytes/embryos frozen.

Main results and the role of chance: During the study period, 47 women underwent a total of 61 social fertility preservation cycles (22% of preservation cycles during the study period). A total of 19 cycles were performed during 2017, as compared to only one cycle in 2011.

Seventy five percent of women were > 35 years old when the first cycle was performed, mean age at first cycle was 36.4 ± 2.1 years.

The majority of women (89%) were single, 95% of them had no children and 23.3% had ovarian reserve markers indicating reduced ovarian reserve prior to the first cycle.

Seventy percent of the women underwent only one cycle, 12% had two cycles and 8% ≥ 3 cycles.

Antagonist protocol was used in 95% of patients.

The mean number of retrieved oocytes at first cycle was 14.8 ± 11.9, with a mean of 11.9 ± 9.9 oocytes being frozen. Four women choose embryo freezing rather than egg freezing.

Only 21% of women at first cycle, yeilded ≥ 20 frozen eggs.

One woman, 34 years old, yeilded 39 frozen eggs.

25% of the cycles ended in freezing less than 5 oocytes.

Limitations, reasons for caution: The main limitation is the retrospective nature of the study and the small sample size, which may limit its power.

Wider implications of the findings: Women do not exploit their full elective preservation options, most probably due to economic burden. Women who wish to perform social freezing should be encouraged to do so prior to 35 years old, and preferably more than one cycle, in order to maximize the chances for a future live birth.

Trial registration number: not applicable.

P-532 Assessing the effects of ovarian tissue ultra-rapid cryopreservation by vitrification on primordial-stage oocytes

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Study question: Is immediate post-thaw assessment of the effects of ovarian tissue vitrification on primordial-stage oocytes accurate?

Summary answer: Assessing ovarian tissue immediately after thawing does not reveal the full extent of cryodamage caused to primordial-stage oocytes by ultra-rapid cryopreservation procedures.

What is known already: To date, the optimal laboratory protocol for ovarian tissue cryopreservation remains unclear. Although slow freezing is the currently standard procedure for ovarian tissue cryopreservation, there is a need to further develop cryopreservation techniques in order to reduce the degree of cryodamage. Ultra-rapid cryopreservation by vitrification has significantly advanced in the last decade and recent studies have suggested equivalent or even better results for ovarian tissue preservation. However, high concentration of cryoprotectants used in vitrification may cause high oocyte toxicity. Observing the effects of vitrification on the primordial-stage oocyte level is important in designing vitrification protocols that will minimize cellular damage.

Study design, size, duration: This was an *in vitro* mouse model study. Six intervention groups were employed, namely fresh, no *in vitro* culture group; fresh, 24 hours *in vitro* culture group; cryo-group 1, no *in vitro* culture; cryo-group 1, 24 hours *in vitro* culture; cryo-group 2, no *in vitro* culture; and cryo-group 2, 24 hours *in vitro* culture.

Participants/materials, setting, methods: Rec8 transgenic mice were used to develop an assay to assess primordial-stage oocytes damage following ovarian tissue vitrification. All animal procedures were approved by the UK Home Office and were carried out in accordance with '3Rs' principle – replacement, reduction and refinement. Cell death – using TUNEL assay and immunofluorescence analysis of activated Caspase 3 –was assessed at two time points: immediately after thawing and after 24-hours *in vitro* culture of thawed ovarian tissue strips.

Main results and the role of chance: Assessment of cell death was determined by a Staurosporine-validated assay that involved TUNEL and Caspase-3 labelling. We found that the proportion of primordial-stage oocytes staining positive for TUNEL and/ or Caspase-3 was higher in vitrified/ thawed ovarian strips analyzed immediately after thawing than in untreated (control) ovarian strips. This increase in the number of apoptotic primordial-stage oocytes was noted regardless of the cryopreservation media used and was evident in all biological repeats. Similar results were noted following 24 hours *in vitro* culture of untreated and treated ovarian strips. Comparison of the binomial proportion of primordial-stage oocytes staining negative for TUNEL and Caspase-3 and positive for TUNEL and/ or Caspase-3 showed a statistically significant difference among the six

intervention groups; $\chi^2(5) = 93.14$, p<0.0005. Post-hoc analysis showed that after 24 hours *in vitro* culture of vitrified/ thawed ovarian tissue strips, there was a statistically significant increase in the total number of primordial-stage oocytes that stained positive for TUNEL and/ or Caspase-3 for cryo-group 2 but not for cryo-group 1 nor for the untreated group. This finding was further supported by quantitative analysis of primordial-stage oocyte DNA fragmentation using the TUNEL assay for each of the six intervention groups; Kruskal-Wallis test: $\chi^2(5) = 326.749$, P<0.001.

Limitations, reasons for caution: Although our model allows cellular assessment of vitrification effects, the use of a mouse *in vitro* model constitutes a study limitation. Significant species differences limit the transferability of our findings beyond the mouse model. Moreover, protection mechanisms available from the mouse organism were eliminated due to the *in vitro* methodology.

Wider implications of the findings: To improve the effectiveness of ovarian tissue cryopreservation, cryodamage caused to primordial-stage oocytes should be minimized. Our *in vitro* mouse model and validated cell death assay could be used as a prototypes for a human ovarian tissue model that will allow optimization of ovarian tissue freezing for fertility preservation.

Trial registration number: N/A.

P-533 Influence of breast cancer prognostic factors on in-vitro maturation outcomes in patients seeking urgent fertility preservation

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Study question: Do the prognostic and predictive factors for breast cancer (BC) influence *in-vitro* maturation of immature oocytes (IVM) outcomes in patients undergoing fertility preservation (FP)?

Summary answer: Patients with SBRIII tumors had significantly less cryopreserved oocytes and HER2 overexpression was correlated with a decreased risk to cryopreserve less than 5 mature oocytes.

What is known already: Overexpression of HER2, Scarff-Bloom-Richardson (SBR) grade, triple negative status as well as high Ki67 expression represent prognosis factors for BC and are involved in the management of BC patients. On the other hand, concerns exist about the potential impact of cancer status on ovarian reserve and function. Previous studies analysing Controlled-Ovarian Stimulation results for FP in cancer patients have shown conflicting findings. Nevertheless, there is no data on the potential impact of BC status and prognostic factors on IVM outcome in women undergoing urgent FP.

Study design, size, duration: From November 2013 to December 2016, we prospectively studied 321 BC patients, aged 18-41 years, and candidates for oocyte cryopreservation following IVM. Number of oocytes recovered, maturation rate and total number of cryopreserved oocytes were assessed.

Participants/materials, setting, methods: Ovarian reserve markers (Antral-Follicle Count (AFC) and serum Anti-Mullerian Hormone (AMH) levels) and IVM outcomes were compared according to BC prognostic factors (Ki67 > 20%, progesterone and/or estrogen receptors status, HER2 status and SBR grade). Then, logistic regression analysis was performed to determine the variables that could be independently associated with poor IVM outcomes determined as maturation rate < 60 % or number of mature oocytes cryopreserved < 5.

Main results and the role of chance: Overall, the mean age of the population was 32.3 ± 4.1 years. Mean AFC and serum AMH levels were 22.8 ± 13.9 follicles and 3.8 ± 3.1 ng/ml, respectively. AMH serum level was significantly lower in case of triple-negative BC as compared to ER/PR/HER2 status positive cancer (3.1 ± 2.6 ng/ml *vs* 4.0 ± 3.3 ng/ml, p = 0.02).

The mean number of recovered oocytes was 10.2 ± 9.1. After a mean maturation rate of 58 ± 26%, 5.8 ± 5.3 matured oocytes were cryopreserved per cycle. The number of retrieved and cryopreserved oocytes were significantly decreased in patients presenting SBRIII tumor as compared with SBRI or II (9.6 ± 8.7 *vs* 11.7 ± 9.8, p = 0.02 and 5.4 ± 5.4 *vs* 6.0 ± 5.8 p = 0.02 respectively).

Multivariate statistical analysis showed that HER2 positive status was positively correlated with a mean maturation rate < 60 % (OR: 0.54; 95% CI (0.30-0.97)). Ki67, SBR classification or hormonal status was not correlated with poor IVM outcomes.

Limitations, reasons for caution: The main limitation of our study is the lack of data concerning the true competence of these cryopreserved oocytes and subsequently the impact of BC prognostic factors on pregnancy rates following utilization IVM oocytes remains unknown.

Wider implications of the findings: BC prognostic factors could play a role in IVM outcome. Moreover, HER2 is likely to be involved in oocyte maturation process. Further investigations are needed to better understand these mechanisms and to objectively assess the real potential of IVM oocytes after cryopreservation.

Trial registration number: N/A.

P-534 Determination of ovarian primordial/primary follicle density in 1002 ovarian tissue biopsies and amh concentrations in 824 serum-samples for a better reflection of the ovarian reserve

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Study question: Enables the follicle density of primordial/primary follicles a better reflection of the ovarian reserve in cortex pieces of patients who underwent cryopreservation of ovarian tissue in terms of fertility protection?

Summary answer: Nomograms of follicle density FD and Anti-mullerian hormone AMH are similar in adulthood but different in childhood, as well the overall correlation of both parameters.

What is known already: Several parameters are suggested to estimate the ovarian reserve. These include the female age, the concentrations of FSH and Inhibin B, the antral follicle count AFC and the concentration of Anti-mullerian hormone AMH. Comparative studies have shown that AMH and AFC best reflect the response to gonadotropins. However, the antral follicles are only a small fraction of the total amount of ovarian follicles and AMH is already known to be a poor prognostic factor for the ovarian reserve in childhood.

Study design, size, duration: This retrospective observational study included 1.002 females (28,1 y ±6.9, 3-45 y) with malignant (n = 955) and benign (n = 47) diseases who cryopreserved tissue in a single center as part of a national fertility preservation program. Patients with ovarian surgery or genetic predispositions for a reduced ovarian reserve had been excluded. Follicle densities (FD) (n = 1.002) and AMH concentrations (n = 824) were evaluated from 03.2011 to 09.2016.

Participants/materials, setting, methods: Three standardized 2-mm biopsies, obtained from different areas of prepared ovarian cortex were collected, FD analysis was performed after tissue digestion and calcein staining (counting of fluorescent/viable PROF/PRIF follicles). Serum AMH was measured (before gonadotoxic therapy starts) using the AMH Gen II ELISA Kit. Statistical nomograms of FD/AMH were drawn in relation to age as well statistical correlation analyses of FD and AMH in two age groups (≤ 25 y/≥ 25 y).

Main results and the role of chance: First-time that a nomogram of FD as a function of age and in correlation with the AMH concentration in serum-blood is published. Thus, the cortex ovary reserve was analyzed directly and

not on the basis of known surrogate parameters. AMH concentrations were lower in childhood, increased to a peak in early adulthood and decreased afterwards. In contrast, FD were highest in childhood and decreased with increasing age. Our correlation analysis of FD with AMH, the marker with the highest prognostic prediction probability for an ovarian response, allowed us to estimate to what extent the AMH concentration correlates with the density of PRIF/PROF follicles. In the age group ≥ 25 y there was a significant better correlation of AMH and the FD ($r = 0.3199$, $p < 0.0001$) compared to the younger group (≤ 25 y), $r = 0.0539$, $p = 0.4022$, probably due the fact that the ovarian volume and surface area are lower in childhood.

Due the limited prognostic value of AMH for the FD, it should be questioned, if in terms of transplantation of ovarian tissue the evaluation of the individual FD is a more valid tool to estimate the amount of ovarian tissue needed for transplantation.

Limitations, reasons for caution: This study showed the FD in single cortical biopsies. Although the analysis and averaging of three biopsies increases the accuracy of the analysis, it is still not possible to accurately determine a heterogeneous FD.

Wider implications of the findings: The analysis of ovarian reserve by FD rather than surrogate parameters is one of the strengths of this study. Nevertheless, as ovarian FD was measured, an estimation of total concentration of follicles requires a surface correction regarding small size ovaries in childhood and the larger size ovaries in young adulthood.

Trial registration number: not applicable.

POSTER VIEWING
Nursing and midwifery

P-535 Fertility nursing education and career progression framework

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Study question: Fertility nursing is a specialist area of practice where nurses are at the forefront of an emerging care setting, do we need to provide an educational framework to support this?

Summary answer: Fertility nursing encompasses the care and practices undertaken by any registered nurse/midwife providing fertility care, an educational framework is paramount for those individuals.

What is known already: Fertility nurses have increased levels of responsibility. Political drivers, workforce requirements and nursing care require multidisciplinary collaboration; whilst it is recognised that not every nurse/midwife will aspire to masters level advanced practice, all are expected to reach the maximum potential expected of their role in the context of competence and knowledge.

Equally the need for nurses to become specialists in their particular field of practice, to enhance overall service provision, through clinical leadership and be recognised as expert practitioners includes understanding the socio economic and political dimensions to delivering care, and ensuring service meets the needs of those seeking fertility care.

Study design, size, duration: A career progression and education framework has been constructed by senior fertility nurses, professional stakeholders and peers. The framework has been published and shared with professional stakeholders for review. The framework is a publication and booklet for fertility nurses and Health care assistants throughout the UK and contains tools for self assessment for competency. The project was the focus of the group for 2017 and has taken a total of 5 months to complete.

Participants/materials, setting, methods: The project group consisted of 12 core contributors, 3 external advisers and a wider group of stakeholders. The work was mainly constructed by individuals of the core group and forwarded to the group chair for amalgamation and proofing. Teleconferences and face to face meetings took place to discuss project content and for any amendments to be made. Each member of the core group were responsible for separate parts of the document.

Main results and the role of chance: This Fertility Nursing Education and Career Progression Framework is intended to facilitate a conversation, and enable the building of a career pathway for all nurses and healthcare assistants in fertility services. The aim is to ensure development and progression of knowledge and expertise towards enhancing the quality of service provision in Fertility Nursing Care.

This framework could also be used by individual nurses to assess their ongoing competence, and prepare for re validation.

All newly appointed nurses and HCA's should have an identified mentor to support their continuing professional development.

This Fertility Nursing Education and Career Progression Framework is intended to inspire individual nurses and HCA's to progress their career to continue to provide a quality service for those seeking fertility advice and management across the care provision.

Limitations, reasons for caution: This project serves as a reminder that anyone providing care should not undertake a procedure unless competent to do so, whilst considering how they can best ensure competence and confidence to carry out activities that expands their scope of practice.

Wider implications of the findings: Negative - Professional bias from other members of the MDT, increased costs for employers and increased training needs.

Positive - Improves patient care and satisfaction, Morale boosting, better skill mix, provides a key pathway for career progression.

Trial registration number: not applicable.

P-536 Development of a quality of life causal model for men undergoing fertility treatment

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Study question: The purpose of this study was to create a QOL (Quality of Life) cause-and-effect model for male patients undergoing infertility treatment in Japan.

Summary answer: The QOL was influenced by psychological Distress, lack of Spousal Support, prolonged infertility period as well as the male factor in a QOL cause-and-effect model.

What is known already: Patients undergoing infertility treatment become stressed from the physical burden of treatment, and their QOL also declines. An overview of 73 studies revealed that even though the mental health problems among the men were no higher than the general population, infertile men experienced infertility-specific anxiety and socially isolated men were more vulnerable to severe anxiety. Researchers from the USA identified life stress as a factor in reducing men's fertility; two or more stressful life events in the past year negatively affected men's sperm motility and morphology however, they found that job strain had no impact on sperm parameters.

Study design, size, duration: A descriptive quantitative cross-sectional questionnaire survey.

During the study period April to August 2016, we distributed 411 questionnaires to clients eligible for participation. A total of 332 (80.8%) were returned, and 321 were usable for analyses.

Participants/materials, setting, methods: This quantitative cross-sectional survey used a self-report questionnaire for 411 infertile male patients with and without male factor infertility from four human reproductive clinics in Japan. The four scales measured the main outcomes: FertiQoL (Japanese version), Psychological Distress, Social Support (spousal and workplace). The data were analyzed with descriptive statistics, multiple regression analyses and structural equation modeling.

Main results and the role of chance: Returned questionnaires were 332 (80.8%). Male factor cause of infertility was significantly associated with the FertiQoL scale score ($F = 5.174$, $p < 0.001$). The QOL was significantly lower in those with male factor infertility than in other causes or unknown cause. The three significant predictors of QOL were: (i) distress ($\beta = -0.53$, $P < 0.001$); (ii) Spousal Support ($\beta = 0.25$, $P < 0.001$) and (iii) infertility period ($\beta = -0.09$, $P < 0.05$). The structural equation modeling revealed the same factors were related to QOL.

Limitations, reasons for caution: Of the 600 fertility clinics in Japan only four urban facilities for data collection were used and were selected by a convenience sample instead of a random sample. The diagnosis and the details of the disease condition of infertile men with male factors were not investigated.

Wider implications of the findings: Infertile male factors, mental anguish, less spousal support, and longer period of infertility were associated with poorer quality of life for infertile men. These results suggested that focusing on infertility causes, distress conditions, length of infertility period, and support for couples' partnership are needed to maintain QOL during treatment.

Trial registration number: not applicable.

P-537 Evaluation of psychological distress in male patients undergoing assisted reproductive technologies (ART) treatments

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Study question: Are men undergoing ART treatment more emotionally distressed, in comparison to a reference population?

Summary answer: ART male patients seem to present lower emotional maladjustment and higher resources to cope with infertility than the reference population.

What is known already: The psychological characteristics of men coping with infertility and ART are understudied. A previous report analyzed men using an online infertility support group and found that they were feeling sidelined and marginalized in the process and in the way fertility professionals responded to them. DERA is a validated instrument of 48 questions with a 5-point Likert scale format developed to measure maladjustment and resources to affront maladjustment in both women and men. The validation study was performed in 490 patients, including 220 infertile men. We now investigate emotional distress and coping resources in heterosexual men undergoing ART with their partner.

Study design, size, duration: Cross-sectional study performed on 33 men between May 2017 and January 2018. Participants completed the DERA questionnaire at the end of their treatment. We evaluated the emotional maladjustment as well as the individual and interpersonal adaptive resources men may have for coping with ART. The sum of personal and interpersonal resources indicates the adaptive resources. The sum of the remaining answers indicates the emotional maladjustment.

Participants/materials, setting, methods: Participants were Spanish male patients undergoing IVF ($n = 22$) or IUI ($n = 11$) with their partner. In 6 cases donor sperm was used (1 IVF and 5 IUI). The study was proposed to each patient by an ART nurse. The participants filled in the questionnaire on the day of the ET or IUI.

Main results and the role of chance: The mean age of participants was 36.2 ± 4.7 (range 29-52). The age of their female partner was 35.4 ± 3.5 (range 28-41). For 8 patients (75.8%) it was their first ART cycle, while 2 participants (6%) had children at the time of treatment. The sub-score of maladjustment was, on average, 51.3 ± 14.7 (range 27-90), with $P_{50} = 49$; the sub-score of personal resources 41.7 ± 4.7 (range 30–54), with $P_{50} = 42.0$; the sub-score of interpersonal resources 45.1 ± 4.7 (range 35-55), with $P_{50} = 45.5$ and the adaptive resources 86.7 ± 7.7 (range 70-109), with $P_{50} = 86$. Overall, our results (P_{50}) indicate lower maladjustment and higher resources to cope than those of the reference population of the questionnaire (54-55, 41-42, 43 and 84 for, emotional maladjustment, personal resources, interpersonal resources and adaptive resources, respectively).

When focusing on patients who needed donor sperm for their treatment, we observed a slightly higher maladjustment (53.0 ± 8, $P_{50} = 49.5$), and lower personal, interpersonal and adaptative resources (37.5 ± 4.7, $P_{50} = 35$, 40.8 ± 5.0, $P_{50} = 41.5$, and 78.3 ± 6.7, $P_{50} = 81.0$, respectively); these results should be however confirmed in a bigger cohort.

Limitations, reasons for caution: The main limitation of this study resides in the relatively low number of patients included, in line however with several studies regarding male ART patients.

Wider implications of the findings: Male patients accessing ART might benefit from individual psychological counseling and support; the impact of using donated sperm on male patients' emotional distress should be explored further in future investigations.

Trial registration number: Not applicable.

P-538 Self-care (behavioral intention) during pregnancy and postnatal depression in females who conceived with in vitro fertilization (IVF)

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Study question: This study compared the incidence of postnatal depression between patients conceiving with in vitro fertilization (IVF; IVF group) and naturally (natural pregnancy group).

Summary answer: At 1 month after delivery, the IVF group showed a more marked tendency to develop postnatal depression compared with the natural pregnancy group.

What is known already: Using the Edinburgh Postnatal Depression Scale (EPDS) (full score: 30) developed by Cox et al. (1987), Okano et al. (1996) defined females showing a score of 9 or higher at 1 month after delivery as EPDS-positive, with a cut-off point of 8/9. Manabe et al. (2001) developed the Self-care Scale for Pregnant Females (full score: 160), consisting of 4 subscales: <prevention and early discovery of abnormalities in pregnancy (40)>, <preparation for delivery and mothering (40)>, <dietary habits (40)>, and <care in daily life (40)>.

Study design, size, duration: Among the 529 patients who had delivered in our facility within the period between May and December 2017, 457 and 72 were classified into the natural pregnancy and IVF groups, respectively. At one month after delivery, we evaluated the 529 patients using the 30-point EPDS revised by Okano. Furthermore, at week 12 of pregnancy, we examined the self-care consisting of 4 subscales in the Self-care Scale for Pregnant Females of Manabe.

Participants/materials, setting, methods: In both groups, those showing an EPDS score of 8 or lower and that of 9 or higher were defined as EPDS-negative and -positive, respectively. Subsequently, in the IVF group (12), scores from the Self-care Scale for Pregnant Females (<prevention and early discovery of abnormalities in pregnancy>, <preparation for delivery and mothering>, <dietary habits>, and <care in daily life>) during pregnancy (at week 12 of pregnancy) were compared between EPDS-negative (5) and -positive (7) members.

Main results and the role of chance: Compared with the natural pregnancy group, the IVF group showed significantly higher EPDS scores, with a markedly higher rate of being EPDS-positive. Furthermore, on comparing EPDS-positive and -negative members of the IVF group, the former showed significantly higher

<care in daily life>-related scores from the Self-care Scale for Pregnant Females, suggesting that they faced more marked stress when performing <care in daily life> as part of self-care during pregnancy. In addition, the duration of outpatient treatment was longer among EPDS-positive than -negative members, although the difference was non-significant. There were no significant differences in parity or a history of miscarriage.

Limitations, reasons for caution: The presence/absence of postnatal depression represents the psychological status of patients who conceived naturally or with IVF at 1 month after delivery. Similarly, their scores from the Self-care Scale for Pregnant Females represent their psychological status at week 12 of pregnancy. Stress assessment is based on an individual's perception.

Wider implications of the findings: Compared with the natural pregnancy group, the IVF group showed a more marked tendency to develop postnatal depression (higher EPDS scores). Furthermore, on comparing scores from the Self-care Scale for Pregnant Females, EPDS-positive of the IVF group showed significantly higher scores related to <care in daily life>.

Trial registration number: None.

P-539 Multidisciplinary gestational surrogacy care in absence of a legal framework

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Study question: How to setup gestational surrogacy care at a medical setting, in the absence of a legal framework?

Summary answer: In absence of legislation for surrogacy, thorough criteria, evaluation by and cooperation within psychology, reproductive medicine, midwifery and obstetrics are necessary to limit related risks.

What is known already: In many European countries, surrogacy is not officially allowed nor prohibited. Consequently, the persons involved are not legally protected and are exposed to several risks.

The woman who gives birth is recognized as legal mother until an adoption procedure is completed and approved in court. This implicates that theoretically the surrogate may claim motherhood and intended parents can abandon their child.

Furthermore obstetrical and fetal complications and emotional distress may occur.

Partially due to the absence of a legal framework, hospitals have a responsibility to ensure a successful outcome. Legislating gestational surrogacy is strongly adviced by fertility centres to diminish risks and responsibilities.

Study design, size, duration: In 2014, a core surrogacy team was installed at an academic fertility centre. The team consists of a gynecologist, psychologist and a midwife, all with a fertility subspecialisation. In order to implement a gestational care program, at first a literature study was conducted, secondly team visits to other surrogacy providers were scheduled and finally the team consulted a lawyer specialised in family law.

Participants/materials, setting, methods: Our research indicated that surrogacy care requires a broader approach than just conventional fertility care. Therefore, gynecologists and midwives, subspecialised in obstetrics joined the team to ensure a complete approach and follow-up, from screening to handing over of the newborn. The surrogacy-program includes a thorough screening procedure, based on clear criteria for acceptance, a clinical pathway and a detailed description of the role of each stakeholder, in order to reduce possible risks.

Main results and the role of chance: We provide a program based on altruistic surrogacy by performing IVF-treatment using only gametes of the intended parents and single embryo transfer. Only surrogacy projects where a long-standing history of friendship or kinship with the surrogate is observed are accepted.

First both intended parents and surrogate are referred to a lawyer specialised in family law where the absence of a legal framework, all risks and rights, and the adoption procedure are fully explained.

The reproductive gynecologist analyses medical indication and fertility status and is responsible for medical care and IVF-treatment.

During several consultations, the psychologist evaluates resilience, stability, motivation, interpersonal and marital relationships, coping strategies, indications of psychopathology and personality measures of intended parents, surrogate and her partner.

An obstetrician performs an obstetrical screening of the surrogate aiming to exclude any potential risk during pregnancy or childbirth.

When screening is completed, a report is submitted to the hospital's ethical committee for approval of the surrogacy treatment.

During the entire process, psychological support is provided by the psychologist assisted by the midwife. The midwife is responsible for navigating and counseling the patients, discussing the birth plan, obstetrical care and guidance during handing over after birth.

Limitations, reasons for caution: Not applicable.

Wider implications of the findings: Surrogacy is a complex process demanding a structured approach. A team coordinator who acts as single person of contact and manages all communication and cooperation is primordial to maintain patient centered care. Therefore, a midwife with reproductive and obstetrical background is best placed in order to achieve a favourable outcome.

Trial registration number: Not applicable.

POSTER VIEWING
Psychology and counselling

P-540 Religion, spirituality and faith: What is the real impact on assisted reproduction?

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Study question: Is there any impact of the patient's faith, religion and spirituality on the outcomes of intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: There is a significant relationship between patient's religion, spirituality and faith on ICSI outcomes.

What is known already: Worldwide, more than eight in ten people identify with a religious group. Many patients seek religion or spirituality to relieve suffering and find a source of strength to cope with the disease. It has long been documented a positive association between religious involvement and better adaptation to medical illness as well as increased level of well-being and quality of life. Lately, there has been considerable interest in exploring the impact of the spiritual and religious issues on the health of people with infertility diagnosis, however nothing is known about the influence of these factors on the results of Assisted Reproduction.

Study design, size, duration: This prospective study included 877 patients undergoing intracytoplasmic sperm injection (ICSI) cycles from April/2016 to June/2017. Patients filled a questionnaire with information on faith, religiosity, and spirituality. The results of the questionnaires were correlated with: patient's age, number of retrieved oocytes, fertilization rate, number and rate of high quality embryos, cycle's cancelation, and pregnancy result using One way ANOVA, followed by Bonferroni post hoc test and linear regression models.

Participants/materials, setting, methods: The study was performed in a private university-affiliated in vitro fertilization center. Women were asked about their religion; if they attend meetings of their religions, and in which frequency; if they keep in touch with leaders of their religions or if they pray, and in which frequency; whether their faith contributed to the decision to go through the treatment; and in a scale, from 0 to 10, how did they believe in the treatment success.

Main results and the role of chance: Patients were divided into: Catholics (n = 457), Spiritists (n = 114), Evangelicals (n = 138), Other (n = 52), and No

religion (n = 98). Eighty patients were not willing to respond to the questionnaire. The significant effects of the religions on ICSI outcomes are described in Table 1. An increased pregnancy rate was observed in patients who reported including infertility diagnosis and treatment in their prayers (31.4% vs. 5.5%, p = 0.039). The high-quality embryos rate increased among patients who answered that their faith contribute to their decision to undergo infertility treatment (38.,9% vs. 28.4% p = 0.012) and those who belief in the treatment's success (RR: 0.001–0.0013, p = 0.022), while it decreased if they affirmed that their faith was affected by their infertility diagnosis (29.9% vs. 39.2% p = 0.050) or that religiousness had negatively affected the decision to undergo infertility treatment (8.24% vs. 36.8%, p = 0.010).

Limitations, reasons for caution: This questionnaire is subjective and may not reliably reflect the actual potential of the patient's faith and degree of religiosity/spirituality. Further bias may come from how prayer, spirituality and faith are understood, duration or types of prayer, or conditions in which prayers, contact with leaders, or religious meetings are performed.

Wider implications of the findings: Spirituality plays a role in adjusting psychological aspects of an infertile patient. Given that prayer or other spiritual approaches are safe and low-risk strategies, ART professionals need to be aware of the use of these strategies as non-pharmacological, non-invasive adjunct therapies.

Trial registration number: None.

P-541 The unwillingness to be in contact with the painful experience of infertility: experiential avoidance as a mediator in relationship between infertility-related stress and depressive symptoms

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Study question: Does experiential avoidance play a mediating role in the relationship between infertility related stress and depressive symptoms?

Summary answer: Representations about parenthood were associated with depressive symptoms indirectly, throughout the association with the impact of infertility in women's life and use of experiential avoidance.

What is known already: Experiential avoidance or psychological inflexibility is described as encompassing two related components: unwillingness to remain in touch with aversive private experiences (including bodily sensations, emotions, thoughts, memories, and behavioral predispositions), and action to change aversive experiences or the events that trigger them. It has been suggest that experiential avoidance can be seen as a vulnerability factor for the development and maintenance of psychopathology. Studies addressing experiential avoidance in people dealing with infertility are scarce but couples with an infertility diagnosis pursuing medical treatment presented significantly higher scores of experiential avoidance when compared to fertile couples and couples applying for adoption.

Study design, size, duration: Cross sectional study. Data were collected online through self-report instruments between February 2016 and June 2016. Inclusion criteria were age (18 years or older), and an infertility medical diagnosis. The study was conducted in a sample of 124 infertile women pursuing medical treatment.

Participants/materials, setting, methods: A sample of 124 infertile women pursuing medical treatment completed online the following standardized self-report measures: Fertility Problem Inventory (FPI), Acceptance and Action Questionnaire (AAQ-II), and the depression scale of the Depression, Anxiety and Stress Scale (DASS-21). Patients were invited to participate through a post on the national patients association, gave their informed consent and were asked to fill in the questionnaires. The mediation model analysis was tested with the SPSS Process macro.

Main results and the role of chance: The model examined the direct and indirect effect of representations about parenthood on depressive symptoms, by modeling the mediation effects of representations about the importance of parenthood on the impact of infertility on participants' life domains and of this on experiential avoidance. The direct effect of representations about parenthood on depressive symptoms was nonsignificant (effect = .04, SE = .02, *p* = .127). The indirect effect of representations about the importance of parenthood on depressive symptoms, while modeling the influence of the intervening variables, was statistically significant (Indirect effect = 0.03, 95%CI: [.015; 050]). An alternative model considering that people facing infertility that highly value parenthood tend to avoid situations and feelings related to children and this may be associated with higher impact of infertility on life domains (e.g. social isolation, poor sexual relationship), which would be associated with depressive symptoms was also tested. The result on the indirect effect of this serial multiple mediation model included 0 and therefore was not significant (Indirect effect = 0.01, 95%CI: [.00; 03])

Limitations, reasons for caution: The findings rely on cross-sectional and self-report data. Even though a sequential mediation effect was tested, the framework to establish the sequence of the variables is a theoretical one, and not a temporal one. The online recruitment tends to recruit more educated participants, with easy access to online platforms.

Wider implications of the findings: Results suggest that the tendency of infertile women to avoid thoughts, emotions and situations associated with parenthood, as a way of trying to escape from the infertility painful experience, induces higher suffering. Consequently, the core emotional regulation process of experiential avoidance should be addressed by psychologists in the clinical context.

Trial registration number: Not applicable.

P-542 The implications of the religious/spiritual beliefs and practices among commercial gestational carriers in the United States for international surrogacy arrangements

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Study question: What are the implications of the religious/spiritual beliefs and practices of commercial gestational carriers (GCs) in the United States for mental health evaluations in international surrogacy arrangements?

Summary answer: Discussing religion/spirituality within a mental-health evaluation serves a GC's psychological best interests and may help to match them with intended parents (IPs) from other countries/cultures/religions.

Table 1 The effect of patient's religion on ICSI outcomes, controlled for patient's age.

	Patient's religion					
	Catholic	Spiritist	Evangelical	Other	No religion	p
Fertilization Rate	82.0 ± 1.7[a]	89.9 ± 9.3[b,c]	87.0 ± 3.5[e]	72.4 ± 3.7[b,d,f]	70.6 ± 3.5[b,d,f]	**0.007**
High quality embryos	2.6 ± 0.2[a]	3.6 ± 0.4[b]	3.0 ± 0.3[b,d]	0.9 ± 1.3[c]	1.2 ± 0.6[c]	**0.043**
High quality embryos rate	32.6 ± 2.3[a]	47.1 ± 3.3[b]	45.1 ± 4.3[b]	36.3 ± 9.7[c]	43.1 ± 5.1[b]	**0.043**
Pregnancy Rate	32.9%[a]	58.0% [b]	33.9%[c]	31,2.%[a]	23,0%[d]	**0.047**

a≠b, c≠d, e≠f

What is known already: Religious customs and beliefs influence both the practice and prohibition of surrogacy around the globe. It has been theorized that religious and spiritual themes are more likely to emerge among altruistic (unpaid) surrogates. Little is known about the religious/spiritual beliefs and practices of commercial surrogates in the United States (US) and how these beliefs influence the process of surrogacy. No studies to date have examined how incorporating a discussion on religious/spiritual beliefs and practices might contribute to both the psychological evaluation process of potential GCs and the matching process with IPs who are coming from other countries and cultures.

Study design, size, duration: This is a qualitative research study using a grounded theory approach. 17 GCs raised in diverse denominations of faith were recruited via an ad posting from across the United States to participate in this study. GCs were interviewed using in-depth, semi-structured interviews that were conducted via Skype. Interviews lasted between 60-90 minutes and took place over a 4-month period (January through April, 2017).

Participants/materials, setting, methods: 17 GCs had a total of 28 surrogate pregnancies (36 surrogate children). 76% (n = 13) of participants worked with international IPs. Participants came from a variety of religious backgrounds (Mormon/LDS, Catholic, Baptist, Christian). GCs were recruited via an ad posting on websites frequented by carriers and through surrogacy agencies. In-depth, semi-structured interviews were conducted via Skype. Interviews were audiotaped and transcribed verbatim. Grounded theory of analysis of data included line-by-line coding and emergent themes were identified.

Main results and the role of chance: Themes emerging from the narrative data included Gestational Surrogacy as a Religious/Spiritual "Calling"; Motivation for Surrogacy Rooted in Faith Beliefs; Gestational Surrogacy as Providing Existential Meaning and Purpose to Life; Role of Religious/Spiritual Beliefs in Relationships with IPs. Some GCs felt their surrogacy experiences were part of "God's plan" that shaped their future trajectory providing existential meaning to life. GCs felt that their decision to become a surrogate was "doing the will of God" and that they were "furthering God's kingdom". GCs felt they were providing a positive role model for their children by modeling Christian values. In the words of one GC (when asked whether it was important for mental health professionals to ask about religious/spiritual issues in the psychological evaluation): "My religion is very important to me - it's part of who I am - it's part of who I've become - I definitely think this is something that should be talked about."

Unexpected findings included reticence on the part of some GCs in sharing their belief system in their psychological evaluation for fear of being excluded based on their beliefs or feeling that their beliefs would be seen as psychopathology.

The role of chance in these results is minimal.

Limitations, reasons for caution: This is a qualitative study and, therefore, the generalizability of the findings should be treated with caution.

Wider implications of the findings: Findings suggest that religious/spiritual narratives are not the exclusive domain of altruistic surrogates. Discussing religion and spirituality can enhance the psychological evaluation and counseling of potential GCs and may also help in matching GCs to IPs, particularly when they are international arrangements representing diversity in religious beliefs and practices.

Trial registration number: Not Applicable.

P-543 Somatising the desire toward a child among Hong Kong Chinese women undergoing IVF: The mediating role of anxiety

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Study question: Does anxiety mediate the effect of importance of childbearing on physical symptoms among Hong Kong Chinese women undergoing IVF?

Summary answer: Anxiety fully mediates the effect of importance of childbearing on physical symptoms among women undergoing IVF after controlling for demographic and clinical factors.

What is known already: Anxiety is a common emotional reaction among women undergoing IVF. The unpredictable and uncontrollable nature of IVF fuels anxiety toward its process and outcome. Anxiety could be somatised and manifested in physical symptoms such as fatigue, headache, insomnia, and muscle pain. The stake and therefore the anxiety toward the IVF treatment could be heightened if the couple regards having a child as essential to themselves, their relationship and the family. Hence, this study examined whether perceived importance of childbearing escalates physical symptoms of somatization, and whether anxiety mediates the relationship between such attitude and physical distress.

Study design, size, duration: This analysis utilized the baseline data of a randomized controlled trial that compared the effectiveness of a body-mind-spirit intervention in reducing the anxiety of women during the two-week wait before pregnancy test against two control conditions (health education and spiritual intervention). 225 participants who provided complete response to the items required in this analysis was included. The baseline data was collected in between 2015 and 2017 from the Queen Mary Hospital, Hong Kong.

Participants/materials, setting, methods: Women who were going to undergo IVF were recruited. The importance of childbearing was measured by a 4-item scale; anxiety was assessed by the State-Trait Anxiety Inventory (State scale); physical symptoms were evaluated by the 14-item physical distress scale of Body-Mind-Spirit Well-Being Inventory. The mediation analysis was conducted with PROCESS macro in SPSS with 5000 bootstrapped samples, and with women's and husbands' age, previous ART treatment, subfertility years, subfertility type, and causes of subfertility controlled.

Main results and the role of chance: Perceived importance of childbearing was positively correlated with state anxiety ($r=.31$; $p<.001$) and physical distress ($r=.20$, $p<.001$), and physical distress was positively correlated with state anxiety ($r=.35$, $p<.001$). After controlling for demographic and clinical characteristics, childbearing importance was significantly associated with physical distress in the total effect model, B = 0.69, SE = 0.23, [95%CI of B = 0.22-1.15], p=.0038. However, when state anxiety was added to the model, state anxiety was significantly related to physical distress, B = 0.70, SE = 0.14, [95%CI of B = 0.42-0.98], $p<.001$, and the association between childbearing importance and physical distress was no longer significant ($p>.05$). The indirect effect was statistically significant, B = 0.36, SE = 0.10, [95%CI of B = 0.20-0.59]. In other words, full mediation by state anxiety was found in the effect of childbearing importance on physical distress. The analysis was repeated by interchanging the role of physical distress and state anxiety, such that physical distress became the mediator. However, although the indirect effect was statistically significant, the mediation was partial as the direct effect from childbearing importance to state anxiety continued to be significant too. Hence, the former model was regarded as a more parsimonious representation of the relationship among childbearing importance, state anxiety and physical distress. Statistical significance was indicated by a p-value below 0.05.

Limitations, reasons for caution: Physical distress was evaluated by self-report of the participants. Recall bias and self-selection bias may reduce the generalizability of the findings.

Wider implications of the findings: Interventions that aim at reducing anxiety during IVF treatment should take into account personal value and expectations attached to childbearing and the somatization of such concerns. Our findings also highlight the interaction among values, emotions and physical distress, and provide empirical foundation for multi-component interventions that address these aspects.

Trial registration number: HKUCTR-1984

P-544 Typology of family involvement and fertility-specific Quality of Life among Chinese couples with infertility: A cluster analysis

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Study question: To identify subgroups of Chinese infertile couples based on their experiences with family emotional supports, economic supports and interference.

Summary answer: Four relatively distinct subgroups regarding family involvement were identified, namely, intrusive, detached, emotionally responsive, and economically supportive categories.

What is known already: Family involvements, largely divided into supports and interference were found to have mixed effects on wellbeing of married couples. As the primary social support reservoir, family supports served as protective factors for couples to overcome psychosocial distress caused by infertility. However, overwhelming interferences and unwanted inquiries from social networks could turn into detrimental stressors for married couples, especially during experiences of infertility. There are limited evidences regarding the typology of family involvements experienced by infertile couples, which constraint the development of family-oriented psychosocial services.

Study design, size, duration: A cross-sectioned study was implemented during October to December 2016. Current study recruited 429 infertile couples in a reproductive center affiliated to a public hospital in Beijing, China. Eligibility criteria for inclusion were couples who were (1) legally married; (2) medically diagnosed with infertility and (3) undergoing assisted reproductive treatments. After obtaining written consents, infertile couples were invited to take questionnaires separately and independently to avoid dyadic interference.

Participants/materials, setting, methods: Despite demographic information, family involvement scale and Fertility-specific Quality of Life were used to measure key variables. Mean age of wives and husbands were 34.21 ± 6.22 and 32.50 ± 5.12, respectively and couples had been married for six years approximately. More than half of participants received tertiary education (62.9%) and 69.4% had a full-time job. In terms of the infertility etiology, there are 252 female factor, 250 male factor, 170 both factor and 148 unexplained factor infertility.

Main results and the role of chance: Cluster analysis identified four subgroups regarding emotional responsiveness, economic supports and interference, namely, intrusive (44 pairs), detached (92 pairs), emotionally responsive (107 pairs), and economically supportive (178 supports) groups. There were significant between-group differences regarding FertiQoL scores ($F = 3.09$, $P<0.05$ for husbands; $F = 4.58$, $P<0.01$ for wives). Multiple comparison results indicated wives in intrusive subgroups show significant lowest emotional QoL, social QoL and total FertiQoL scores comparing to their counterparts in other three subgroups. For social QoL, husbands in detached subgroups show significantly lower scores than husbands in emotionally and economically supportive subgroups. Moreover, husbands in emotionally supportive subgroups show higher emotional QoL and total FertiQoL scores than husbands in intrusive and detached subgroups. In terms of relational QoL, both wives and husbands in emotionally and economically supportive subgroups reported significantly higher scores than their counterparts in detached and intrusive subgroups. No significant between-group differences on mind-body QoL for couples.

Limitations, reasons for caution: Current study carried several limitations. First of all, measures used to evaluate family supports and interference was universal, rather than being specific to infertility. Secondly, cross-sectional research design adopted by current study was constrained to draw causal inferences. Finally, self-selection bias might limit generalizability of results found in this study.

Wider implications of the findings: Despite person-centered and dyadic approach in infertility counseling, family-oriented interventions should be designed to improve quality of life for couples during experiences of infertility. Given the significance of emotional supports, it is extremely important to extend the therapeutic alliance through educating extended families regarding emotional competence and skills.

Trial registration number: Not applicable.

P-545 The mediating role of self-compassion between parental interference and quality of life among Chinese infertile couples: An actor-partner interdependence model

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Study question: This study aims to test the mediating role of self-compassion between the relationships between parental interference and quality of life (QoL) among Chinese infertile couples.

Summary answer: Self-compassion partially mediated the negative relationships between in-law/family-of-origin interference and QoL for both husbands and wives. No partner effects have been found in current study.

What is known already: Infertility, as a collective family crisis, could involve actions of the entire family. Studies conducted in Chinese context found the overwhelming attentions and unwanted inquiries from the extended families could cause psychosocial distress for infertile couples. Parental interference became a risk factor for the wellbeing of infertile couples, though differences between in-law and family-of-origin interference wellbeing still remained inadequate. Moreover, a dearth of studies has identified self-compassion as a mediating factor between family supports and psychological wellbeing; however, few studies explore its mediating role between interference as the opposite of supports, and psychosocial outcomes, particularly in infertility context.

Study design, size, duration: A cross-sectioned study was implemented during October to December 2016. Current study recruited 431 infertile couples in a reproductive center affiliated to a public hospital in Beijing, China. Eligibility criteria for inclusion were couples who were (1) legally married; (2) medically diagnosed with infertility and (3) undergoing assisted reproductive treatments. After obtaining written consents, infertile couples were invited to take questionnaires separately and independently to avoid dyadic interference.

Participants/materials, setting, methods: Despite demographic information, family involvement scale and Fertility-specific Quality of Life were used to measure key variables. Mean age of wives and husbands were 34.23 ± 6.21 and 32.49 ± 5.11, respectively and couples had been married for six years approximately. More than half of participants received tertiary education and 69.9% had a full-time job.

Main results and the role of chance: Current study adopted actor-partner interdependence mediation model (APIMeM) to investigate the mediating role of self-compassion in the negative relationships between parental interference (in-law/family-of-origin interference) and QoL among infertile couples. For the in-law interference, APIMeM results confirmed two significant mediation effects of self-compassion in the negative relationships between in-law interference and QoL for both husbands and wives ($b=-0.057$, $p<0.01$). In terms of family-of-origin interference, APIMeM results also showed two significant actor effects for both husbands and wives ($b=-0.07$, $p<0.001$). However, no partner effects have been found in all APIM models. These significant actor effects indicated that the mediating effects of self-compassion on the associations between parental interference and QoL only existed at the individual level, rather than the intra-couple level.

Limitations, reasons for caution: Current study carried several limitations. First of all, measure used to evaluate family interference was universal, rather than being specific to infertility. Secondly, cross-sectional research design adopted by current study was constrained to draw causal inferences. Finally, self-selection bias might limit generalizability of results found in this study.

Wider implications of the findings: To mitigate the negative effects of family interference, family-oriented psychosocial education should be developed to prepare extended families to support in a way that suited infertile couples' needs. Given the mediating role of self-compassion, counseling and interventions should aim at awaking and improving couples' inner strengths during experiences of infertility.

Trial registration number: Not applicable.

P-546 Effects of infertility on quality of life in Greek infertile women

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Study question: Can the infertility and its treatment affect the quality of life of infertile women?

Summary answer: The experience of infertility is highly stressful for women. In general, women have lower levels of fertility -related to quality of life.

What is known already: Infertility influences all the dimension of women life. Social and family pressure, well-being,change in marital relationship and in sexual satisfaction can lead to impairment in quality of life.Attention to their quality of life guarantees stability and satisfaction with life and establish an effective relationship between patient and her treatment team.Quality of life has become one of the most crucial issues nowadays and is seen as one of the measurable criteria for evaluation of treatment.Promotion of quality of life in infertile women requires a deep understanding of their problems from different aspects.

Study design, size, duration: We included 192 infertile women,who were attended the clinic Institute of Life, under at any stage of treatment, at the beginning of treatment and at the day of embryo transfer, from April to November 2017.

Participants/materials, setting, methods: This is a cross- sectional study in which 192 infertile women were (aged 30-45) participated.The subjects,who visited the Institute of life clinic, were selected through random sampling.Data were collected using a questionnaire which had two sections:demographic information and FertiQol questionnaire which measured the quality of life. The collected data were analyzed using SPSS version 16.

Main results and the role of chance: The result showed that were no significant difference between quality of life of infertile women and sociodemographic factors. A regression analysis was conducted and showed that age is characterized as a predictor of infertility.Age has be noted as a factor which is significant with infertility, on the other hand education of infertile women has be noted as a non significant factor that affects infertility in accordance with regression analysis of the present study.

Limitations, reasons for caution: We have only included women who were undergoing in vitro fertilization (IVF) treatment in the study.Due to practical reasons their partners did not participate.The result of the study may be influenced by the small sample size.

Wider implications of the findings: Data were obtained only from one clinical site. The present study would has a huge interest if also record couples or even men opinion about infertility.

Trial registration number: non clinical trial.

P-547 Health and wellbeing of adults conceived by IVF: a comparison of those who do and don't participate in a follow up study

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Study question: Will there be measurable differences in characteristics of participants and non-participants that could bias the results of a clinical review follow up study?

Summary answer: Based on questionnaire data obtained by telephone several years before, factors influencing participation in the current clinical review were not major confounders.

What is known already: As there is wide belief that common adult health problems have their origins in early life, the IVF population might be at an increased risk through epigenetic mechanisms. In our earlier telephone interview-based study of this cohort, differences between the IVF and a spontaneously conceived control group were found, i.e. increased respiratory conditions, such as asthma and hay fever, and increased hospital admissions in childhood and adolescence. In terms of clinical review, there have been few studies to date of adults conceived by ART but a small number report on cardiometabolic outcomes and suggest a difference.

Study design, size, duration: This retrospective cohort of 24-35 year olds was recruited for clinical follow up in 2016-2017. 530 IVF-conceived and 490 age and sex-matched, spontaneously conceived controls were invited to participate.

Participants/materials, setting, methods: Participants were those who had completed a telephone interview in an earlier study in Victoria, Australia, and who were willing to come to the hospital for a clinical review, which took approximately three hours, encompassing assessments of cardiovascular structure and function, cardiometabolic profile, respiratory function and growth. A comparison of characteristics (e.g. health, sociodemographic, psychosocial) of participants with non-participants was possible using the questionnaire data collected in the initial telephone interview study.

Main results and the role of chance: Of the 179 (33.8%) IVF-conceived and 81 (16.5%) controls who participated in the follow up study, females were more likely to participate than males (58% of IVF and 69% of controls). Active decline occurred in 21% of both groups and the remaining proportion did not respond to three invitations to participate. The following key variables demonstrated no significant differences between participants and non-participants within both the IVF and control study groups: number of hospitalisations in childhood, chronic illness, asthma, allergies, mental health, gestation at birth, mother's age and parity, BMI, perceived age at sexual maturity, fertility concerns, quality of life, financial status, educational attainment (level of schooling). In the IVF group, participants were younger than non-participants when initially interviewed (61% <20 years compared with 48%, $p = 0.01$) and fewer worked >37 hours/week (36% vs 46%, $p = 0.05$). In the control group, participants had more allergies than non-participants (26% vs 19.3%, $p = 0.03$), were less likely to be in a relationship (41% vs 58%, $p = 0.03$), and had more anxiety/behaviour problems (32% vs 21.5%, $p = 0.04$). In both groups, among those who had completed school, non-participants had a lower final average education score than participants ($p = 0.01$).

Limitations, reasons for caution: Comparisons are based on self-reported data without validation, but the same errors in reporting are likely to be seen across all groups. Also, there has been a time lag between the telephone interview and clinical review (5-6 years) and we have no knowledge of that intervening period in non-participants.

Wider implications of the findings: Although participation was lower than expected, the similarity of characteristics between participants and non-participants reduces concern about selection bias. Furthermore, knowing what the few differences between participants and non-participants are, enables sensitivity analyses. We therefore expect that the clinical review will contribute substantially to knowledge of long-term safety of IVF.

Trial registration number: not applicable.

P-548 Yoga as an adjuvant to enhance the outcome of IVF treatment

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Study question: To study the effect of Yoga on clinical pregnancy rates in women undergoing frozen embryo transfer.

Summary answer: This study suggests Yoga can be an adjuvant to improve the pregnancy rates in couples undergoing frozen embryo transfer.

What is known already: The distress of the diagnosis and the treatment of infertility is a known, well established fact. It can be confounding variable which can lead to poor treatment compliance, and hence outcome. Yoga is system of neuromuscular retraining which helps the organism to learn the relaxation response and a faster regaining of homeostasis after stress. Utilization of the techniques may help woman to cope with the roller coaster of psychophysiological states accompanying diagnosis and management of infertility.

Study design, size, duration: The study was conducted from 01.01. 2016 to 31.12.2017 at a private infertility centre. 150 women were randomly allocated to two groups. Group A (N = 77) attended 3 months of Yoga, thirty sessions- involving asana (exercises) and pranayama (regulated breathing) before undergoing frozen embryo transfer and Group B (N = 73) underwent frozen embryo transfer after 3 natural cycle without any Yoga adjuvant. Primary outcome was clinical pregnancy rates. Secondary outcomes were change in psychological test scores.

Participants/materials, setting, methods: Women with primary infertility > 5 years, who did not conceive in the first IVF cycle and were to undergo frozen embryo transfer in subsequent cycle were enrolled.Age > 38 years, Fibroid > 5 cm, either side hydrosalpinx, adenomyosis were excluded. Psychological assessment was done at baseline (S1) and after 3 months (S2) before ET using Hamilton Depression Rating Scale (HAM- D), Hamilton Anxiety Rating Scale (HAM-A) and FertiQol.

Main results and the role of chance: The Clinical Pregnancy rates were significantly better in Group A compared to Group B(49.35% vs 32.87%: p = 0.04). There was a significant S1 to S2 reduction after YOGA therapy in Hamilton Depression rating Scale (HAM- D), Hamilton Anxiety Rating Scale (HAM-A) (p< 0.001 for HAM-D and p<0.001 for HAM-A).The improvement in quality of life as seen on FertiQol but not statistically significant.

Limitations, reasons for caution: The study was done on a single centre, replication with more subjects and in different centres is needed.

Yoga encompasses a heterogeneity of techniques, there is a need to develop a standardised protocol suitable for the infertility patients which is simple, safe and effective.

Wider implications of the findings: Integrating **yoga** as an adjuvant to infertility treatment management may provide a simple, economic non invasive method which can enhance the outcome of the IVF treatment. It is cost effective and safe.

Trial registration number: MCDH/2016/54.

P-549 Fertility knowledge of Japanese university students who aspire to be schoolteachers in the future

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Study question: The aim of this study was to clarify the fertility knowledge of Japanese students who just entered the education department of one university.

Summary answer: Fertility knowledge among education students is insufficient, except for questions related to aging and smoking as risk factors.

What is known already: It is obvious that aging, STDs, obesity and smoking have a negative influence on fertility. Many previous studies reported that knowledge about fertility, reproduction and infertility among high school and university students is insufficient. Very limited research investigating fertility knowledge and attitudes toward fertility education among Japanese schoolteachers has been conducted. One study showed that the majority of teachers do not have enough fertility knowledge and are not confident in teaching this topic to students. However, the fertility knowledge of younger people who aspire to become schoolteachers in the future has not been clarified.

Study design, size, duration: We conducted a quantitative, cross-sectional questionnaire survey of first-year education department students in April 2017 at one university in the Kyusyu area. Questionnaires were distributed to all 291 students in an introductory psychology class that is required for all first-year education students. Only those who consented to participate in the survey were asked to complete the questionnaire.

Participants/materials, setting, methods: Although the response rate was 100%, those who were not first-year education students were excluded, leaving 252 participants.

The questionnaire asked about sociodemographic variables, desire to have a child, life planning experience, and daily life habits. To measure fertility knowledge, including the reproduction system and infertility, the Cardiff Fertility Knowledge Scale (13 items) and six original questions were used. To assess the relationship between fertility knowledge, gender and other variables, chi-square analysis was conducted.

Main results and the role of chance: 96.1% (242) of participants were 18-19 years old and 60.3% (152) were women. Although only 8.7% (22) currently had a sex partner, nearly 90% desired to have a child in the future. Approximately 70% experienced life planning in the past and almost 60% were interested in their own fertility.

The accuracy of knowledge about the effects of aging and smoking on fertility was comparatively high (71-90%). On the other hand, less than half answered the question about STDs as a risk factor of fertility correctly. As for questions concerning infertility (definition, frequency, gender ratio of cause), accuracy was not high (39-52%). The accuracy of the timing of pregnancy during the menstrual cycle was under 30%. Although over 60% correctly answered the question concerning the frequency of sperm production, only 4% correctly answered the question about the frequency of primordial egg generation.

As for fertility knowledge and gender, the accuracy of the question 'A woman who never menstruated is still fertile' among women was significantly higher than men. For the question about the frequency of sperm production, men had a higher accuracy rate than women. However, men's accuracy rate for the question about menopause was significantly lower than women.

Limitations, reasons for caution: The limitation of this study is that all participants were selected from only one university in the Kyusyu area in Japan. Therefore, caution should be taken when generalizing the results to the general population of Japanese university education department students.

Wider implications of the findings: From the present results, in order for younger people to have a child in the future when they desire, it is essential to give them accurate fertility knowledge. Therefore, first, schoolteachers should have accurate fertility knowledge to teach students. This might contribute to decreasing infertile patients due to age.

Trial registration number: not applicable.

P-550 Psychological influence of azoospermic male infertility diagnosis among men about to start in-vitro fertilization (IVF) treatment using donor sperm

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Study question: To compare psychological stress among men undergoing assisted reproductive technology (ART) with donor sperm compared to stress among men undergoing ART with own sperm.

Summary answer: The depression scores among males with donor sperm used as compared to males using their own sperm were significantly higher. The marital satisfaction was same.

What is known already: Studies comparing women and men found that women react more strongly overall to infertility. Some studies also suggest that men's experience of infertility is connected to threats to their masculinity. So, it was thus anticipated that even after donor sperm ART, male factor infertility could influence men's psychological well being. The aim of this study was to investigate whether a diagnosis of male azoospermic infertility had any influence on their experience of anxiety, depression and spousal relationship well being as compared to male partner in couples where the diagnosis was male, female, mixed or unexplained infertility.

Study design, size, duration: A cross sectional study was conducted with 100 couples attending the Out Patient Department of Akanksha IVF, Delhi for the first IVF cycle from January 2017 up to December 2017. 50 of these were diagnosed with azoospermic male infertility requiring donor sperm (Group 1), while the remaining 50 were diagnosed with other causes of male infertility,

female infertility, mixed or unexplained infertility using husband sperm (Group 2). Data were analyzed using SPSS software version 20.

Participants/materials, setting, methods: The study population comprised of 100 male partners who consented to participate in the study. A questionnaire was given to male partners at start of the ART cycle. They were asked to answer without communicating with their partners. They filled questionnaires on Beck's Anxiety Inventory (BAI), Beck's Depression Inventory (BDI) and Couple Satisfaction Index-16 (CSI-16). Data was analyzed. Means, standard deviation and ranges were used in descriptive statistics. Mann-Whitney test was performed for the scores.

Main results and the role of chance: The age and duration of infertility were analyzed in both the groups and the following values were ascertained. The mean age in the group of males requiring donor sperm (Group 1) was 36.86 ± 4.40 years and the mean age in the other group, (Group 2) was 36.76 ± 5.10 years. The mean value of duration of infertility in the donor treated sperm was 6.20 ± 3.07 years while the duration of infertility in Group 2 was 5.52 ± 3.79 years. The p-value of comparisons of age and duration of infertility in both the groups is 0.917 and 0.664 respectively. So, the demographic variables of both the groups were comparable.

The Mann-Whitney test was performed on the Beck's Anxiety Inventory score, Beck's Depression Inventory score and the Couple Satisfaction Index-16 scores of both the groups. The U score was 931.5 for the comparison of the Beck's Depression Inventory in the two groups, with a p-value of 0.028, signifying greater depression scores in the donor sperm usage group. The anxiety levels, analyzed by BAI, were concluded to be non significantly different ($U = 1065.5$, $p = 0.203$). The CSI-16 scores depicting marital relationship was also comparable ($U = 1202.5$, $p = 0.743$).

Limitations, reasons for caution: The infertility treatment are not covered under insurance in India, so, the study included subjects who were financially secure, well educated and from an urban background, a wider study in a public setup would be more descriptive of the general population.

Wider implications of the findings: The psychological state of men with azoospermic infertility needs to be given more consideration and may need counseling.

Trial registration number: MCDH/ 2017/6

P-551 Can men's dyadic coping strategies make the difference? The role of man's dyadic coping strategies for his and his partner's adjustment to infertility

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Study question: Does the association between the impact of infertility stress and marital and emotional adjustment is mediated by dyadic coping (DC) by oneself and by partner?

Summary answer: Significant indirect effects were found between infertility stress impact and marital adjustment through DC strategies by the self, for men, and by partner, for women.

What is known already: In previous literature, emotional and marital adjustment to infertility showed great variability. Research is now focused in examining factors that predict a better adjustment. Individual coping strategies were found to explain partially this variability in infertile individuals' adjustment. Nevertheless, in other fields of expertise, DC strategies were associated with dyadic adjustment and distress levels, being even a stronger predictor of relationship quality than individual coping strategies. As infertility is a dyadic experience, it seems plausible that strategies triggered inside the relationship to cope with stressors (DC strategies) influence couples' adjustment, but until now this association has not been studied.

Study design, size, duration: The study was cross-sectional and included 67 heterosexual couples with infertility (n = 134 participants). Participants were recruited in a public fertility treatment unit.

Participants/materials, setting, methods: Participants are 67 couples that were referred to a reproductive medicine unit for fertility problems. They were consecutively recruited by the researcher. Written informed consent was obtained. Self-report measures assessing sociodemographic data, infertility-related stress (Fertility Problem Inventory), dyadic coping (Dyadic Coping Inventory), dyadic adjustment (Dyadic Adjustment Scale – Revised) and depression and anxiety symptoms (Hospital Anxiety and Depression Scale) were filled out by participants.

Main results and the role of chance: The final sample was composed by 67 couples, with a response rate of 73%. Couples were on average in a relationship for approximately 6 years ($M = 5.98$; $SD = 3.10$) and were trying to conceive for approximately 3 years ($M = 3.01$; $SD = 1.67$). Mean age of participants was 34.67 and 32.73, for males and females, respectively. Most participants were highly educated and had medium socio-economic status. There is an indirect effect of the impact of infertility in one's life on marital adjustment through men's perceived dyadic coping efforts employed by the self (Estimate: .181; 90% BCCI -.356, -.061) and women's perceived dyadic coping efforts of the partner (Estimate: -.236; 90% BCCI -.422, -.126). These models explained 38% and 30% of marital adjustment variance for women and men, respectively. Regarding the emotional adjustment of infertile couples, infertility stress impact had an indirect effect only on depressive symptoms through men's dyadic coping by oneself.

Limitations, reasons for caution: Due to the small sample size and the cross-sectional design, the results must be analysed with caution. Moreover, the sample comprised mainly highly educated individuals, from urban areas, which restrain the generalization of findings to all infertile couples' population.

Wider implications of the findings: The results highlight the importance of men's dyadic coping strategies for the marital adjustment of couples, as well as for men's emotional adjustment. Findings emphasize the importance of involving men in the fertility treatment process, reinforcing the dyadic nature of infertility processes.

Trial registration number: not applicable.

P-552 Endometriosis and/or wish for a child: Implications on partnership, sexuality and psychological well-being

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Study question: Goal was to investigate the relationship between psychological well-being and partnership of women with and without endometriosis and/or wish for a child and their partners.

Summary answer: Study emphasizes the importance of partnership for psychosocial well-being. Participants having EM and no desire for children scored the highest on stress, depression and anxiety.

What is known already: Endometriosis (EM) is a common, gynecological, inflammatory disorder, often associated with severe dysmenorrhea, pelvic pain, dyspareunia and infertility. It is well known that partnership positively influences the course of various diseases. Furthermore, a positive relationship between partnership and perceived stress can be found in infertility treatment, with partnerships being able to buffer high levels of stress associated with treatment. However, there are still few studies on the reciprocal effects of endometriosis on partnership and sexuality.

Study design, size, duration: A quantitative study was devised, approved by the Ethics Committee of the Heidelberg Medical Faculty. The study took place from September 2016 to December 2017 with N = 108 participants.

Participants/materials, setting, methods: Participants completed a questionnaire with items regarding endometriosis (EHP-5, Endometriosis Health Profile), wish for a child (Screen-IVF), partnership (SHP, Swiss Household Panel), sexuality (FSFI, Female Sexual Function Index), psychosocial well-being (DASS, Depression Anxiety Scales), pain (DSF, German Pain Questionaire) and sociodemographic items. All women undergoing laparoscopy during their treatment at Heidelberg University Women's Hospital and their partners were invited to participate in the study. Data were analyzed using independent t-test analysis and ANOVA.

Main results and the role of chance: In total, N = 58 women (Ø age: 30.9) and N = 50 men (Ø age: 35.0) participated in the study. 93.4% lived in partnership (Ø 7.0 years) and 9.3% already had children. 87.6% had a present wish for a child, on average since 3.1 years. EM was clinically confirmed in N = 78 (72.2%) participants respective their partners. Of all subjects, 57.5% had high school or university graduation. The sample was split in three groups: Participants with EM and wish for a child (EM+C+; N = 63), participants with EM and without wish for a child (EM+C-; N = 15) and participants with no EM but wish for a child (EM-C+; N = 30). Sociodemographic variables did not differ between the groups, besides group EM+C- was younger than the other groups (p≤.001). The groups differed significantly from each other in terms of partnership satisfaction (p≤.080), with group EM+C+ having the highest and the men of group EM+C- the lowest partnership satisfaction (p≤.001). Group EM-C+ had the highest intercourse frequency (p≤.034) and group EM+C- reported most dyspareunia (p≤.001). Satisfaction with sexual intercourse was comparably high in all groups. Furthermore, the groups differed in terms of perceived stress (p≤.004), anxiety (p≤.002) and marginal in depression (p≤.068), with group EM+C- reporting the highest scores.

Limitations, reasons for caution: Generalization of findings is limited due to small sample and the unequal distribution of groups, based on the difficulty of diagnosing women with endometrioses outside of family planning. Furthermore, participants in this sample had an above-average high educational background.

Wider implications of the findings: The study highlights the importance of partnership for psychosocial well-being. Participants with EM and no desire for children showed lower partnership satisfaction, but higher scores on stress, depression and anxiety, compared to participants with EM and desire for children. The causal relation of these factors needs to be explored further.

Trial registration number: None.

P-553 Mothers to be without a partner - current data on a German-wide documentation on issues raised by single women in fertility counselling

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Study question: What do we know about women without a partner seeking fertility counselling in Germany?

Summary answer: Our study shows that of all documented fertility counselling sessions with individuals approx. a quarter (26%) were used by women without a partner.

What is known already: Delaying child birth is a common phenomenon in many industrial countries. In Germany in 2015, 51% of all first-time-mothers were 30 years or older. In order to fulfil their wish for a child, an increasing number of women decide to build a family even though they lack a partner. So far, there is only little international data regarding women considering solo mothership and there are no studies in Germany.

Study design, size, duration: After the development of evaluation instruments, a first German-wide online assessment of counselling sessions was carried out between 2015 and 2017. The study included 517 valid documentations from psychosocial fertility counsellors and 197 feedback forms from women and men with the desire for a child who took up professional counselling.

Participants/materials, setting, methods: The study was conducted in cooperation between the State Institute for Family Research at the University of Bamberg, the Institute of Medical Psychology at Heidelberg University Hospital and the German Society for Fertility Counselling. Data was collected via an online questionnaire addressed to all German professionals offering psychosocial fertility counselling and to all men and women who sought counselling.

Main results and the role of chance: In 26% of all counselling sessions with individuals (n = 282), women stated that childlessness was the result of a lack of partner. These women were aged between 26 and 48 yrs. with a mean age of 38 yrs. 79% of them planned pregnancy with the assistance of medical treatment and sperm donation. Though most intended to use sperm donation only, approx. 5% already had concrete plans for using either both egg and sperm donation or solely embryo donation. Nearly 14% of the women who had already worked out concrete plans for using reproductive assistance, or had already started treatment, took advantage of treatment possibilities abroad. However, during counselling, nearly 33% of all single women discussed the possibility of treatment abroad. 61% of all women without partner were interested in specific forms of medical treatment of which 33% were egg or embryo donation and 2.5% social freezing. The most important issues during counselling were as follows: 84% managing future parenthood; 75% managing emotional distress; and 61% information regarding legal regulations. This data shows the need for comprehensive counselling addressing both current (such as legal information and emotional distress during treatment) and future issues (such as parenting a child after donor conception).

Limitations, reasons for caution: We only report on those women without partner who took up counselling; other single women may have different needs and perspectives. We also only examined those opting for medical treatment and not for private solutions. Some women of the target group may not have been addressed by an online survey.

Wider implications of the findings: The data shows that approx. a quarter (26%) of individuals seeking fertility counselling are women without a partner. Analysing the collected data enables counsellors to develop needs-orientated offers for women planning solo motherhood.

Trial registration number: not applicable.

P-554 Effects of family-centered postpartum care for postpartum depression among previously infertile couples

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Study question: The purpose of this study was to develop and evaluate a family-centered postpartum care for postpartum depression among previously infertile couples.

Summary answer: For husbands, the mean score of EPDS in the experimental groups had significantly lower than that of the control groups after intervention.

What is known already: Postpartum depression (PPD) is a significant mental health problem that occurs more frequently in the first 4 weeks postpartum and also may occur later or during the first postpartum year. The correlation between paternal and maternal depression was positive, but little is known about the effects of family-centered postpartum care for postpartum depression among couples receiving assisted reproductive technology.

Study design, size, duration: This is a randomized controlled trial for previously infertile couple who will be divided into experimental group and control group based on Edinburgh Postpartum Depression Scale (EPDS≧10 points, either husband or wife)checked 4 weeks after delivery. There were 180 couples recruited in the screening stage. Forty-nine couples were randomized assign into two groups. The experimental group consists 21 couples and the control group consists 19 couples in the final stage.

Participants/materials, setting, methods: Experimental group will receive health education CD/booklet at the 5th week, and telephone consultation for 20 minutes during 6th-9th weeks after delivery. The self-administer questionnaire is composed of subjects' profiles, Edinburgh postpartum depression scale, self-esteem questionnaire, Brief parenting stress questionnaire and social support questionnaire. Generalized estimating equation(GEE) will be used for multi-variant time-series data analysis by SPSS/PC

window 19.0 to evaluate the effects and difference of long-term change with the intervention.

Main results and the role of chance: For husbands, the mean score of EPDS in the experimental groups had significantly lower than the control groups after intervention(Exp(B) = 0.12, 95%c.I. = 0.025-0.569, p<0.05). But, for wives, the mean score of EPDS in the experimental groups had not significantly lower than the control groups after intervention(Exp(B) = 0.457, 95%c.I. = 0.165-1.268, p>0.05). The couples still had high demand for information of postpartum care during 3 to 6 months after delivery. The most helpful education method perceived by husbands in 3th and 6th months was booklet(86%), and then telephone consultation(62%) and booklet(62%). The most helpful education method perceived by wives in 3th and 6th months was telephone consultation(86%), and then telephone consultation(72%).

Limitations, reasons for caution: The study was performed by a self-administered questionnaire which might involve a recognition problem in meaning or definition of questions asked.

Wider implications of the findings: Through this study, a family-centered postpartum care for postpartum depression in previously infertile couple model can be developed as a reference for care and policy.

Trial registration number: not applicable' for non-clinical trials

P-555 Cortisol diurnal profile in women with high anxiety may be associated with progesterone levels in infertile women

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Study question: Can anxiety be associated with an impaired reproductive function in women with infertility?

Summary answer: Women with anxiety symptoms above the normal range may have altered diurnal cortisol rhythm, especially in the situations where progesterone levels are low.

What is known already: The effect of stress in infertility has been addressed in previous studies, but findings are still inconsistent. In large cohort of couples trying to conceive, it was found that preconception stress, assessed by alpha amylase, was associated with a longer time to pregnancy, but the mechanism of this effect remains unclear. Also, it has been suggested that ovulation may be linked with stress, although this hypothesis needs further research.

Study design, size, duration: This study is part of a prospective study evaluating biopsychological correlates in couples with infertility. The design of the current study was cross-sectional. Participants were women from heterosexual couples attending a reproductive medicine unit for infertility diagnosis. Written informed consent was obtained from all participants.

Participants/materials, setting, methods: Women were consecutively recruited by the researcher at their first visit at the unit. Progesterone was measured at the 23rd day of the cycle. Five samples of saliva were collected in the previous day for determination of salivary cortisol, to obtain the diurnal profile: at awakening, +30', +45', at 16 h and by bedtime. In the same day, symptoms of anxiety were assessed using the Hospital Anxiety and Depression scale.

Main results and the role of chance: The final sample was composed by 44 women. Mean age of participants was 31.31 (SD = 3.17). Most participants were highly educated and had medium socio-economic status.

Women were grouped in "low" and "high" anxiety according to the cutoff score for the HADS for the anxiety scale (7). Groups based upon the progesterone level were dividing using the cut off value of 9 ng/ml (<9=likely inadequate luteal function, ≥9=suggestive of adequate luteal function). Results showed that there was a significant interaction effect between the diurnal cortisol profile, levels of anxiety (low vs. high) and progesterone level (<9=likely inadequate luteal function, ≥9=suggestive of adequate luteal function) (F = 2.848; p = 0.05). Further analysis revealed differences across the groups (F = 4.750, p=.038). The diurnal profile of the cortisol was equal across the two groups of progesterone levels in women with low anxiety. In the group of high anxiety, the group with progesterone levels suggestive of inadequate luteal function had a different profile, with peaked cortisol values at the third cortisol measurement.

Findings highlight that women with anxiety symptoms above the normal range may have altered diurnal cortisol rhythm, especially in the situations where progesterone was low.

Limitations, reasons for caution: Main limitation was the small of number of patients that were included in the final sample. In addition, the study was cross sectional, and therefore no causal inference can be established. Finally, the absence of absolute cut off scores for defining ovulation may affect the consistency of the findings.

Wider implications of the findings: The findings challenge the notion that there is a linear association between stress and infertility. Our findings suggest that psychobiological stress is associated with the progesterone output, although future prospective should clarify the predictive effect of psychobiological stress in ovulation.

Trial registration number:

P-556 Preliminary data on communication to parents of donor-conceived offspring regarding the risks of inherited diseases

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Study question: How to manage the communication of genetic risks to parents? What's the impact of this risk on the desire for other children using donor gametes?

Summary answer: Description of our experience on communication of genetic risks to parents with donor-conceived offspring and how this information influences the wish for a consecutive child.

What is known already: Little is known about the communication of newly identified genetic risks in donor-conceived offspring and the preferences and needs of their parents. More studies on risks of inherited diseases in donor-conceived offspring focus on medical evaluation and genetic screening and their limitations and challenges. Studies show an international variability in genetic evaluations, education and informed consent processes (Sims et al., 2010; Isley & Callum, 2013; Dondorp et al., 2014; Isley et al., 2016).

However, there is limited knowledge about the wish for a second child using the same donor with a (minor) genetic risk (Squires & Simoglou, 2013).

Study design, size, duration: Between June 2015 and December 2017, a qualitative study was performed with 27 parents who have one or more children, conceived using gametes from anonymous sperm donors (n = 23), oocyte donors (n = 3) or both (n = 1). The genetic risk presented after the donors had been qualified for donation. All parents were treated in our fertility unit with gametes from our internal or from an external bank. Twenty two parents had a wish for a second child.

Participants/materials, setting, methods: Semi-structured interviews were performed with the parents at our Medical Genetics unit, asking them about: (1) the way they were informed about the genetic risk, (2) their perceptions of the communication by health-care providers, (3) their perceived risk, and if applicable (4) how they would like to realise their wish for a second child.

Main results and the role of chance: Twenty one parents were informed about a minor genetic risk by the fertility department when seeking treatment to conceive a second child, 4 parents without seeking treatment, and 2 parents received an e-mail from an external sperm bank. Both parents informed by e-mail, were upset and preferred being informed face-to-face. In the group with the wish for a second child, 2 parents were aghast and worried that they would

never know the risk if they didn't ask for the treatment. Eleven parents (40%) experienced the risk-communication as emotionally taxing, 10 parents (37%) felt comfortable during this contact. Two parents (7%) regarded the communication as unnecessary; both of them have a wish for a second child. Independent of the medical risk, 55% perceived the risk as high, 14% as moderate and 33% as low.

Of the parents wishing for a second child who perceived the risk as high, 7 had doubts (58%) about the way to fulfil their wish, 3 (25%) asked for a different donor, while 2 (17%) asked for the same donor. With a low risk-perception, 6 (67%) wanted to use the same donor, 2 (22%) had doubts and 1 (11%) wanted to use a different donor.

Limitations, reasons for caution: The small sample size undermines the representability of the results to a whole population. Variation in how future parents were counselled upon intake may affect how they experienced the communication. Because of the descriptive study-design findings may be open for interpretation.

Wider implications of the findings: This research may be a pre-cursor to future research. The issues reported can be used to achieve a higher quality of care. These findings may be a starting point for developing guidelines on counselling, exploring the (ir)relevance of minor genetic risks for parents with donor-offspring and the impact on secrecy.

Trial registration number: No randomized controlled trial.

P-557 Obsessive symptoms and the need of gamete donation as predictors of treatment dropout in infertile patients

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Study question: Can psychological symptoms and medical diagnosis be a predictor of dropout in infertile patients?

Summary answer: In infertile patients high scores of obsessivity and the need of gamete donation could predict the possibility of dropout.

What is known already: Infertility diagnosis and treatment is a burden for some patients and this relates to psychological symptoms that have an impact on their quality of life and medical treatments. There is an agreement in the literature that psychological burden is referred as one of the reason of treatment dropout but there are no studies about specific symptoms related to this abandonment. As far as we know, there are no publications regarding treatment discontinuation and the need of gamete donation.

Study design, size, duration: Prospective and relational study part of a wider research program in psychology and infertility from the CER Medical Institute from Buenos Aires, Argentina. Over a period of six months, from March-August 2017, 187 patients completed a questionnaire.

Participants/materials, setting, methods: 187 new patients from infertile couples, 120 women(Age: x̄ 38.21 +/-5.18 years) and 67 men(Age: x̄ 39.61 +/-7.42 years) agreed to complete the Symptom Checklist-90-R(SCL-90-R) (Derogatis, 1994), a self-reported questionnaire, in the waiting room before their first appointment at the fertility clinic.The SCL-90-R is a standardized instrument that assesses a wide range of current psychological symptoms. Medical data is obtained from clinical records. Logistic regression was made with the SPSS 24. Statistical significance = $p < 0.05$.

Main results and the role of chance: In the total sample, the likelihood of the patient dropping out was significantly predicted by: a) levels of obsessivity, $\beta = 1.32$, SE=.66, Wald(1) = 6.157, $p < .05$, and, b) gamete donation (female or male), $\beta = 1.26$, SE=.48, Wald(1) = 6.96, $p < .01$. Both higher levels of obsessive symptoms and the need for a gamete donation increase the odds of dropping out. When looking at women, the need effect of gamete donation remain significant, $\beta = 1.32$, SE=.62, Wald(1) = 4.58, $p = .03$. On the other hand, in the men sample, obsessivity significantly predict drop out, $\beta = 6.13$, SE = 2.99, Wald(1) = 4.19, $p = .04$.

Limitations, reasons for caution: It was a small sample and limited in time so results should be generalized with caution.

Wider implications of the findings: This study shows that the impact of knowing the need of gamete donation and obsessive symptoms could predict treatment discontinuation in infertile patients.It would be helpful to be aware of this in fertility clinics in order to incorporate a psychopathological screening to provide patients with help and improve treatment adherence.

Trial registration number: N/A.

P-558 The evaluation of patients' experience with manual vacuum aspiration: Comparison with other methods of miscarriage managements and infertility procedures

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Study question: How do women perceive manual vacuum aspiration (MVA) in comparison with other methods of miscarriage management and infertility procedures?

Summary answer: Even though MVA was associated with the highest pain score in comparing with infertility procedure, women highly accepted MVA procedure.

What is known already: Previous studies reported that MVA was as effective as electric vacuum aspiration and it had fewer side effects than the use of misoprostol. However, it is not known the efficacy and acceptability of MVA in comparing with three other measures of first trimester miscarriage.

Study design, size, duration: This comparative cohort study took place in a university based teaching hospital with recruiting 55 women underwent MVA over a 12 months period. The data of women underwent suction curettage (n = 53), medical evacuation (n = 59) and expectant management (n = 58) were retrieved from our previous randomized controlled trial.

Participants/materials, setting, methods: Patient's psychological status was systematic assessed through various instruments including impact of event (IES), state-trait anxiety inventories (STAI), Beck depression inventory (BDI), general health questionnaire (GHQ), and client satisfaction scale (CSQ). Women were asked to rate their pain score of (1) MVA, (2)oocyte retrieval (OR), (3) hysterosalpingogram (HSG), (4) embryo transfer (ET). The number of days of bleeding and abdominal pain was recorded by a symptom log sheet.

Main results and the role of chance: As regarding the pain experience of infertility procedures, women at MVA procedure (6.4 ± 1.7) had significantly higher ($p = 0.000$) pain level than HSG (5.1 ± 2.8), OR (4.5 ± 3.0) and ET (0.9 ± 1.8). However, there was no difference in client satisfaction level. Among all groups, there was no difference in IES, BDI, GHQ and CSQ scores. In comprising the 4 different methods of miscarriage management, the anxiety score in women who underwent MVA (54.9 ± 9.4) was significantly lower ($p = 0.041$) than that in the expectant group (60.8 ± 13.0), and found similar to suction curettage (58.5 ± 10.6) and medical management group (59.7 ± 12.9). The duration of bleeding (in days) of MVA group (9.7 ± 4.1) was significantly shorter than the medical (15.1 ± 6.6) and expectant (12.8 ± 5.8) management group, but there was no difference from suction curettage group (10.7 ± 5.8). Four groups of women had no difference in the duration (in days) of abdominal pain. The complete evacuation rate of MVA group (94.5%) was significantly higher than medical (71.2%) and expectant (79.3%) management group, but there was no significantly difference between MVA and suction curettage group (98.1%).

Limitations, reasons for caution: Due to the comparative cohort study design, the finding should be interpreted with caution.

Wider implications of the findings: Even though MVA group had similar client satisfaction level compared with other methods of miscarriage management, it was also associated with the highest pain score. Therefore, further study is required to examine methods to reduce the pain during MVA is deemed desirable.

Trial registration number: not applicable.

P-559 Counselling in decision making by couples for assisted reproductive technology – A key role in quality care

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Study question: How couples decide for fertility treatment and what is the role of counselling?

Summary answer: Key factors in deciding for fertility treatment are finance, society pressure and prognosis. Counselling can help couples to choose the appropriate treatment.

What is known already: Coping with the emotional challenges of infertility can be difficult for couples. More stressful is the task of decision making in seeking fertility treatment. When they embark on assisted reproductive treatment (ART) they may experience many different emotions, from joy and excitement to grief and disappointment. This can be emotionally and physically challenging, Clinicians need to develop the skills to communicate about the treatment modalities available to be able to reduce the emotional suffering, to prepare them for fertility treatments, and explore the options and implications when making decisions about starting, changing or stopping treatments.

Study design, size, duration: Longitudinal study was performed at fertility centre in Delhi, India where 900 patients were interviewed during the year, January 2017 - December 2017, who consented to participate in study. They were divided into two groups: Group A (n = 348) who were ready for ART and Group B (n = 552) who did not accept ART due to factors like finance, social pressure and prognosis explained for the ART. Data were analyzed using SPSS software version 20.

Participants/materials, setting, methods: Group B was counselled for accepting ART. 333 patients accepted ART after counselling while 219 did not accept ART. Chi square test was applied to find out the role of counselling and extent to which finance, social pressure and prognosis influence the decision of couple for accepting or rejecting ART treatment.

Main results and the role of chance: Chi-square test for independence was calculated comparing frequency for accepting ART treatment in patients with social pressure. A significant interaction was found (X^2 [1] = 12.83, $p<0.05$) and Cramér's phi coefficient = 0.476 which shows strong association that is 71.43% of them accepted ART treatment after counselling as compared to 28.57% who did not accept.

Similarly significant interaction was found between prognosis of ART and accepting ART after counselling (X^2 [1] = 5.79, $p<0.05$). In Patient with good prognosis ,79.17% of them accepted ART treatment after counselling as compared to 20.83% who did not accept and patient with poor prognosis ,44.45% still opted for ART after counselling and Cramér's phi coefficient = 0.34 which shows moderate association.

Chi-square test for independence was calculated comparing non- modifiable factor finance with accepting or rejecting ART after counseling. Although patients accepted ART after counseling but was found to be not significant.

In Conclusion, we found in our study counselling helped significantly in couples for accepting ART treatment when they were effected by peer pressure and worried about final outcome of ART. Counselling may have a role in patients with financial problems also but finance is limiting factor for accepting ART.

$P<0.05$ was considered significant.

Limitations, reasons for caution: Although it is prospective study, it has limitation of small sample size.

Wider implications of the findings: When couples first meet with specialist, most of them have not made any decisions and are seeking information and advice. Understanding how couples reach consensus about accepting reproductive treatment and counseling appropriately holds promise to improve quality of care and increase long-term decisional satisfaction for patients and their partners.

Trial registration number: MCDH/ 2017/7

P-560 Fertility- and early menopause-related information needs of young breast cancer survivors

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Study question: What are the fertility- and early menopause-related information needs and preferences of Dutch young breast cancer survivors?

Summary answer: Extensive information about fertility is desired by young breast cancer survivors, especially on the risk of infertility, possible treatments and long-term consequences of early menopause.

What is known already: Approximately 10-15% of women diagnosed with breast cancer are in their reproductive age. Treatment can impair their fertility, which is disturbing since half of them desire to have a subsequent pregnancy after treatment. Current guidelines focus on informing breast cancer patients on the possibility of reduced fertility mainly prior to treatment, because this is the moment to decide about fertility preservating options. However, it is equally important to address information needs on fertility and possible early menopause after treatment has been completed and the wish to conceive becomes topical.

Study design, size, duration: A qualitative study consisting of semi-structured in-depth interviews with professionals and patients. In May 2015 13 professionals (oncologists, surgeons, gynaecologists, nurses) were interviewed about their experiences with information provision concerning fertility during follow-up. Based on these results a topic guide has been developed, which was used in 18 interviews with patients between April 2016 and December 2017. Furthermore, after each interview, patients composed a priority list with the five most important items of information provision.

Participants/materials, setting, methods: Interviewed professionals were active in the Radboud university medical center (Radboudumc) and four peripheral hospitals in the Netherlands. Interviewed patients were treated in the Radboudumc or Rijnstate Hospital. Eligible for participation were women between 20-45 years at the time of the interview who had completed their initial treatment, i.e. surgery and chemo- or radiotherapy. The number of interviews was determined by data saturation. Data were analyzed in accordance with grounded theory.

Main results and the role of chance: The risk of infertility was discussed with most of the women before starting treatment, although half of them weren't satisfied with the information they received. After treatment had ended, also the majority of participants indicated that they received too little information and many reported having brought up the topic themselves. Most women looked up fertility and early menopause online and described struggling to find reliable information specific to their situation. They expressed the need for an information tool on fertility and early menopause aimed at young breast cancer survivors. Among other items, this tool should include information such as the chance of still being fertile and when to expect their menstruation cycle to resume. They want to know the risks of pursuing a pregnancy, for themselves as well as for the future child. When is the best moment to do so and what contraception should be used in the meantime? They seek advice on how long they should try to conceive before approaching a fertility specialist. With regard to early menopause, they want to know which symptoms to expect and especially what the long term risks are for their general health. They value advice on dealing with these problems.

Limitations, reasons for caution: Limitations include focusing on breast cancer survivors and a shortage of quantitative data. We started to provide quantitative data through patients' priority lists, but given the sample size statistic measurements couldn't be performed. It would be interesting to confirm data in a larger population also including other types of cancer.

Wider implications of the findings: In-depth insight in the information needs of young breast cancer survivors concerning fertility and early menopause was obtained. In the following months information materials will be developed

and subsequently tested within this population. This supports professionals to improve information provision to meet the needs and preferences of their patients.

Trial registration number: not applicable.

P-561 Impact of infertility diagnosis on male sexuality and self esteem

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Study question: Does infertility diagnosis impact on male sexuality and self esteem?

Summary answer: Diagnosis of infertility does not seem to affect male self esteem. However, in couples presenting with combined infertility factors, male partners showed orgasmic dysfunctions.

What is known already: Studies show that the diagnosis of infertility, specially male infertility, negatively interferes in male auto esteem, confidence and sexuality.

Study design, size, duration: Cross sectional, descriptive study.

Participants/materials, setting, methods: One hundred twenty two men during in vitro fertilization were included, between March 2017 and December 2017. Sexual Quotient- male version- (SQ-M) was used to evaluate sexuality and Rosenberg Self-Esteem Scale to evaluate self esteem. In associations involving diagnosis, fixed factor analysis of variance was used. In associations involving sexual dysfunctions, Fisher's exact test was used. Adopted $p = 0.05$ statistically significant.

Main results and the role of chance: 27% of couples presented male, 50% female and 22,1% combined infertility factor. There was no association between self esteem and infertility factor ($P = 0.851$). In couples presenting with combined infertility, male partner had difficulties in achieving orgasm ($P = 0.043$). In couples with male infertility, male partner showed higher self confidence in sex ($P = 0.045$) than in couples with female or combined factors. No associations were found between sexuality/auto esteem and duration of infertility, previous pregnancies or education.

Limitations, reasons for caution: I n our sample, the majority of the couples presented female infertility, what might have generated a sample bias.

Wider implications of the findings: Diagnosis of infertility has a negative impact on male sexuality. In those couples psychological follow up should be encouraged.

Trial registration number: Not applicable.

P-562 'myFertiCare': quantitative evaluation of an online application for infertile couples

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Study question: Is it feasible to develop and successfully implement an online application to support couples undergoing ICSI with surgically retrieved sperm during their treatment trajectory?

Summary answer: myFertiCare, an online application providing personalized and interactive features has been quantitatively evaluated. Since it meets Human, Organizational en Technological aspects, successful implementation is concluded.

What is known already: The Internet is an important and low threshold source of information and support for couples undergoing ART. We hypothesized that an online application can be a suitable instrument to support couples during their full treatment trajectory. By providing personalized information and offering the opportunity of communication, it is possible to decrease infertility-specific anxiety and stress, increase knowledge and enhance patient-centred care.

Study design, size, duration: A quantitative study in which 314 couples were invited to evaluate 'myFertiCare'. A questionnaire was based on the HOT-fit framework, assuming a 'fit' between Human, Organizational and Technological factors is needed to successfully implement an eHealth tool. Therefore, it consisted of validated questionnaires covering these domains. Moreover, attention was payed to the effect on patient knowledge, experienced patient-centeredness and treatment burden. Non-users of myFertiCare received an adjusted questionnaire, to discover motivations for not using.

Participants/materials, setting, methods: The questionnaires were distributed between June and August 2017 in the Radboud university medical center in Nijmegen, the Netherlands. Eligible for participation were couples undergoing ICSI with surgically retrieved sperm, who had had the opportunity to use myFertiCare during their treatment trajectory. Each couple received one questionnaire together. Both couples that did and did not actually use myFertiCare could participate. Furthermore, user statistics were analyzed.

Main results and the role of chance: Response rate was 48%. After exclusion, 142 questionnaires were included for analysis. Participants appreciated myFertiCare on human, organizational, as well as technological aspects. At the human and technology domain, myFertiCare showed good system usability, high user satisfaction and good information and interface quality. The visualized treatment trajectory was the most appreciated functionality of the application. At the organizational domain, the information provided to patients about the application appeared to be complete. Unfortunately, non-users of the application often didn't recall receiving information about the possibility to use myFertiCare. Among the health care providers, knowledge about all opportunities myFertiCare offers to couples could be improved.

myFertiCare turned out to improve knowledge about treatment of infertility, to increase the degree of experienced patient centered fertility care and to decrease the experienced treatment burden.

User statistics showed that myFertiCare was mainly used by the female partner, and most activity was seen at the beginning of treatment.

Limitations, reasons for caution: Although we conclude that myFertiCare has been successfully implemented, we also assessed some pitfalls. This provides us the opportunity to further develop the application based on patients' needs and preferences and to improve the infrastructure surrounding myFertiCare, so that all couples are optimally aware of the opportunities myFertiCare offers them.

Wider implications of the findings: Since myFertiCare has been successfully implemented, the use of the application can possibly be expanded to other fertility treatments, and also to other fertility clinics, both in the Netherlands and abroad. Further research can be done to explore possibilities of using the application format in other medical departments.

Trial registration number: not applicable.

P-563 Factors affecting the disclosure management of infertility of expatriate women in Hong Kong

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Study question: What are the emotional and socio-cultural factors affecting women's decisions to disclose their infertility to various members of their social support network?

Summary answer: Various Infertility-related stigmas affected women's disclosure decision. Women tended to consider their infertility disclosure from a two-pronged approach – protection and productivity.

What is known already: Infertility affects up to 15% of reproductive-aged couples worldwide with negative psychological, physical, social and economic impacts. Women experienced the higher stress as well as feelings of shame and guilt due to their infertility. Studies showed that higher perceived social support could ameliorate the emotional distress for women with infertility. However, existing evidence has revealed a perplex relationship between social support and women's disclosure patterns as social stigma associated with infertility would negatively influence women's decision of disclosure. Therefore, this

study explored the complex relationship between a woman's social support network and the extent to which infertility-related information was disclosed.

Study design, size, duration: This study employed a qualitative design with semi-structured individual interviews. As a methodological innovation, this study applied Infertility Disclosure Map (IDM) to enact a visual, tangible representation of woman's social support network and the extent of their disclosure of infertility-related information. Six participants were recruited. The interviews were conducted between March 2015 and March 2016.

Participants/materials, setting, methods: Participants were English-speaking expatriates living in Hong Kong aged 18 or above. They were diagnosed of primary infertility and were undergoing ART procedures in Hong Kong at the time of the study. Exclusion criteria included inability to give written informed consent, condition with cognitive impairment or major psychiatric disorders. Participants were recruited from a locally-based, online social networking forum, targeting English-speaking expatriates in Hong Kong. The interviews were audio-taped, transcribed and thematically analysed with NVivo.

Main results and the role of chance: Six female participants aged between 33 and 40 were recruited. They had varies ethnic backgrounds including Caucasian, Chinese and Latin/Asian. Three of them had one children and the other had no children. The duration of infertility ranged from 1.75 to 6.5 years. Consistent with existing findings indicating that many women have experienced stigmatization from their infertility, five of the six participants experienced some forms of stigmatization. However, perceptions of stigma among participants were divergent, which included: infertility is the woman's fault; infertility is a disease; voluntary childlessness is shameful; and age discrimination on the desire for biological children. Extending the existing models that explain women's disclosure decision-making, the findings illustrated a two-pronged approach utilized by participants in deciding whether to conceal or disclose information about their infertility – protection and productivity. Protection of self, spouse and even the recipients of the information so as to avoid pain, worry and embarrassment was a significant consideration that prompted participants to withhold information. Productivity, namely the usefulness of the disclosure to participants was the other key component in participants' decision-making process. The usefulness includes emotional support from others, tangible support to facilitate treatment, information gathering, and altruistic purpose of helping others with infertility.

Limitations, reasons for caution: Major limitations include the small sample size and insufficient representation from individuals from different cultural backgrounds.

Wider implications of the findings: With a deeper understanding of the complex reasons and emotions influencing women's decisions to disclose their infertility to various members of their social support network, counsellors and other helping professionals could better assist the women to manage their infertility treatment as well as improving their psychological wellbeing.

Trial registration number: Not applicable.

P-564 Psychological wellbeing and relational stress of women with a predicted poor ovarian response at the start of IVF treatment

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Study question: Do women predicted to have a poor ovarian response (POR) at the start of IVF treatment report higher psychological and relational stress, than non-POR women?

Summary answer: Prior to IVF treatment the predicted POR group showed higher psychological difficulties (relational stress) than women without a predicted poor ovarian response.

What is known already: The Bologna Criteria are used to predict a poor response to ovarian stimulation based on age, ovarian response and number of oocytes retrieved in a previous cycle. It is estimated that between 5.6% and 35.1% of women undergoing ART will exhibit a POR. Although it is known that poorer prognosis is associated with higher stress during treatment, suggesting that women with POR may need additional/tailored psychosocial support, to date no studies have examined associations between a POR classification/diagnosis and psychological adjustment.

Study design, size, duration: This cross-sectional case-control study is part of a longitudinal study of infertile couples undergoing IVF in a private clinic between February 2013 and September 2015.

Participants/materials, setting, methods: 257 women (POR = 50; non-POR = 207, based on the Bologna criteria) filled out questionnaires (response rate 65%) on well-being (FertiQoL and FPI) and relational stress (ECR, Commitment Inventory, ENRICH), after physicians informed patients about POR. 16 out of 50 of the cases reported a history of POR. The two groups were compared on well-being and relational stress using multivariate analysis of covariance, controlling for cause and duration of infertility. Effect size was expressed by partial eta squared.

Main results and the role of chance: Wilks's λ was significant, $F(1, 241) = 2.34$, $P < 0.01$, $\eta p^2 = .10$, showing a difference between the two groups. Contrast analyses revealed that patients with POR reported lower quality of life on the relational domain ($z = 9.35$, $p < .01$, $\eta_p^2 = .04$); lower level of commitment on couple identity and relational agenda ($z = 4.76$, $p < .05$, $\eta p^2 = .02$; $z = 3.94$, $p < .05$, $\eta p^2 = .02$, respectively); higher anxiety and avoidance in close relationships ($z = 6.48$, $p < .05$, $\eta p^2 = .03$; $z = 4.27$, $p < .05$, $\eta p^2 = .02$, respectively); and higher FPI sexual stress ($z = 8.13$, $p < .01$, $\eta p^2 = 0.03$), than non-PORs women.

The group difference remained significant after controlling for cause and duration of infertility, $F(1, 241) = 1.99$, $P = 0.02$, $\eta p^2 = .09$.

Limitations, reasons for caution: The questionnaires were completed immediately after the POR diagnosis and the influence of preexisting levels of infertility stress cannot be excluded. Moreover, uncontrolled factors explaining the association between POR status and distress could have been relevant. Finally, no measures of mental health disorders, such as anxiety or depression, were collected.

Wider implications of the findings: POR status is associated with higher relational stress and the cause of this association could be due to the awareness of their risk of a POR. The findings provide support for the importance to address specific relational issues in women with POR at the early stage of ART treatment.

Trial registration number: Not required.

P-565 A pregnancy loss in the first fertility treatment is a postitive predictor of birth in the following treatment

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Study question: What is the chance of a birth in the second treatment after a pregnancy loss (PL) compared to no pregnancy in the first treatment?

Summary answer: The chance of a birth in the second treatment is significantly higher after a PL compared to a negative pregnancy test after the first treatment.

What is known already: When infertile couples experience an early PL during fertility treatment, it is generally considered to be a positive predictor of future birth because the couple were able to achieve a pregnancy. Previous studies on this topic in the ART population are conflicting. A study in 12,174 Chinese patients found no difference in birth rate in the second treatment after a PL compared to negative pregnancy test. In contrast, a PL in the first treatment was a positive predictor of a birth among 112,549 UK patients in the two following treatments (compared to no pregnancy in the first treatment).

Study design, size, duration: A Danish national cohort study of 32,975 infertility patients including total register data on their first two fertility treatments. We used data from the mandatory Danish ART register and included treatments from 1994 to 2012. Both intra-uterine inseminations and ART treatments were included and treatments with egg-donation were excluded.

Participants/materials, setting, methods: Detailed data from the first and second treatment in every Danish patient attending both public and private Danish fertility clinics were retrieved. An accurate registration of births was obtained by cross-linking treatments with the Danish National Birth Register by the personal social security number. A positive s-hCG without a resulting birth was categorized as a pregnancy loss. Data is presented as percentages and differences were accessed using the Pearson chi-square test and one-way ANOVA.

Main results and the role of chance: Outcome of the first treatment was devided into three groups; 1) no pregnancy (n = 26,852, 81.4%), 2) pregnancy loss (n = 3092, 9.4%) and 3) birth (n = 3031, 9.2%). Mean maternal age differed at time of first treatment between the groups 1) 32.9 years, 2) 32.9 years and 3) 31.2 years, respectively (p<0.0001). In the second treatment, only 17.3 % (n = 4640) in group 1 achieved a birth compared to 22.7 % (n = 702) and 23.8 % (n = 722) in group 2 and 3, respectively, which also was significantly different (p<0.0001). The reoccurrence of another pregnancy loss in the same couple (group 2) in the second treatment was 11.7% (n = 362) compared to the first occurrence of a pregnancy loss in the second treatment in group 1) 8.6% (n = 2304) and 3) 11.0% (n = 333), (p<0.0001).

Limitations, reasons for caution: This is a nationwide study and the analyses are restricted to the first encounter with fertility treatment, irrespective of type of treatment. Current results are not reflecting the treatment regime as a whole.

Wider implications of the findings: Our study confirms that a pregnancy loss compared to a negative pregnancy test is a positive predictor of birth in the following treatment. The findings can not be explained by maternal age and will be useful when counselling patients who experience a pregnancy loss in their first treatment.

Trial registration number: Not applicable.

P-566 Stress and depression among couples with recurrent pregnancy loss - focusing both on the male and the female partner

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Study question: What is the prevalence of psychological stress and moderate/severe depression in couples with recurrent pregnancy loss (RPL)?

Summary answer: Male partners revealed no increased frequency of stress and depression, but we confirmed our previous finding of significantly increased frequencies among female partner with RPL.

What is known already: In a previous large study of women with RPL, we have shown that psychological stress and major depression are significantly more common among women with RPL than other pregnancy planners. Emotional distress among male partners is much less studied, but one study investigated 76 male partners of RPL using the Beck Depression Inventory and found that 14,9% of the men were considered mildly depressed.

Study design, size, duration: The study is a cross-sectional study, where we compared the prevalence of stress and depression among 226 men and 342 women in RPL couples referred for evaluation and treatment at the Danish national RPL Unit from 2015-2018. We defined RPL as three or more pregnancy losses before 12 weeks' gestation. Results among men were compared to a Danish population study performed in 2000.

Participants/materials, setting, methods: From March 2015, all couples have been asked to complete the Major Depression Index (MDI) and Cohen's Percived Stress Scale (PSS) as part of standard evaluation before first consultation. Of the invited patients, 226 men (57%) and 342 women (80%) participated.

Main results and the role of chance: Of the men in the RPL couples 5/226 (2.2%) had a score on the MDI corresponding to moderate/severe depression, as did 27/342 (7.9%) of the participating women.

In a Danish population study from 2000 using the same psychometric scales, 2.2% of the men aged 20-79 years reported symptoms corresponding moderate/severe depression. The prevalence of moderate to severe depression among men in RPL couples was not different from the Danish background population (P = 0.96).

The prevalence of moderate/severe depression among the female partners of RPL couples is comparable to our previous results in a different cohort of RPL women (8.6%) (P = 0.73). In our previous study the response rate was 69 % compared to 80 %.

Among the men in the RPL couples 26/226 (11.5%) reported a high stress level (defined as ≥ 19 on the PSS scale), as did 97/338 (28.7%) of the women. When compared to the stress levels reported in our previous study on a RPL female cohort we find a significantly lower frequency of high stress level (P < 0.001). The lower occurrence of high stress level in the most recently collected cohort maybe explained by selection.

Limitations, reasons for caution: The response rate among men is relatively low compared to women; this may bias the results. We are unaware if some of the non-responders suffer from depression or high stress level. Furthermore, the comparison group is a population sample and we are thus unaware if they suffer from reproductive problems.

Wider implications of the findings: To our knowledge, this is the first larger scale questionnaire study of emotional distress among men with RPL. We did not find a high prevalence of neither stress nor moderate/severe depression. Furthermore, we confirmed previous findings regarding stress and depression among women with RPL, highlighting the emotional toll of RPL.

Trial registration number: N/A.

P-567 Quality of life of transgender women and men during hormonal treatment

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Study question: Does quality of life change during cross-sex hormonal treatment in transgender individuals and are quality of life aspects comparable with non-transgender individuals?

Summary answer: Quality of life in transgender individuals improves during cross-sex hormonal treatment.

What is known already: Social understanding and support for transgender individuals have steadily improved, but knowledge about their socio-psychological development is scarce. There are few studies that show better physical and mental health after hormonal treatment or sexual reassignment surgery but aspects such as socio-economic integration, self-perception and acceptance have been neglected.

Study design, size, duration: Since no suitable questionnaire was available, we developed a transgender questionnaire. Validation of the questionnaire was performed by repeated completion after one week. The questionnaire includes demographic data, 45 questions about trans-identity, physical, mental and sexual wellbeing as well as social background and acceptance. Validation of the questionnaire was performed with item-total correlation, internal consistency (Cronbach's α) and test-retest reliability (intraclass correlation coefficient). Chi^2Test, Wilcoxon- test and independent sample t-tests were used for statistical analysis.

Participants/materials, setting, methods: Transgender individuals receiving hormonal treatment in the University hospital of Innsbruck and matched controls were included. Transgender individuals answered the questions twice, representing the time point before and after start of treatment whereas the controls answered it once except those who validated the questionnaire.

Main results and the role of chance: So far 26 transgender individuals and 44 controls were included. Median age of transgender individuals was 25,8 (min-max: 18-50) years, 26,7 (min-max: 18-52) years in controls. Quality of life improved during hormonal treatment in transgender individuals. This was mainly true for self-confidence and physical perception. We also observed less

discrimination and more support from the social environment after at least 6 months of hormonal treatment.

Limitations, reasons for caution: Our study was conducted by a limited number of participants. Transgender individuals may already experience a different quality of life due to social acceptance and access to medical facilities.

Wider implications of the findings: Hormonal treatment options for transgender individuals raise the urge to better understand and support transpersons. Our study shows a possible way to monitor and optimize quality of life and psychosocial aspects during hormonal treatment.

Trial registration number: not applicable.

P-568 Remote access to embryo images and video during their cycle enhances a patients In Vitro Fertilisation (IVF) experience

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Study question: Does providing patients remote access to images and video of their embryos developing during an IVF cycle enhance their experience?

Summary answer: The majority of patients surveyed indicated that viewing their embryo images during cycle enhanced their experience of IVF treatment.

What is known already: With the advent of online self-diagnosis and in turn patients understanding and expecting certain service standards during their treatment, there is a shift in how medical providers are adjusting their interactions and delivery of treatment to patients. Patients who have a tangible image from ultrasound scans respond better and feel more informed about their treatment options than those without an image (Carlin et al 2014). The advent of time-lapse imagery of embryo development allows not only scientists to view in great detail the growth of an embryo but opens up opportunities for patients to have access to this information.

Study design, size, duration: Patients undertaking an oocyte retrieval were recruited on a voluntary basis over an 8 month time period from May-2017 to January-2018. Patients who participated were sent an optional survey to complete after embryo culture but before pregnancy outcome. A total of 149 responses were collated from 358 patients who participated in the trial. The survey consisted of a number of questions on usability, enhancement of IVF experience and communication using a 5 level Likert scale.

Participants/materials, setting, methods: Participants were recruited on a voluntary basis to trial an embryo viewing portal through a phone or PC application (Grow™, Genea Sydney). This app allows patients to log on and view their embryos growing in-situ via a direct link with image collating software Geri Connect using the Geri embryo monitoring incubator (Genea Biomedx, Sydney). Still and time-lapse video were uploaded at specific time points and patients received ongoing communications from embryologists regarding their embryo development.

Main results and the role of chance: 149 patients took part in the trial and completed the survey. The average age of the recruited cohort was 35.9, with 62% of patients falling into the <38 age group and 38%in the > = 38 age group. First cycle patients accounted for 39% of the recruited cohort and the remaining 61% had at least one previous cycle either at Genea or elsewhere. Overall, 85% of participants either strongly agreed or agreed that their IVF experience was enhanced by having access to their embryo images during treatment. Technical issues in the usage of the application and a requirement for greater information were the main contributors to the 15% of either neutral or negative scores. Feedback collected during the survey collation pointed towards a variety of reasons for this enhancement with inclusion of partner in the cycle, giving greater understanding and education, feeling more connected with treatment and also provides an element of feeling more in control during treatment. Patient feedback continues to be collected and assessed to improve the patient experience whilst undergoing treatment for infertility.

Limitations, reasons for caution: The cohort of patients that elected to take part in the trial may have been a positively biased group. No data has yet been collected to determine if negative pregnancy outcomes would impact the score of enhancement.

Wider implications of the findings: The introduction of an application for patients to view their developing embryos enhances the delivery of information by providing greater education and allowing patients to feel more involved across their treatment as well as providing a more personal experience thereby aligning IVF with trends in medical management of patients.

Trial registration number: Not applicable.

POSTER VIEWING
Reproductive (epi)genetics

P-569 Is mitochondrial DNA quantification (NGS) a useful tool for embryo viability assessment?

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Study question: Is there a relationship between human blastocyst mitochondrial DNA (mtDNA) content and female age, embryo chromosome ploidy and clinical pregnancy?

Summary answer: This study demonstrates no association among mtDNA score, female age and pregnancy outcome. However, a relationship between mtDNA score and embryo ploidy was observed.

What is known already: Recently, there are controversial publications about the clinical relevance of mtDNA quantification in human blastocysts. The increase of mtDNA may be a sign of elevated metabolism related to high energy requirements in stressed embryos.

Some studies found no association between this parameter and chromosomal ploidy, maternal age or pregnancy outcome. However, some laboratories, based on their evidence, apply this quantification as an implantation potential biomarker between euploid embryos.

Different methodologies have been used for mtDNA assessment, principally next generation sequencing (NGS) platforms and real-time polymerase chain reaction (qPCR), with consistent results between them.

Study design, size, duration: A cohort of 384 blastocysts obtained from 144 couples, with an average female age of 35 years old (21-45), undergoing preimplantation genetic testing for aneuploidies (PGT-A) were retrospectively included. The data was collected between April 2016 and December 2017 from a single center. Mosaic embryos (between 20%-80%) were excluded from this analysis.

Participants/materials, setting, methods: Trophectoderm biopsies from 384 blastocysts (PGT-A) using NGS (Veriseq-Illumina) were assessed.

For each sample, reads were aligned to the human reference genome (hg19) and the mtDNA genome separately. Reads mapped to the mtDNA were divided by the number of reads that mapped to the nuclear DNA. The resulting values were mathematically adjusted by correction factors previously reported for embryo ploidy and gender.

mtDNA scores were analyzed regarding embryo aneuploidy, maternal age and clinical pregnancy (T-test).

Main results and the role of chance: Regarding all blastocysts analyzed, 189 (49%) were euploid and 195 (51%) aneuploid. The amount of mtDNA was significantly higher in aneuploid embryos ($P = 0.002$). However, the amount of mtDNA was not different between embryos from younger (<38 years, n = 147) and older women (≥38 years, n = 41) ($P = 0.2161$).

During the period covered, 46 normal embryos were transferred after thawing, resulting in 25 ongoing pregnancies (54%). The mtDNA score from euploid blastocysts that achieved a clinical pregnancy was no significantly different from those that did not result in an ongoing pregnancy ($P = 0.4118$).

A mathematical adjustment was performed in order to correctly normalize the relative mtDNA content regarding embryo gender and chromosome ploidy.

From our in-house data, mtDNA score was only statistically associated with chromosomal aneuploidy suggesting that high levels of mtDNA may have a negative impact resulting in an inadequate chromosome segregation.

Limitations, reasons for caution: This is a retrospective study. Despite NGS approach for mtDNA quantification has been validated by others methodologies, these techniques should be applied in order to confirm these results.

Wider implications of the findings: This study contributes evidence that mtDNA quantification in euploid embryos have no clinical impact in pregnancy outcome. The results demonstrate that mtDNA levels are independent to female age. However, mtDNA score reveals a strong association with embryo aneuploidies.

Trial registration number: Not applicable.

P-571 Impact of microfluidic sperm sorting on embryo quality and comprehensive chromosome screening outcomes of couples with repeated implantation failure

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Study question: What is the impact of the microfluidic sperm selection technique for IVF/ICSI on embryo quality and euploidy rates in couples with Repeated Implantation Failure (RIF)?

Summary answer: When microfluid chip-sorted spermatozoa is used for IVF/ICSI, a higher number of top quality blastocysts and better euploidy rates were observed compared to standard selection.

What is known already: Aneuploidy is the most common type of chromosome abnormality and the leading cause of implantation failure in humans. Oocyte and spermatozoa equally contribute to embryonic aneuploidy however, womens' age is the well-known factor. We are still unable to modify the quality of oocytes, however several sperm selection techniques have gained attention. Being one of those, microfluidic sorting technology has been proposed in order to pick the best spermatozoas with better genomic integrity and physiological quality. It is of interest whether utilizing this technique will reveal better embryo morphology and euploidy rates in couples with RIF undergoing pre-implantation genetic screening (PGS).

Study design, size, duration: This was a retrospective analysis of assisted reproductive technology (ART) cycles performed at the Centrum Clinic IVF Center located in Ankara, Turkey between 2016 and 2017. In total, data from 171 patients were obtained for the analysis that accounts for 491 embryos. In group 1 (STANDARD), a total of 127 cycles and 366 embryos, in group 2 (CHIP) 44 cycles and 125 embryos were evaluated in terms of embryology data and euploidy rates.

Participants/materials, setting, methods: Only patients with complete patient records on clinical, IVF/ICSI cycle characteristics, and PGS (Next Generation Sequencing) analysis could be included in the retrospective analysis. Main indication for PGS was RIF history of couples. Women with age > 43, BMI > 35, with documented uterine abnormalities, trombophilia and azoospermic males were excluded. In CHIP group, microfluid sorted spermatozoas were obtained for IVF/ICSI. On the day of biopsy, 5–10 trophectoderm cells were sent to the laboratory.

Main results and the role of chance:

Table 1 Basal characteristics, stimulation outcomes and embryologic data.

	STANDARD (N = 127 cycles, 366 embryos)	CHIP (N = 44 cycles, 125 embryos)	*p* value
Female age (years)	37.3 ± 4.8	37.4 ± 4.9	NS
AMH (ng/ml)	1.69 ± 1.4	1.8 ± 1.6	NS
FSH (mIU/mL)	8.8 ± 4	7.8 ± 2	NS
TSH (IU/mL)	2.06 ± 0.9	2.1 ± 1.2	NS
Sperm concentration (mil./ml)	22 (1-130)	29 (1-120)	0.03
Total motility (A+B+C) %	46.8 ± 19	48.5 ± 17	NS
DFI % (TUNNEL Assay)	23.5 ± 14	17.1 ± 7.9	NS
Total duration (days)	11.2 ± 2	11.3 ± 1	NS
M2 oocytes (n)	4 ± 2.7	5.4 ± 6.5	NS
2PN (n)	3.7 ± 2.6	4.3 ± 3.5	NS
No of cleavage embryos	3.6 ± 2.4	4.1 ± 3.1	NS
No of blastocysts	3.0 ± 2.1	3.5 ± 2.7	NS
No of top quality blastocysts (5AA, 5AB, 5BA, 4AA)	0.8 ± 1.4	1.6 ± 2.7	0.04
Euploid blastocyt %	7	23	<0.001
Mosaic embryos %	2.7	4	NS
Cost USD (excluding IVF/ICSI, including PGS and/or chip-sorting)	864.5 ± 557	1436 ± 645	<0.001

According to the univariate linear regression analysis;

– female age (β: 0.09, p = 0.24)
– sperm concentration (β: 0.09, p = 0.23)
– collected oocytes (β: 0.2, p = 0.008
– top quality blastocyst formation (β: 0.2, p = 0.001)
– microfluidic chip sorting (β: 0.35, p<0.001) parameters were found to influence (p<0.25) **embryo euplodiy** (%, dependent variable).

When backward multivariate linear regression analysis was performed, chip sorting was the only significant parameter (p = 0.002), whereas top quality blast formation did not reach enough significance (p = 0.06).

Limitations, reasons for caution: Major limitations are retrospective design, limited patient number, lack of DFI data following chip sorting and pregnancy outcomes of the groups. Chip-sorting is not a routine in our center and offered to those with RIF history only, because it comes with a significant cost. Moreover, literature data is very limited.

Wider implications of the findings: Successful euploid-blastocyst formation is the consequence of a perfect fertilization and mitosis. When comparable women age and number of oocytes are considered, chip sorted spermatozoas may influence our results in terms of better blastocyst formation and euploidy when DFI is relatively high (>15% in both groups). Cost should be considered.

Trial registration number: Not applicable.

P-572 Role of male factor in embryo aneuploidy

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Study question: Are sperm quality parameters relevant predictors of embryo aneuploidy?

Summary answer: Sperm apoptosis levels and male age are significantly correlated with different types of embryo aneuploidy in egg donor cycles.

What is known already: Embryonic chromosomal aneuploidy plays an important role in human reproduction causing implantation failures, miscarriages and a wide variety of genetic syndromes. While contribution to embryo aneuploidy has been largely attributed to female factor, focusing on maternal age, male role keeps uncertain. Infertile men often display unconventional semen parameters, nonetheless, wider implications in relation to aneuploidy have not been clarified. There are few studies in which sperm parameters are considered to be involved with embryo aneuploidy. To our knowledge, there is an absence of female factor-controlled studies.

Study design, size, duration: This is a retrospective observational study including data from a total of 102 egg donation cycles undergoing Preimplantation Genetic Testing for Aneuploidy (PGT-A) at blastocyst stage and comprehensive analysis of sperm quality parameters between January and October 2017. Egg donor cycles are optimal to study male factor avoiding maternal effect. Paternal influence on embryo aneuploidy and clinical outcomes were explored in all cases.

Participants/materials, setting, methods: 102 male patients aged 25-64 (41,74 ± 6.9) undergoing egg donation cycles with PGT-A participated in the study. Comprehensive sperm analysis including volume, concentration, total count, motility, morphology, vitality, proportion of apoptotic spermatozoa and DNA integrity was performed in all cases. All embryos were cultured to blastocyst stage, biopsied and PGT-A tested by Next Generation Sequencing (Illumina VeriSeq protocol). Analysis of the association between the different male parameters studied, embryo aneuploidy and clinical outcomes was performed.

Main results and the role of chance: In the study group, no influence of conventional semen parameters on embryo aneuploidy was found. Differences in sperm total count, motility, morphology, vitality, sperm DNA integrity did not impact embryo aneuploidy levels.

Interestingly, in normozoospermic samples, sperm apoptosis significantly correlated with the proportion of aneuploid embryos presenting sex pair abnormalities. Sperm presenting high levels of live apoptotic spermatozoa produced a significantly increased number of embryos with aneuploidies affecting the sex-chromosomes (Spearman Test, $R = 0.348$, $p = 0.044$).

Additionally, male age was found to influence the presence of complex aneuploid embryos, i.e. with 2 or more chromosomes affected (Spearman correlation Test, $R = 0.246$, $p = 0.044$). Men over 40 y.o. showed 1.5× more embryos with several aneuploidies than younger men (46.46%±6.39 vs. 31.15% ±5.98, U-Mann Whitney, $p = 0.0037$).

Finally, a significant influence of sperm apoptosis was also observed beyond preimplantation embryo development, affecting the likelihood of pregnancy after single euploid embryo transfer. The group of males that failed to achieve pregnancy presented significantly higher levels of live apoptotic spermatozoa compared to the ones who accomplished a positive beta (13.40%±1.25 vs. 9.04%±0.972, Student's t-test, $p = 0.004$), and an ongoing 12-weeks pregnancy (12.61%±1.19 vs. 8.15%±1.02, Student's t-test, $p = 0.004$)

Limitations, reasons for caution: The retrospective and unicentric design of this study may be a reason for caution. It is important to note that the number of patients included was limited and should be increased to confirm the results. Further prospective studies are needed to validate our findings.

Wider implications of the findings: Evidence shown raises the question of whether male age and sperm apoptosis should be taken into account as predictive markers for cases at risk of embryo aneuploidy and whose outcomes may be improved through PGT-A. Corrective methods are strongly recommended to palliate sperm apoptosis even if levels are not pathological.

Trial registration number: not applicable.

P-573 Haplotyping and copy-number profiling of single cells by massive parallel sequencing

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Study question: Provide a high-throughput and comprehensive methodology for haplotyping and copy-number profiling single cells to overcome the limitations of SNP arrays by using massive parallel sequencing.

Summary answer: Genotyping-by-sequencing allows for accurate concurrent haplotyping and copy-number profiling of single cells and can be used for preimplantation genetic testing (PGT).

What is known already: PGT embodies not only the diagnosis of disease alleles for Mendelian disorders (PGT-M), but also allows detection of aneuploidies and structural rearrangements genome-wide, PGT-A and PGT-SR, respectively. Genome-wide haplotyping of single cells became feasible through karyomapping. We recently developed haplarithmisis that enables concurrent haplotyping and copy-number profiling of single cells using SNP arrays. These methodologies are being implemented as a generic method for PGT-M in many IVF laboratories. SNP arrays come at a stable cost and the resolution is limited. To provide comprehensive screening of a single cell whole-genome sequencing is necessary, which is currently too expensive and computationally heavy.

Study design, size, duration: To address the limitations of SNP array and the cost of sequencing, we exploit a reduced representation sequencing strategy on a single cell by performing genotyping-by-sequencing (GBS). Proof-of-principle experiments were performed on single cells from HapMap cell lines. For all embryo samples informed consent was given.

Participants/materials, setting, methods: Three different restriction enzymes were tested to digest DNA and reduced representation libraries were generated. Sequencing was performed on an Illumina HiSeq2500 system. Downstream analysis was evaluated and optimized to allow haplotyping with the interactive web application HiVA, which is based on haplarithmisis. Subsequently, an internal validation was performed by comparing GBS with SNP array. Furthermore, nine embryos in three families with different PGT-M indications were analysed.

Main results and the role of chance: For the HapMap samples, a total of 7 single cells of two individuals was used to assess genome-wide haplotype accuracy. Haplotypes from both GBS and SNP array data were generated through HiVA. The concordance rate reached 99% for both maternal and paternal haplotypes. In the internal validation, for a total of 9 embryos from three families with different indications for PGT-M, we obtained 100% accuracy.

Limitations, reasons for caution: This study is limited by the sample size.

Wider implications of the findings: We present a novel NGS-based methodology for haplotyping single cells through massive parallel sequencing. By applying a reduced representation sequencing methodology and the advancing sequencing technologies, we envision that this methodology will a valuable alternative to SNP array technology for genotyping, haplotyping and copy number profiling of single cells.

Trial registration number: not applicable.

P-574 Feasibility of artificial intelligence for predicting live birth from a blastocyst image

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Study question: Can artificial intelligence (AI) applied toward blastocyst images have strong potential to predict the probability of becoming an alive neonate?

Summary answer: Classifiers using AI applied toward a blastocyst image have strong potential to show the probability of an alive neonate being the outcome.

What is known already: None of the features of morphological structures have been conclusively assessed as having prognostic value for the further developmental competence of oocytes. Preimplantation genetic testing for aneuploidy to examine chromosomal profiles is an invasive technique for the embryo, which bring with it considerable ethical arguments. The chromosomal profile of the biopsy specimen does not always represent the profile of the rest of the embryo.

Study design, size, duration: Logistic regression, naive Bayes, neural network, random forest, nearest neighbors and deep learning were examined as machine learning method of AI. The images of the blastocyst of live birth (n = 80) and of abortion because of chromosomal abnormalities (n = 80) were used in retrospective study. The 10-fold ross validation method was used for making classifiers.

Participants/materials, setting, methods: We developed a system for using the machine learning approach of AI to predict the probability of live birth from a blastocyst image. An image was obtained from the fifth day after oocyte retrieval. The off-line images of which training data were augmented were transferred to the AI, then the classifier by the AI was made as the accuracy was higher and the variances were lower as much as possible.

Main results and the role of chance: The logistic regression with L2 regularization was the best among the above mentioned machine learning methods. Only a few seconds are needed to complete analysis of each image. The accuracy, sensitivity, specificity, positive predictive value and negative predictive value for predicting those in the abortion category were 0.619, 0.600, 0.638, 0.626 and 0.629, respectively. Area under the curve of the receiver operator curve was 0.594 ± 0.045 (mean ± standard error). Estimated probability of belonging to the live birth category was found significantly related to the probability of live birth ($P<0.02$).

Limitations, reasons for caution: Though the logistic regression showed the best result among the six machine learning methods examined, further investigation in the future might prove other machine learning methods superior.

Wider implications of the findings: This method does not harm the embryo, which can be transferred after establishing the prediction. It offers economic and time savings for patients and/or clinical institutes, gives quick and efficient diagnosis of the classification.

Trial registration number: not applicable.

P-575 Preimplantation genetic screening (PGS) with next generation sequencing (NGS) in recurrent spontaneous abortion patients (RSA); does it help?

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Study question: Does PGS using NGS increase the live birth rate in recurrent spontaneous abortion patients?

Summary answer: PGS using NGS successfully increases the live birth rate in recurrent spontaneous abortion patients.

What is known already: Advances in PGS with the newest NGS technology, considered the main technique for selecting embryo to result in healthy pregnancy, has the ability to accurately know the ploidy status and select an embryo prior to implantation thereby removing aneuploidy, which is one of the most common cause of recurrent spontaneous abortion, as the cause of spontaneous abortion in the equation for RSA. RSA patients are considered the best candidates for PGS however, limited data exist about the effectiveness of this technique.

Study design, size, duration: This retrospective study included a total of 161 patients with a history of recurrent spontaneous abortion undergoing artificial reproductive technology at our hospital from the year 2015 to 2017. We included 67 patients in the PGS group and 94 patients in the control no PGS group. The comprehensive chromosome screening was done using NGS.

Participants/materials, setting, methods: All patients underwent controlled ovarian stimulation and oocytes retrieval. In the PGS group, ICSI, trophectoderm biopsy and blastocyst cryopreservation was performed before single frozen balanced/euploid embryo transfer detected by NGS molecular cytogenetic analysis. For the no PGS group, IVF or ICSI was followed by single or double embryo transfer with fresh or frozen embryos. The primary outcome was live birth.

Main results and the role of chance: After 209 cycles on 161 patients, a total of 70 embryos were transferred (0.97 per cycle) in the PGS group, of which 37 (53.6%) implanted, resulting in a live birth rate of 47.8%. In the no PGS group a total of 166 embryos were transferred (1.21 per cycle), of which 48 (28.9%) implanted, resulting in a live birth rate of 26.5%. The higher live birth rate in the PGS group was statistically significant ($p<0.05$). The analysis revealed aneuploidy in 123/240 (51.25%) of blastocyst tested in the PGS group, among which 31(12.9%) had single chromosome loss (monosomy), 24 (10.0%) displayed single chromosome gain (trisomy), 21/240 (8.75%) had dual chromosomal abnormality, 13 (5.42%) showed complex chromosomal abnormality and 34/240 (14.2%) were observed with mosaicism.

Limitations, reasons for caution: This study is a single center retrospective study with a small study population. Any findings can only be indicative and the improved live birth rate after PGS with NGS should further be investigated in a larger population.

Wider implications of the findings: Our findings show that PGS with NGS improves the live birth rate in recurrent spontaneous abortion patients. If confirmed, these results could encourage the routine use of PGS with NGS to reduce the time to achieve pregnancy and avoid the physical and emotional burden of a miscarriage.

Trial registration number: Not applicable

P-576 Whole exome sequencing identified of MEI1 mutation causing human non-obstructive azoospermia

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Study question: What's the genetic etiology of non-obstructive azoospermia (NOA) in a consanguineous Han Chinese family?

Summary answer: A novel homozygous missense mutation in *MEI1* gene, c.1585 T>A, might be the genetic cause of NOA in this family.

What is known already: *Mei1* knockout mice have been reported to present with NOA and only two polymorphic alleles (SNP3 (C1791A) and SNP4 (C2397T)) of the human *MEI1* gene were associated with azoospermia. No causative mutation has been identified in *MEI1* in men with NOA.

Study design, size, duration: Genomic DNA samples from a consanguineous Han Chinese family, 50 sporadic NOA patients (with normal 46, XY karyotype and no Y-chromosome microdeletions) and 200 fertile male controls with randomly matched age (32 ± 2.7 years) who had at least one child were obtained.

Participants/materials, setting, methods: Whole exome sequencing was performed to identify the genetic cause of NOA in two affected brothers of a consanguineous Chinese family. Histological analysis was performed for the biopsied testicle sample in the proband. Mutation screening was performed using Sanger sequencing for 50 NOA patients and 200 fertile male individuals, and *in silico* analysis was conducted for functional characterization of the mutation. Expression analysis of various tissues of human and mouse was performed by using RT-PCR.

Main results and the role of chance: We identified a homozygous missense mutation (NM_152513.3: c.1585 T>A, p.Phe529Ile) in a new gene, *MEI1*, which co-segregated with the NOA phenotype in this family. The identified missense mutation results in the substitution of a conserved phenylalanine residue with isoleucine in the SCOP domain of MEI1, which might resulted in protein misfolding. Histological analysis demonstrated a lack of spermatozoa in the proband's seminiferous tubules due to meiotic arrest. Expression analysis showed that *MEI1* is expressed specifically in adult human and mouse testis. For mutation screening, we only detected one heterozygous mutation of the c.1585 T>A in *MEI1* in a cohort of 200 fertile controls. We did not find any association between the two polymorphic alleles (SNP3 (C1791A) and SNP4 (C2397T)) of the human *MEI1* gene and NOA.

Limitations, reasons for caution: All novel putative disease-causing mutations may only be clinically reported as 'variants of uncertain significance' (VUS), unless and until the mutation is functionally characterized and more patients with a similar phenotype are discovered with the same mutation.

Wider implications of the findings: To the best of our knowledge, this is the first report of the identification of *MEI1* as a new causative gene with an autosomal recessive mode of inheritance for human NOA.

Trial registration number: Not applicable.

P-577 Novel inactivating mutations in the FSH receptor cause premature ovarian insufficiency with resistant ovary syndrome

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Study question: What is the genetic aetiology of three resistant ovary syndrome (ROS) pedigrees from thirteen Chinese Han families with non-syndromic premature ovarian insufficiency (POI)?

Summary answer: Four novel mutations of the FSHR gene were the genetic aetiology of these three ROS families, respectively.

What is known already: To date, only 15 FSHR-inactivating mutations have been described in women affected by POI, each of them is a missense mutation.

Study design, size, duration: Thirteen Han Chinese families with POI were recruited from the Reproductive and Genetic Hospital of CITIC-Xiangya. Genomic DNA samples from the thirteen portends and their family members were obtained.

Participants/materials, setting, methods: To identify the genetic cause of POI in the affected women from these families, the proband in each family was subjected to whole-exome sequencing and the putative disease-causative variations were confirmed by Sanger sequencing. Bioinformatic and in vitro functional analyses were performed for the functional characterization of the FSHR mutations, which aimed to address the clinical relevance of the three novel point mutations.

Main results and the role of chance: Four novel mutations, two homozygous mutations (c.419delA(p. Lys140Argfs*16), c.1510 C>T(p. Pro504Ser)), and a compound heterozygous mutation (c.44 G>A(p. Gly15Asp) and deletion of exons 1 and 2) of FSHR gene were identified in the three non-syndromic POI-with-ROS families. A donor splice site mutation in STAG3 (c.1573 +5 G>A) and a missense mutation in DMC1 (c.106 G>A) were identified in two of the thirteen families, one in each family. Bioinformatic analysis predicted that the three novel point mutations in FSHR are deleterious and are associated with POI in the three families, which was confirmed by in vitro functional analysis in which FSH-induced cAMP production was abolished for all receptors.

Limitations, reasons for caution: All novel putative disease-causing mutations may only be clinically described as 'variants of uncertain significance' unless and until more patients with a similar phenotype are discovered in the same mutation.

Wider implications of the findings: To the best of our knowledge, c.419delA is the first report of a truncation mutation of FSHR, and c.44 G>A is the first missense mutation in the signal peptide-encoding region of FSHR causing POI. Our findings expand the mutational spectrum of FSHR and are valuable for the genetic diagnosis of POI.

Trial registration number: not applicable.

P-578 The effect of paternal trans fatty acid diet on expression pattern of PPAR genes in the ovary of rat offspring

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Study question: Does the intake of trans fatty acids in paternal diet affect on body weight index and also expression pattern of *PPAR* metabolic genes in the ovary of rat offspring?

Summary answer: Paternal intake of trans fatty acids will increase the female offspring's body weight in rats and decrease the expression of *PPAR* genes in their ovary.

What is known already: Studies on maternal exposures and the risk of diseases in the offspring is growing. But paternal associations are often not considered. Trans fatty acids in industrial oils have harmful effects on human health and cause to significant changes in expression pattern of several metabolic genes. PPARs are nuclear receptors that involve in many metabolic functions in different organs including ovary.

Study design, size, duration: The study included 20 adult female Wistar rats (5 in each groups) that their fathers were fed in the following groups for 60 days. C: Standard diet group (control group), CT: The trans fatty acid group, E: The group contains vitamin E, ET: The group containing vitamin E + trans fatty acid.

Participants/materials, setting, methods: ovary of rat offspring was isolated. Total RNA was extracted from ovary using TRIzol reagent. A Nanodrop (Thermo Fischer) was used to quantify total RNA extraction and cDNA synthesis was performed. Using designed primers the mRNA expression of *PPAR* family genes were measured by real time polymerase chain reaction. Expression data was analyzed by SPSS- one way ANOVA.

Main results and the role of chance: There was a significant difference between the mean body weight of the groups under study ($F(3,36) = 10.24$; $p<0.001$). According to the findings, the mean body weight of the CT and ET groups were significantly higher than the E and C groups from 5 and 10 wk of age, respectively. The mRNA expression levels of *PPAR* genes were decreased in ovary tissue in group CT, and increased in group E, although these differential expressions were only significant for the *PPARγ* member gene in group E ($p<0.05$). There were no significant differences in *PPARs* in group ET.

Limitations, reasons for caution: In order to have robust data concerning the effect of trans fatty aicds on expression of metabolic genes, an even larger dataset would be required.

Wider implications of the findings: We imply that excessive consumption of trans fatty acids can have bad genetic/epigenetic effects on human reproduction, and vitamin E can diminish this consequence by its antioxidant function.

Trial registration number: NA.

P-579 Next-generation sequencing (NGS) based preimplantation genetic testing for products of reciprocal translocation (PGT-TR) improves diagnosis of the patients by verification of their classical karyotyping results

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Study question: Can results of next-generation sequencing based preimplantation genetic testing for products of reciprocal translocation (PGT-TR) be useful for verification patient's postnatal karyotype result?

Summary answer: Molecular karyotyping of embryos provides information that helps to localize the breakpoints more accurately, leading to verification of classical karyotype results of the patients.

What is known already: Classical banding karyotype and fluorescence in situ hybridization (FISH) are classical techniques used for detection and description of reciprocal translocations. The application of molecular techniques, e.g. aCGH and NGS allows for description of unbalanced products of chromosomal rearrangements in higher resolution. The patients who are carriers of balanced reciprocal translocations routinely are identified by classical karyotype and results are provided to the laboratory conducting preimplantation genetic testing during course of IVF treatment. These results serve as the reference information in the prediction of localization and size calculation for putative products of translocations.

Study design, size, duration: Study was performed from December 2016 to January 2018 and included 352 cases of reciprocal translocations. PGT-TR outcomes were analyzed for presence and localization of the breakpoints based on patients' classical karyotype results provided to the laboratory.

Participants/materials, setting, methods: Biopsy samples were subjected to whole genome amplification (WGA) and whole genome library were constructed. Ion Personal Genome Machine System was used for whole genome sequencing. The Torrent Suite Software was used to generate BAM files. The BAMs files were uploaded to the Ion Reporter Software used for genotyping. Results with MAPD value (the Median of the Absolute values of all Pairwise Differences) less than 0.2 were qualified for further interpretation.

Main results and the role of chance: The localization of breakpoints in molecular karyotyping were overlapped with classical karyotyping in 668 out of 704 positions. Remaining 36 spots, which is 5.1% of all breakpoints analyzed, were subjected to further investigation specifically. The differences in localization ranged from 5 to 26 mega bases (Mb). In case when data derived from two or more embryos and re-localization of the breakpoint's coordinates affected of classical band descriptions laboratory ask the clinics for verification provided result of postnatal karyotype. All verifications resulted in new description concordant with molecular coordinates obtained during preimplantation testing.

Limitations, reasons for caution: The reliability of this technique may be lower in case of rearrangements where the breakpoints are located near telomeres or centromeres what may have effect on precision of established coordinates.

Wider implications of the findings: The application of next generation sequencing in preimplantation genetic testing for chromosomal rearrangements enhances the quality of diagnosis in the infertility clinics and have potential effect on reproductive risk calculation and prognosis to the patients.

Trial registration number: Not applicable.

P-580 Comparison of mitochondrial DNA content in euploid and aneuploid embryos at cleavage stage and blastocyst stage with NGS

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Study question: Is the mitochondrial DNA-content (mtDNA) and its dynamics in cleavage stage and blastocyst stage embryos different in euploid and aneuploid embryos when measured with NGS?

Summary answer: mtDNA-content is comparable in euploid and aneuploid embryos at cleavage stage, however, it is significantly higher in aneuploid embryos at blastocyst state.

What is known already: Mitochondria and their DNA (mtDNA) are lately increasingly investigated regarding their ability as a potential marker for embryo viability and implantation chances and an increased number of mtDNA-copies in the blastocyst seems to be associated with decreased implantation-rates. Replication of mtDNA is not initiated during the first 3 days of embryo development and because of cell division, mtDNA-content is expected to decline from cleavage to blastocyst stage biopsy. Increased mtDNA-numbers at blastocyst stage are found with advanced female age and in aneuploid embryos. However, so far, data on the mtDNA-content in euploid and aneuploid embryos at cleavage stage is rare.

Study design, size, duration: Retrospective, blinded, observational study including 35 patients (118 embryos) which underwent preimplantation genetic testing (PGT-A) from August 2016 to January 2017. This study validated the chromosomal status of D5 embryos previously diagnosed on D3 and determined the mtDNA-content. Double biopsy (D3 and D5) was performed to all embryos that reached blastocyst stage on D5 and were not selected for transfer (including surplus euploid embryos that could not be vitrified according to the UAE law).

Participants/materials, setting, methods: Infertile patients with normal karyotype undergoing preimplantation genetic screening with fresh oocytes and with ≥5 MII or more than 4 embryos to biopsy on D3. For embryo biopsies at D3, only embryos with ≥5 nucleated blastomeres and < 25 % fragmentation and for blastocyst biopsy, only hatching blastocyst were evaluated. Relative values of mitochondrial DNA were directly obtained from the software and were analyzed using IGenomix algorithm for day 3 and day 5 biopsies.

Main results and the role of chance: After genetic screening at cleavage stage, there were 70 aneuploid and 48 euploid embryos. In the group of aneuploid embryos, mean mtDNA-content on D3 was 50.8 with a range from 31.7 to 99.4, a 95% confidence interval from 47.1 to 54.4 and a standard deviation (SD) of 15.3. In the group of euploid embryos, mean mtDNA on D3 was 51.1 with a range from 35.2 – 99.6, a 95% confidence interval from 47.3 to 54.8 and SD of 13.0. No significant correlation between mtDNA on D3 and the chromosomal status of the embryos was found ($p = 0.628$).

After blastocyst biopsy, the results of the genetic screening revealed 51 aneuploid and 62 euploid embryos. In the group of aneuploid embryos, mtDNA-content was 22.8 with a range of 12.2 – 48.4, a 95% confidence interval of 20.8 to 24.9 and SD of 7.3. In the euploid embryos, the results were 20.6 for the mean mtDNA-content with a range of 12.8 to 45.4, a 95% confidence interval of 19.0 – 22.2 and SD of 6.2. A significant difference in mtDNA on D5 between aneuploid and euploid embryos ($p = 0.034$) was found with Mann-Whitney test.

Limitations, reasons for caution: It is a bivariate analysis (aneuploidies versus mtDNA-content), and not controlled by other covariates that could potentially bias the relationship we are studying. At blastocyst stage, 5 embryos with trophectoderm Mitoscore values above 1.000 were considered as "outliers" and therefore excluded from the comparison between euploid and aneuploid embryos.

Wider implications of the findings: The fact that mtDNA-content is similar in euploid and aneuploid cleavage stage embryos, however significantly higher in aneuploid blastocysts supports the idea that mtDNA-content might have a direct impact on chromosome segregation during embryo development. It seems that embryos under energetic stress are forced to increase the biogenesis as compensation.

Trial registration number: not applicable.

P-581 Whole-genome methylomic profiling of iPSC-derived granulosa-like cells and adult granulosa cells implicates novel pathophysiological pathways of polycystic ovarian syndrome

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Study question: Were the epigenetic aberrations (esp. DNA methylation) involved in the pathophysiology of polycystic ovarian syndrome (PCOS)?

Summary answer: Common differentially methylated pathways were found between iPSC-derived granulosa-like cells (GLCs) and adult granulosa cells (GCs), which might be related to the pathophysiology of PCOS.

What is known already: Although the heritability of PCOS is considered as high as 70% from the twin study, there is no definite candidate gene found. Epigenetic aberrations had been found to be involved in the pathogenesis of metabolic disorders such as type 2 diabetes and could possibly also contribute to the etiology of PCOS.

Study design, size, duration: This is a case-control study conducted from 2014-2016. Eleven patients of PCOS and 4 control subjects who underwent in-vitro fertilization treatment were recruited for the collection of ovarian GCs. Another 2 patients of PCOS and 2 control subjects were recruited for the collection of skin fibroblasts.

Participants/materials, setting, methods: Luteinized ovarian GCs were collected during oocyte retrieval and were purified by Ficoll gradient and cell culture. Patient-specific iPSC were established from skin fibroblasts and were then differentiated into GLCs using multistep approaches with cocktails of growth factors. Whole-genome DNA methylation profiles were obtained using Infinium Methylation EPIC Beadchip in both adult GCs and iPSC-derived GLCs. Other validation methods included real-time PCR, western blotting, immunofluorescence, flow cytometry, and in vivo and in vitro differentiation assays.

Main results and the role of chance: We demonstrated a successful derivation of PCOS-specific iPSCs from fibroblasts of women with PCOS by applying the safer, non-viral episomal reprogramming and differentiate them into ovarian GLCs through a cocktail of growth factors. Gene expression analyses revealed the GC specific markers including anti-Mullerian hormone (AMH), type 2 AMH receptor, follicle-stimulating hormone (FSH) receptor, luteinizing hormone (LH) receptor and estrogen synthetase cytochrome P450 19A1 (CYP19A1) all expressed in differentiated GLCs derived from PCOS and non-PCOS iPSCs. When integrating the results of whole-genome DNA methylomic analysis in iPSC-derived GLCs and adult GCs, a commonly overexpressed cAMP-response element binding protein (CREB) pathway in the PCOS group was found. Western blot also revealed a higher expression of CREB binding protein in the iPSC-derived GLCs and adult GCs in the PCOS group. The aberration of DNA methylation could explain the hereditary trait and the susceptibility to environmental influence of PCOS. The methylomic aberrations existed after cell reprogramming and re-differentiation, and this might suggest that the development origin of PCOS could start from very early stage.

Limitations, reasons for caution: The population of PCOS is highly heterogeneous and our case number is small. Further validation in a larger cohort is necessary.

Wider implications of the findings: Our findings not only provide new perspectives on the pathophysiology and therapeutic choices of PCOS, but also provide new knowledges on the stem cell and epigenetic research.

Trial registration number: Nil.

P-582 Optimized NGS based protocol for the detection of small duplications/deletions in preimplantation embryos from carriers of balanced translocations and inversions

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Study question: Is it possible to develop an accurate, automated and fast Next Generation Sequencing (NGS) based Preimplantation Genetic Testing for structural rearrangements (PGT-SR) protocol?

Summary answer: NGS can be applied to identify duplications and deletions ≥6 Mb in carriers of balanced translocations/inversions offering high sensibility and specificity in trophectoderm biopsies.

What is known already: Chromosome translocations are the most frequent structural chromosomal abnormalities in humans. In carriers of balanced translocations and inversions, small duplications and deletions can arise because of adjacent meiotic segregation. The presence of inter-chromosomal effect has been described inducing a higher risk of aneuploidies for chromosomes not involved in the rearrangement. Array-CGH (comparative genomic hybridization) and FISH (fluorescence in-situ hybridization) have been used to detect these alterations. Currently, NGS is widely applied in PGT for aneuploidy (PGT-A) and to increase the resolution for its application in PGT-SR, improved NGS protocols are needed to detect deletions/duplications ≥6 Mb.

Study design, size, duration: From July to January 2017, an optimized NGS protocol to detect unbalances ≥6 Mb was developed and validated using 10 Coriell cell lines with deletions of known sizes (5.8-20 Mb). To test the sensibility and specificity of the protocol a set of experiments using pools of 4-6 cells mimicking trophectoderm biopsies was set up (n = 38). Then, 136 trophectoderm biopsies with unbalances of expected sizes according to the reported karyotype ranging from 6.4-57.8 Mb were analysed.

Participants/materials, setting, methods: In the cell lines experiments, a total of 38 samples, with a minimum of three samples per cell line were analyzed. A modified Reproseq protocol was employed for genome amplification, library preparation and purification, using Ion Chef™ and S5 sequencer (Thermofisher). A customized workflow was developed using the Ion Reporter software version 5.4 for analysis and interpretation of the sequencing data. Afterwards, 136 trophoblast biopsies were analysed following the protocol developed with cell lines.

Main results and the role of chance: Using the pools of cells with deletions of different sizes, we set the detection limit of our protocol in 6 Mb. The percentage of detection was 100%. In trophoblast biopsies from 35 patients, we analysed 136 embryos and 99.3% of them were informative. The percentage of unbalanced embryos was 44.8% (61/136). Full chromosome aneuploidies for chromosomes not involved in the rearrangements were observed in 38.9% (53/136) of the biopsies: 16.9% (23/136) of them in unbalanced biopsies and 22.1% (30/136) in balanced biopsies. Additionally, this protocol can be completed in 12-13 hours, from the preparation of the sample (lysis, preamplification, amplification, purification, pooling and quantification) to the release of the results.

Limitations, reasons for caution: It could be variability in the number of cells retrieved in the biopsy and their integrity accounting for potential amplification failures or non-informative results. Also, the whole genome amplification could result in false positives/ negatives. Nevertheless, in the validation study no false positives/negatives were observed.

Wider implications of the findings: Formerly, FISH or array-CGH were the techniques used for PGT-SR. These techniques have disadvantages. Here in, we describe an improved, mostly automatized, fast, and accurate protocol for detecting small del/dup up to 6 Mb. An additional advantage is that PGT-A and PGT-SR could be performed with similar equipment.

Trial registration number: Does not apply.

P-583 Exome sequencing in a consanguineous family including spontaneous ovarian hyper-stimulation cases with unknown triggers

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Study question: Ovarian hyper-stimulation can rarely occur spontaneously in patients not undergoing ovulation induction therapies. Could we determine underlying factors via high throughput sequencing?

Summary answer: By using whole exome sequencing, a homozygous autosomal mutation has been identified in two affected sisters in the family.

What is known already: Spontaneous ovarian hyper-stimulation syndrome (sOHSS) is a rare case of OHSS, defined as OHSS appearing in patients with no history of stimulatory infertility treatment. Cases of sOHSS have been reported in women with severe primary hypothyroidism, polycystic ovary syndrome while pregnant, gonadotroph pituitary adenoma, and normal pregnancy. So far, mutations in FSH receptor (FSHr) have been identified only in cases of sOHSS triggered by pregnancy, no causative mutation has been identified for non-pregnant sOHSS cases with unknown triggers.

Study design, size, duration: This is a case control study carried on a consanguinous Turkish family comprising four siblings in which 2 sisters showing well-documented recurrent sOHSS with no currently known triggers. Affected sisters had sOHSS sporadically without pregnancy, TSH levels are normal, no tumors or pituitary adenomas have been detected.

Participants/materials, setting, methods: Saliva samples have been collected from parents and all siblings. Genomic DNA was extracted from saliva using Oragene DNA self-collection kit (DNA genotek, Ottowa, Canada) according to the manufacturer's instructions. Whole exome sequencing of parents and four sisters was performed by the Institute of Genetics and Molecular and Cellular Biology microarray and sequencing platform, member of the 'France Génomique programme'. Suspected mutations were confirmed via Sanger sequencing.

Main results and the role of chance: A homozygous autosomal mutation on chromosome 10 has been identified in two affected sisters. Parents and one of the fertile sisters are carrier while the third fertile sister is wild type. Results were confirmed with Sanger sequencing. The identified mutation alters the wild type donor site and most probably affecting splicing. Functional studies are planned in order to highlight underlying mechanism related to the identified mutation.

Limitations, reasons for caution: Our study contains only one family presenting this very rare form of female infertility.

Wider implications of the findings: Our results provide a new understanding for the pathogenesis of sOHSS and help us to identify underlying trigger in our patients. It may help to define novel therapeutic approach to patients with similar symptoms.

Trial registration number: Not applicable.

P-584 Comprehensive comparison of trophectoderm (TE) and inner cell mass (ICM) by next generation sequencing (NGS)

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Study question: Does the chromosomal information of trophectoderm (TE) accurately predict the chromosomal information of the inner cell mass (ICM)?

Summary answer: The observed discordance rate between TE and ICM was 15.9%. Hence, further exploration on the implication of mosaicism due to mitotic errors is mandatory.

What is known already: The most important cause for implantation failure is chromosomal imbalance in the embryo. Identification of a chromosomally normal embryo is possible due to current techniques available; however, mosaicism can complicate interpretation of the result. Whereas meiotic errors of the oocyte result in uniform aneuploidies, errors occurring during the first three mitotic division of the embryo might lead to mosaicism. TE biopsy is commonly used to infer the chromosomal status of the ICM, hence in case of mosaicism, TE biopsy might not represent the correct chromosomal status of the ICM and could therefore lead to a misdiagnosis of embryo´s chromosomal status.

Study design, size, duration: Observational, blinded study, including 88 embryos, that underwent PGS prior embryo transfer, between August 2016 and January 2017. NGS technique used to compare the chromosomal status of the trophectoderm and the inner cell mass in blastocysts on embryos that were not selected for transfer (including surplus euploid embryos that could not be cryopreserved according to the UAE law).

Participants/materials, setting, methods: Infertile couples with normal karyotype undergoing PGS with fresh oocytes, female partner with age 18 to 45 years and a body mass index of 19 to 30. Only expanded blastocyst with distinguishable ICM were biopsied. The genetic laboratory was blinded regarding the identity of the couple and the origin of the sample.

ICM biopsy was performed as described in the validated procedure by Capalbo et al. 2013.

The patients were counseled and consents were obtained.

Main results and the role of chance: Out of the 88 embryos, in which trophectoderm and ICM biopsy could be performed, 14 embryos (14/88 = 15.9%) had a discrepant chromosomal status between ICM and trophectoderm.

In depth analysis of the embryos with discordant findings of trophectoderm and ICM, in 4 embryos (4/14 = 28.57%; 4/88 = 4.54%) ICM revealed an euploid finding and trophectoderm an aneuploid finding (= false-positive for trophectoderm biopsy). In 3 embryos (3/14 = 21.42%; 3/88 = 3.41%), ICM reported an aneuploid finding and trophectoderm an euploid finding (= false-negative of trophectoderm biopsy). Therefore in total 7 discrepancies (7/14 = 50%; 7/88 = 7.95%) regarding the embryo diagnosis (euploid / aneuploid; aneuploid / euploid) for whole chromosome analysis. The other 7 embryos (7/14 = 50%; 7/88 = 7.95%) had different chromosomal abnormalities in ICM and trophectoderm.

Limitations, reasons for caution: To thoroughly identify the exact rate of mosaicism, more embryos should be included in future studies.

Moreover, the well known methodological artefacts linked to the whole-genome amplification, warrant careful interpretation of the current findings.

Wider implications of the findings: Mosaicism in the embryo results from mitotic errors during the first cleavage divisions and different proportions of the embryo can be affected. Our results of false-positive TE biopsy question the accuracy of aneuploidy results from TE biopsy in regards of being representative for the whole embryo.

Trial registration number: Not applicable.

P-585 Segmental aneuploidy (SA) in oocytes and preimplantation embryos

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Study question: Does the detection of SA reflect a real event or is it an artifact?

Summary answer: Sequential biopsies of the same oocyte or embryo indicate that SA most probably are a real event.

What is known already: SA, the loss or gain of chromosomal fragments, has been detected in ~6% of spontaneous abortions. Some are compatible with life and the affected children carry a range of congenital abnormalities. The current cytogenetic techniques used in preimplantation genetic testing for aneuploidy (PGT-A) have shown that SA occur at appreciable frequencies. However, the possibility exists that whole genome amplification (WGA) may induce artifacts resulting in SA.

Study design, size, duration: This retrospective study includes 285 PGT cycles from January 2014 to December 2017. According to clinical indications, biopsy was performed on polar bodies (PB, n = 66 cycles), blastomeres (n = 169 cycles) or trophectoderm cells (TE, n = 50 cycles). In cases of SAs involving loss or gain in excess of 15 Mb, embryos were excluded from transfer and, depending upon the patients' consent, re-biopsy was performed. The aim was to evaluate the repeated occurrence of SA in following biopsies.

Participants/materials, setting, methods: Whole genome amplification and array comparative genomic hybridization (a-CGH) (Illumina) was done on cells biopsied from oocytes and / or embryos of IVF patients with normal karyotype from our PGT-A program. The chromosome analysis of sequential biopsies was performed at later stages, after the conclusion of the treatment cycle. Re-biopsies was done in 48 cases and included sequential blastomere biopsy, TE and BF.

Main results and the role of chance: The occurrence of SAs was significantly lower in oocytes (4.5%; 13 oocytes over 288 analyzed), compared with 13% in blastomeres (105/821; P<0.001), 11% in TE cells (P<0.005), and 15% in BF (P<0.001). The analysis of sequential biopsies revealed that in 6 of 13 oocytes (46%), SA occurred repeatedly: 2 were reciprocal in PB1 and PB2, while 4 were also detected in the following stages. In blastomeres, SA were confirmed in 11 of 16 samples (69%): 5 had the same SA, 4 had the opposite SA, and 2 cases involved the whole chromosome. In TE cells, we had 19 sets of sequential biopsies, of which 15 were confirmed (79%): 3 had the same SA plus 4 case involving the whole chromosome, and 5 had the opposite SA plus 3 cases involving the whole chromosome. In all, 32/48 SAs detected in one biopsy occurred also in subsequent biopsies as the same anomaly (n = 8), opposite anomaly (n = 15) or involving the whole chromosome (n = 9). Excluding the 9 re-biopsies involving the whole chromosomes, SAs were confirmed in 23/48 subsequent biopsies (48%). The highest incidence of SA was found in chromosomes 1, 2, 9 and 16.

Limitations, reasons for caution: The cytogenetic analysis on blastomeres and TE cells did not take into consideration the occurrence of mosaicism for which each biopsy might be not representative of the whole embryo.

Wider implications of the findings: These data could be relevant for PGT-A, where the detection of de novo SA seems to reveal an event that has either meiotic or mitotic origin. The transfer of embryos carrying SA should be strictly followed-up to gain knowledge on implantation and on the genetic status of any resulting fetus.

Trial registration number: Not applicable.

P-586 MiNtagSNP: A tagSNPs selection algorithm for informativity maximization in Preimplantation Genetic Diagnosis (PGD) through the use of Single Nucleotide Polymorphism (SNP)

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Study question: Could a tagSNP-algorithm be used for finding usefull informative SNPs for a PGD case?

Summary answer: MiNtagSNp is an application for designing single and multi-population genotyping panels based on the linkage disequilibrium of SNPs and additional constraints imposed by the user.

What is known already: PGD has become a standard of care when dealing with stopping the transmission of heritable-diseases. At first sight it seems that the directly sequencing of the disease locus is the best option to discard sick and carrier embryos, but because of the Allele drop-out effect (ADO) the disease allele could be present but silent.

Ancient methods aimed the amplification of polymorphic Short Tandem Repeats (STRs) to detect mutant and wild allele. However, even when the technology has evolved quickly is still deficient to face these kind of problems.

Study design, size, duration: We performed a new tagging-approach that combines the correlation calculations of Linkage Disequilibrium criteria in order to select informative SNPs (Informative success: heterozygous in one of the both parents and homozygous in the other one)

Number of tagSNP selected and the number of informative-tagSNP finding were calculated to evaluate our algorithm.

TagSNPs were obtained for three regions-of-interest and compared with the results obtained by a STRs approach. Time execution, sensitivity and specificity were also evaluated.

Participants/materials, setting, methods: *In-silico* and *in-vitro* approaches were achieved in order to evaluate our algorithm.

For the *In-silico set,* phased SNPs were obtained for the 404 individuals of the European super-population registered in 1000Genomes db.

Sequencing data from 15 trios (parent couple + child) obtained from the Coriell Institute and 15 trios (appropriately informed) from our own laboratory were used to perform two *in-vitro sets.*

Main results and the role of chance: An SNP is a genetic variation when a single nucleotide in the DNA-sequence is altered and kept through heredity thereafter. A tagSNP is a polymorphic site that represent others in a DNA-region; using tagSNP we could ensure that disease embryos may be discard for the transferred phase because not onlythose that shows the disease allele are discard, even those with thehaplotype associated to it. But there is no software specially designed for accurately use them for PGD till now.

MiNtagSNp automates the design of tagSNP genotyping panels with maximum likelihood of informative success, while minimizing the number of tagSNP to assay. This approach makes MiNtagSNP different from other applications that only considers if one SNP can be tag of a region, block, or set of SNPs, without considering if the informativity problem solution is maximized or not.

An extensive experimental study on various datasets including three regions of interest of the evaluation approach shows that MiNtagSNP uses fewer tagSNPs than the state-of-the-art methods, maximizing the prediction accuracy and the informativity problem solution.

Limitations, reasons for caution: Consanguineous couples may have large homozygous regions due to the heritance of the same alleles; these regions could interfere with MiNtagSNP approach.

Wider implications of the findings: We develop an efficient computational framework for tagSNP selection that is able to identify smaller tagSNP sets, than the other studied algorithms, usefull for PGD cases and always outperform in terms of the tagSNP size and number of choosen informativity-SNPs.

Trial registration number: none.

P-587 Aneuploidy rescue in preimplantation embryos

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Study question: How do human embryos try to rescue an aneuploid condition?

Summary answer: One possible mechanism involved in aneuploidy rescue includes the active extrusion of aneuploid cells into the blastocoelic fluid (BF).

What is known already: Embryos try to rescue aneuploidy, although the majority of chromosomal errors are probably unlikely to be corrected, especially if they involve many chromosomes. Accordingly, most of arrested embryos carry multiple chromosomal errors, suggesting that chromosomal abnormalities are tightly related to embryonic arrest. Some embryos with total or partial aneuploidy can form blastocysts that are morphologically indistinguishable from those with a euploid status. Their implantation potential depends on the proportion of aneuploid cells. Several processes can contribute to the self-correction of aneuploidy, including the formation of micronuclei, cellular fragmentation, and apoptosis.

Study design, size, duration: This retrospective analysis includes a subset of 156 embryos from 71 patients with normal karyotype that underwent PGT-A from January 2010 to December 2017. Embryos were biopsied at the blastocyst stage by removing 6-8 trophectoderm (TE) cells and the BF. The aim of the study was to compare the number of chromosome errors in TE-DNA and in the corresponding BF to verify whether the BF could be a sort of collector of aneuploid DNA.

Participants/materials, setting, methods: Whole genome amplification and array comparative genomic hybridization (a-CGH) (Illumina) was done on TE cells. Before vitrification, the BF was collected and stored for later analysis that was performed by operators blinded to the results of the corresponding TE cells. Results were reported on a per-chromosome basis, but cases of segmental aneuploidy (SA) involving loss or gain of chromosomal fragments in excess of 15 Mb were also described.

Main results and the role of chance: Chromosomal results from the analysis of 156 BFs and TEs from the same embryo showed a ploidy concordance of 93.6%, namely 104 cases of total concordance (66.6%), and 42 of partial concordance (27%). The remaining 10 blastocysts (6.4%) had a ploidy discordance. The overall mean number of aneuploid chromosomes was significantly higher in

BFs than in TE cells (1.9 versus 1.3 respectively, P = 0.01). In the cases of partial concordance, this figure was 4.0 versus 2.2 respectively (P = 0.002). Both values were significantly more frequent in the group of partial concordance in comparison to the group of total concordance (1.1; P<0.001). In the 10 discordant cases, 4 were aneuploid for TE cells and euploid for BF, while the remaining 6 blastocysts had the opposite condition. SA were found in 19 embryos (11.4%). In 7 cases they were present in both TE and BF, while in the remaining 15 cases, 8 were in BF (TE was either normal or aneuploid for the whole chromosome) and 4 in TE cells (BF was aneuploid for the whole chromosome).

Limitations, reasons for caution: A higher number of observations is necessary to support the hypothesis that the embryo actively tries to extrude aneuploid cells into the BF.

Wider implications of the findings: The higher incidence of chromosome abnormalities including SA in BFs could reflect a mechanism that embryos have against aneuploidy. The following outcome depends on the grade of mosaicism in the embryo, but also on its capacity to activate a rescue system to gain a euploid, or prevalently euploid, condition.

Trial registration number: Not applicable.

P-588 Presence of genomic dna in extracellular vesicles (EVs) secreted from human embryos: a new source for non-invasive pre-implantation diagnosis?

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Study question: To investigate the nature of DNA contained in embryo-derived EVs and to study whether it could predict the genetic status of the embryo.

Summary answer: Parental mutated and healthy alleles can be detected in embryo-derived EVs using direct and indirect DNA analysis.

What is known already: EVs are heterogeneous populations of endogenous nano- and micro-sized cell-derived membrane vesicles that are released by different cell types and their cargo can affect the phenotype and activity of target cells. Previously we demonstrated, for the first time, that conditioned media from human embryos cultured in vitro between day 3 and 5 of development contain EVs with predominant exosomal nature. The embryonic origin of EVs was confirmed by the presence of specific proteins and RNAs.

Study design, size, duration: This study was conducted to characterize the genetic cargo of EVs released from embryos during in vitro culture. Initially, we characterized the presence of genomic DNA in embryo-derived EVs. Subsequently, we studied the utility of the amplifiable vesicular DNA in the context of non-invasive pre-implantation diagnosis (PGD).

Participants/materials, setting, methods: Embryos undergoing PGD analysis were cultured up to blastocyst stage and underwent trophectoderm biopsy. The spent medium was collected at day 5 of embryo culture, EVs were isolated, lysed and subjected to DNA amplification. The DNA fragmentation and coverage were assessed with TapeStation and Short Tandem Repeats (STR)/Next Generation Sequencing (NGS) analysis. The parental STR-mutation associated analysis and direct gene mutation detection were performed on EV-amplified DNA. Concordance between trophectoderm biopsy and EV-analyses was deduced.

Main results and the role of chance: In EVs derived from grouped embryo culture (n = 4 pools of 50 embryos), STR analysis confirmed the presence of 8 common markers. The DNA in EVs was less fragmented and more enriched compared to the free DNA in the medium. In single embryo culture, only 11out of 24 samples were analysed due to DNA amplification issues. NGS analysis confirmed a good DNA coverage and parental STRs used in PGD linkage analysis were identified. In 11 of samples suitable for analysis, the only maternal allele was detected in 6 out of 11 (54.5%), the only paternal allele was detected in 3 out of 11 (27.3%), whereas both maternal and paternal alleles were present in 2 out of 11 (18.2%) cases. Allele drop-out might be an explanation for these findings possibly due either to the high rate of DNA fragmentation or the limited DNA amplified. In one case, the presence of the maternal mutated allele causing congenital deafness was confirmed in the EV-DNA by the direct analysis of 35delG in CX26 gene.

Limitations, reasons for caution: Improvement of DNA amplification and analyses when utilizing EV-cargo is needed to increase the efficacy of the methodology. Additional clinical data must be obtained before this approach can be evaluated for routine integration into PGD programs.

Wider implications of the findings: The detection of mutated or healthy alleles (maternal and/or paternal) in embryo-derived EVs is encouraging in a non-invasive PGD context.

Trial registration number: not applicable.

P-589 Monopronuclear embryos derived from testicular sperm extraction followed by intra cytoplasmic sperm injection revealed extremely high euploidy rate with next generation sequencing

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Study question: To investigate the incidence and euploidy rate of monopronuclear (1PN) embryos derived from testicular sperm extraction (TESE) followed by intra cytoplasmic sperm injection (ICSI).

Summary answer: TESE-ICSI derived 1PN embryos showed significantly higher incidence than those with ejaculated sperms, however the euploidy rate of TESE-ICSI derived 1PN embryos was extremely high.

What is known already: It has been reported that 1PN embryos were observed around 5% both from in vitro fertilization (IVF) and ICSI, and that blastocyst formation rate of 1PN embryos was significantly lower than that of two nuclear (2PN) embryos (especially in ICSI). Embryos derived from 1PN may be considered abnormal which are unsuitable for embryo transfer. However, a recent study revealed that 1PN embryos contained normal embryos similar to those of 2PN embryos by preimplantation genetic screening (PGS). We asked whether 1PN embryos derived from TESE-ICSI showed similar euploidy rate as ejaculated sperms by next generation sequencing (NGS) because of their immaturity.

Study design, size, duration: A single-center retrospective study was performed. Patients who generated 1PN embryos were asked to participate in this study, and gave written informed consent for the study between April 2016 and November 2017. All 1PN embryos were cultured until blastocyst stage followed by NGS. Finally, embryos reached blastocyst and performed NGS were from TESE-ICSI (n = 5), IVF (n = 20) and ejaculated sperm-ICSI (n = 12). Rates of 1PN and euploidy were compared among three groups.

Participants/materials, setting, methods: The average age of patients who provided embryos was 39.7 years. Trophectoderm biopsy was performed using laser system (Saturn 5 Active™, RI Ltd., Falmous, UK). Whole genome amplification was carried out using the SurePlex DNA Amplification System (Illumina Inc., San Diego, USA), and further chromosome profiles were analyzed by VeriSeq-PGS kit (Illumina). The Chi-squared tests were performed to analyze differences in distribution between the two groups. Statistical significance was defined as P < 0.05.

Main results and the role of chance: The incidence of 1PN embryos by TESE-ICSI (10.1%, 402/3982) was significantly (P<0.001) higher than that of IVF (4.0%, 413/10374) or ejaculated sperm-ICSI (4.2%, 756/18009), while 2PN rate on ejaculated sperm-ICSI (70.7%) was significantly (P<0.001) higher than that of IVF (54.7%) or TESE-ICSI (53.5%). Polypronuclear (3PN or more) incidence in IVF (8.8%) was significantly (P<0.001) higher than that of ejaculated sperm-ICSI (3.5%) or TESE-ICSI (2.5%). Blastocyst formation rate of 1PN embryo was 27.8% (97/349) in IVF, which was significantly (P<0.001) higher than that of ejaculated sperm-ICSI (10.6%, 61/578) or TESE-ICSI (14.2%, 48/337). Trophectoderm biopsy can successfully be performed from 1PN embryos with similar rate among three groups; 100% (20/20) in IVF, 92.3% (12/13) in

ejaculated sperm-ICSI, and 100% (5/5) in TESE-ICSI. The euploidy rate of 1PN embryos was 30.0% (6/20) in IVF, 41.7% (5/12) in ejaculated sperm-ICSI and 100.0% (5/5) in TESE-ICSI. Of all 1PN embryos, female embryos were significantly (P<0.05) higher (62.2%, 23/37) than in male embryos (37.8%, 14/37). The male/female embryo ratio was 9/11 in IVF, 5/7 in ejaculated sperm-ICSI, and 0/5 in TESE-ICSI, respectively. We did not perform embryo transfer of these euploid embryos because these embryos were to be discarded (allowed to be used for this study).

Limitations, reasons for caution: Since this study is a single center retrospective observational study with a small sample size, multi-center larger scale studies are needed to conclude. Since we did not perform other tests to track biparental inheritence of the 1PN embryos, uniparental diploidy owing to endoreduplication of a haploid oocyte cannot be excluded.

Wider implications of the findings: We, for the first time, revealed that TESE-ICSI derived 1PN embryos showed significantly higher incidence and higher euploidy rate than those with ejaculated sperms. Although several mechanisms have been proposed to account for normal 1PN embryos, we speculate premature pronuclear breakdown by immature testicular sperm might occur in the embryo.

Trial registration number: N/A.

P-590 Chromosomal mosaicism in spontaneous abortions: a case-control molecular cytogenetics study of 1280 specimens by interphase FISH

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Study question: What is the contribution of mosaic aneuploidy and polyploidy to spontaneous abortion (SA)?

Summary answer: Aneuploidy/polyploidy was found in 52,8% of 1280 SA specimens; chromosomal mosaicism was observed in 49,1% of abnormal cases.

What is known already: Numerical chromosomal abnormalities are the most common genetic cause of SA during the first trimester. Classical cytogenetic methods have shown that chromosomal abnormalities are found in approximately 15% of SA. Many previous studies have indicated that chromosomal mosaicism frequently contributes to SA and can be potentially associated with intrauterine mortality.

Study design, size, duration: Using interphase FISH with chromosome-specific DNA probes selected according to the involvement in causative chromosome imbalances, we have performed a case-control study of chromosomal mosaicism in spontaneous abortions.

Participants/materials, setting, methods: We have analyzed 1280 chorionic villus samples of SA (first trimester) by interphase multiprobe FISH using DNA probes for chromosomes 1, 9, 13/21, 14/22, 15, 16, 18, X and Y.

Main results and the role of chance: Numerical chromosomal abnormalities were detected in 676 cases (52.8%) of 1280 SA samples. Multiple aneuploidy/polyploidy were observed in 7.5% of abnormal samples (51 samples) or in 4.0% of all the SA samples. Autosomal trisomy was found in 46.5% of abnormal samples (314 samples) or in 24.5% of all the samples. Chromosome X aneuploidy was found in 21.3% of abnormal samples (144 samples) or in 11.2% of all the samples. Polyploidy was found in 20.4% of abnormal samples (138 samples) or in 10.8% of all the samples. In 4.3% of abnormal samples, mosaic autosomal monosomy was observed. Among all the cases of chromosomally abnormal SA, mosaicism was detected in 49.1%. Consequently, mitotic non-dissociation and/or anaphase lagging causing mosaic chromosomal pathology is likely to be one of the most frequent genetic causes of fetal death. Beside highlighting the role of chromosomal mosaicism in intrauterine fetal death, it is shown that chromosomal mosaicism uncovered by interphase mFISH significantly contributes to SA.

Limitations, reasons for caution: Current study is limited to numerical chromosomal abnormalities of chromosomes 1, 9, 13/21, 14/22, 15, 16, 18, X and Y.

Wider implications of the findings: These data demonstrate that chromosomal mosaicism is highly frequent in SA. Furthermore, interphase FISH seems to be a valid approach towards uncovering chromosomal mosaicism in large scale case-control studies.

Trial registration number: Not applicable.

P-591 Application of SMRT Sequencing in preimplantation genetic testing (PGT) for reciprocal translocation carriers

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Study question: Can SMRT sequencing be used in in preimplantation genetic testing (PGT) for balanced reciprocal translocation carriers?

Summary answer: Although it is still needed to be optimized and evaluated, SMRT Sequencing could be another option for PGD, especially for balanced reciprocal translocation carriers.

What is known already: Fluorescence in situ hybridization (FISH)-PGD and comprehensive chromosome screening have been used to identify unbalanced embryos and prevent recurrent spontaneous abortion for reciprocal translocations carriers. Array CGH, MicroSeq, and MaReCs were successfully applied for discrimination of normal embryos and balanced embryos. However, detection of both copy number variants (CNVs) and structural variants (SVs) during PGT in a single sequencing testing remains unsolved. Single Molecule, Real-Time (SMRT) Sequencing enables accurate localization of CNVs and SVs simultaneously with long-read whole genome sequencing. However, its application in PGT is rarely tested.

Study design, size, duration: This proof-of-principle study included totally 2 couples with balanced reciprocal translocation in CITIC-XIANGYA. Besides regular PGT with next-generation sequencing (NGS) and MicroSeq, we performed SMRT sequencing for both translocation carriers and all 6 Day-6 blastocysts.

Participants/materials, setting, methods: Two patients with balanced translocation participated in the study after signing informed consent. We performed SMRT Sequencing on gDNA of the carriers to identify the translocation breakpoints with about 6× coverage. SMRT Sequencing (~10× coverage) and NGS were then performed with the MDA-amplified whole genome amplification (WGA) products. Specific primers spanning the breakpoints were designed to verify the SMRT sequencing results. Results of SMRT sequencing were compared to those of NGS to evaluate its efficiency.

Main results and the role of chance: We successfully detected the breakpoints of the two reciprocal translocations. For SMRT sequencing of the six embryos, both CNVs and target breakpoint could be detected by using blasr-PBhoney. The results of CNVs detected by SMRT sequencing was comparable to that of NGS. The existence of the breakpoints in the embryos was validated by PCR. However, there are numerous SVs are derived from artificial noise in low coverage, e.g. Blunt ligation could induce target DNA self-ligated before adapter ligation; adapter was not recognized in sequencing process. BAMsplit was developed for reads selection and SVs could be screened by using CNV. Each family has 1 unbalanced embryo, 1 balanced embryo, and 1 normal

embryo. The two normal embryos were transferred. Both patients got pregnant. By prenatal diagnosis, 2 babies were confirmed normal without balanced translocation, which consisted with our breakpoint analysis.

Limitations, reasons for caution: Due to current costs of the SMRT sequencing, it is hard to sequencing the whole genome with a high coverage, which may influence the accuracy of PGT. Besides, artificial noise affect the efficiency of SVs detection. This limitation may be solved by developing new methodology of both sequencing and bioinformatics.

Wider implications of the findings: This proof-of-principle study suggest that SMRT Sequencing could be another option for PGD, especially for balanced reciprocal translocation carriers. However, there are still a lot of problems remain to be solved before future clinical application.

Trial registration number: not applicable.

P-592 Comparison of euploid embryo formation after natural or mild stimulation

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Study question: To compare the efficiency of euploid embryos analysed with next-generation sequencing (NGS) after natural and mild stimulation in a preimplantation genetic screening program.

Summary answer: Our study on patients undergoing NGS cycles suggests that natural modified stimulation did not lower the likelihood of euploidy in developing embryos.

What is known already: Mild stimulation cycles aim to achieve multiple follicular recruitment balancing the quantity and quality of oocytes with reduction of health risks to the patient in normoresponder patients. Natural modified protocols assist the production of a single, high quality follicle and therefore egg in low responders. Some papers have suggested that the cumulative pregnancy rate is comparable between natural modified and mild stimulation, however this has not been demonstrated at the present time. In this work, we test the effectiveness of these protocols by examining the percentage of production of normoploid embryos diagnosed with PGS-NGS.

Study design, size, duration: In this retrospective observational research study we included 96 patients undergoing PGS at Create Fertility St Pauls, London. Between 2015 and 2017 in a PGS-NGS program 550 eggs were collected resulting in 136 embryos biopsied. Data related to the stimulation type (natural modified or mild stimulation) was retrospectively analyzed. The outcome measures were fertilization rate, blastocyst formation and euploid rate.

Participants/materials, setting, methods: Patients with normal karyotype undergoing PGS with fresh oocytes, aged between 25 and 49 years old, with a body mass index between 19 and 30 were included. Blastocysts were scored according to the standard Gardner grading system and laser-assisted trophectoderm biopsy was performed on day 5/6 post oocytes retrieval. Preimplantation genetic screening was performed with next-generation sequencing. Chi-squared test was used for statistical analysis with $P<0.05$ considered significant.

Main results and the role of chance: A total of 550 oocytes were collected, 132 "natural modified" vs 418 "mild" respectively. No statistical difference was observed in fertilization rate between the two groups (82 vs 260, NS). A better blastocyst formation was observed in the natural modified group, (27% vs 46% respectively, $p<0.001$). After 5 or 6 days of culture, 136 embryos were analyzed with PGS-NGS (49 vs 87, NS). In 10 samples no results were obtained (3 vs 7, NS). On a total of 126 biopsies with results 18 embryos were euploid (4 vs 14, NS) and 108 were abnormal (42 vs 66, NS) with a euploid rate of 4.88% for natural modified and 5.38% for mild stimulation.

Our results suggest that more embryos are able to grow to blastocyst stage in the natural modified group, in equivalent culture conditions. Secondarily, the percentage of the euploid embryos between the two groups is not statistically different.

These data suggest that natural modified cycles are as effective in producing high quality eggs than mild cycles in their distinctive patient groups, leading to lower embryo wastage and a more efficient use of embryos in IVF cycles.

Limitations, reasons for caution: This study was limited by its retrospective design. Prospective data on the outcomes of embryo transfers is needed to determine their true implantation potential.

Wider implications of the findings: This study showed that euploid rates for blastocysts are not significantly different in natural or mild stimulation cycles. This suggests that the natural modified stimulation approach is an efficient and safe protocol in a program of assisted reproduction.

Trial registration number: not applicable.

P-594 Novel loss of function mutations of follicle-stimulating hormone receptor in a patient with premature ovarian insufficiency identified by infertility gene panel using next generation sequencing

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Study question: Can an infertility gene panel (GP) approach uncover genetic etiology of premature ovarian insufficiency (POI) in a patient with primary amenorrhea (PA) and hypergonadotropic hypogonadism?

Summary answer: Analysis of GP using next generation sequencing (NGS) identified two new rare inactivating mutations of FSHR which are very likely responsible for the POI phenotype.

What is known already: Inactivating mutations of FSHR are an extremely rare cause of POI. Sixteen inactivating mutations of the receptor have been reported so far in POI patients affecting either the extracellular domain (ECD) or the transmembrane domain (TMD) of the FSHR whether in homozygous or compound heterozygous state. In patients with previously characterized FSHR mutations, the phenotype varied depending on the level of inactivation of the receptor. In most cases, patients presented with a primary amenorrhea and streak ovaries. Some mutations were associated with partial or normal breast development, secondary amenorrhea and normal sized ovaries containing preantral and even small antral follicles.

Study design, size, duration: Analysis of a panel of 31 genes implicated in POI was performed in a 32 years old Belgian patient presenting an idiopathic POI with PA diagnosed at the age of 17 and normal breast development. This patient consulted our fertility clinic for assisted reproduction with oocyte donation and gave her written informed consent to be tested for a genetic etiology of POI.

Participants/materials, setting, methods: We performed GP using NGS @ BRIGHTcore in a patient with POI. FSH and AMH levels were respectively 74UI/L and 1.4ug/L. Karyotype and array CGH were normal. Transvaginal pelvic ultrasound and laparoscopy showed small ovaries with no antral follicles. Transient transfection of COS7 cells was performed with a plasmid containing wild-type FSHR cDNA as well as the two novel FSHR variants identified by GP analysis. Cell surface expression of FSHR variants was tested by FACS.

Main results and the role of chance: NGS identified the presence of two new compound heterozygous FSHR missense mutations: c.646 G>A (Gly216Arg) in exon 8 (ECD) and c.1313 C>T (Thr438Ile) in exon 10 (TMD) of the receptor. Both mutations were predicted to be deleterious and/or probably damaging by in silico analysis. The in vitro functional study showed that the expression of both FSHRs variants was barely detectable at the cell surface of transfected COS7 compared to wildtype FSHR. Two experiments showed similar results.

Limitations, reasons for caution: Mutations segregation in patient's family was not evaluated. As FACS experiments used antibodies that recognize an epitope located in the ECD of FSHR, it is possible that the c.646 G>A mutation

alters the epitope recognition by the antibodies without altering the cell surface expression of the receptor.

Wider implications of the findings: FSHR mutations are found in less than 1% of POI patients rendering the single gene analysis approach inefficient. We report new FSHR inactivating mutations in a patient presenting PA and hypergonadotropic hypogonadism demonstrating the interesting contribution of a specific GP using NGS to uncover genetic etiologies of idiopathic POI cases.

Trial registration number: P2016/196/CCB B406201628264

P-595 Higher resolution aneuploidy screening with targeted NGS may increase the pool of transferrable embryos despite inclusion of segmental and mosaic range diagnostic categories

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Study question: How does the higher resolution of next generation sequencing based aneuploidy screening, inclusive of segmental error and mosaic range diagnostic categories, influence the proportion of transferrable embryos in PGT-A cycles?

Summary answer: The higher resolution capability of targeted NGS (tNGS) for PGT-A produces fewer whole chromosome aneuploidy calls. If mosaics/segmentals are considered transferrable, more embryos are available.

What is known already: Euploid embryos, screened with qPCR, have been prospectively demonstrated to produce higher implantation rates than unscreened embryos. Early reports suggest that an embryo diagnosed as euploid with higher resolution NGS platforms produce even higher implantation rates than lower resolution approaches. However, some concern has been raised that inclusion of additional diagnostic categories (segmental and mosaic range) in NGS reports may significantly limit the pool of transferable embryos by reducing the number of euploids available. Given lack of quality data regarding the reproductive potential and long term safety of these embryos, some have suggested limiting their inclusion on clinical reports.

Study design, size, duration: This was a retrospective cohort study of 13,465 embryos from 2,830 cohorts utilizing tNGS based aneuploidy screening at a single IVF center between July 2016 and November 2017. This represented all PGT-A cycles at the center after the reference laboratory switched from qPCR based PGT-A to a tNGS platform. Aneuploidy screening results were compared to a large, published retrospective cohort of 15,169 embryos analyzed with qPCR based PGT-A in 2014 by our group.

Participants/materials, setting, methods: Targeted NGS embryos were placed into one of four categories: aneuploid (whole chromosome aneuploidy present), segmental (only abnormality was deletion/duplication), mosaic range (no other whole chromosome or segmental aneuploidy), euploid. Diagnostic results were compared to published 2014 cohort using qPCR / SNP microarray with respect to 1) Percentage aneuploid diagnoses per embryo and 2) Percentage of cohorts with at least 1 euploid available. Relationship between age and each diagnostic category was evaluated by logistic regression.

Main results and the role of chance: The age of the female partners ranged from 21 to 46 years. The percentage of the 13,495 embryos with aneuploid calls was significantly reduced with tNGS compared to qPCR/SNP microarray (32.4% vs. 41%, $p<0.001$). Mosaicism and segmental rates in tNGS cohorts were 3.6% and 7%, respectively. The no result rate was significantly lower with tNGS than the qPCR/SNP microarray cohort (1.2% vs. 2.8%, $p<0.001$).

A total of 80.7% of the 2,830 tNGS cohorts featured at least one euploid embryo for transfer. The likelihood that a cohort produced all aneuploid embryos increased as the age of the female partner increased ($p<0.001$). However, more than 90% of cohorts produced at least one euploid embryo for transfer for all ages <35 years; 80% of cohorts did so through age 38. It wasn't until age 42 that the majority of cohorts produced no euploid embryos for transfer. The percentage of cohorts in which the only available embryo for transfer was segmental or mosaic was 1.3 and 2.5%, respectively. Thus, 96.2% of cohorts produced a clear clinical strategy (euploid available or all aneuploid embryos).

Female age was strongly associated with whole chromosome aneuploidy. However, there was no association between age and segmental or mosaic range diagnoses.

Limitations, reasons for caution: These data reflect the experience of one PGT-A strategy. While NGS-based methods are now widely used, the amplification strategy, opportunity for concomitant genotyping, bioinformatic customization, and thresholds for intermediate calls can vary widely. This may explain wide variation in reported prevalence of mosaicism. Higher mosaicism rates may alter these conclusions.

Wider implications of the findings: If segmental and mosaic range embryos are considered for transfer, the pool of eligible embryos is increased with tNGS (despite inclusion of additional abnormal diagnostic categories), as whole chromosome aneuploidy calls are less frequent. Data from nonselection studies are needed to define reproductive potential and health outcomes of these embryos.

Trial registration number: Not applicable.

P-596 Clinical implementation of a POI gene panel in idiopathic patients

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Study question: Can a small NGS based gene panel be clinically incorporated in order to identify the genetic cause in idiopatic POI patients?

Summary answer: We have developed a gene pannel consisting of 21 literature evidence based genes which has allowed us to investigate 32 idiopathic POI women.

What is known already: Premature ovarian insufficiency (POI) has been shown to occur in 1% of all women under 40 years old. Already known etiologies are mainly karyotype abnormalities, X fragile premutation, auto-immunity and iatrogenic factors. During recent years, genetic causes, ranging from chromosomal aberrations to single nucleotide alterations, have been shown to contribute to POI, as 31% of POI patients have a familial history. To date, different genetic tools have been used in order to the uncover genetic etiology of POI, but few reports have focused on the contribution of a specific female infertility gene panel in the POI assessement.

Study design, size, duration: We have performed a specific female infertility gene panel by NGS. This GP is restricted to 21 genes implicated in POI and selected according to the evidence based literature findings. Genes where only limited associations have been reported were excluded. This approach allows a more accurate diagnostic route, and an easier clinical workflow in terms of data interpretation.

Participants/materials, setting, methods: We have applied our GP to a cohort of 32 idiopathic POI cases recrutted prospectively from our hospital since june 2016. These patients presented a normal caryotype, no fragile X premutation, no pathogenic nor likely pathogenic CNV at array CGH and no adrenal nor ovarian antibodies. The GP analysis was performed (Capturing Based) on an Illumina HiSeq1500 machine.

Main results and the role of chance: We have identified six rare variants at the heterozygous state (HTs) and one variant at the homozygous state (HOs). Two of them are likely pathogenic and probably responsible for the POI phenotype as they implicate NOBOX and DIAPH 2 genes which inheritance is dominant. The remaining ones are of uncertain significance (VUS): One is homozygous in the XPNPEP2 gene (X-linked dominant inheritance) while the remaining ones implicate the *STAG3*, *HFM1*, *FOXO1* and *MCM8* genes in which the inheritance is recessive.

Limitations, reasons for caution: The implication of the identified variants in the POI physiopathology needs to be further documented by familial

segregation of mutations and phenotypes, as well as functional studies are required. Furthermore, the limited gene set of our panel has to be taken into account.

Wider implications of the findings: Our data shows that a specific GP restricted to a relative small number of genes is useful and rapid genetic tool identifing rare variants in 21,8% of patient. This GP should be integrated as a first line assessment of idiopathic POI idiopathic cases before large GP and Exome sequencing.

Trial registration number: P2016/196/CCB B406201628264

P-597 Preliminary analysis of chromosome abnormalities in inversion preimplantation genetic diagnosis cycles using comprehensive chromosome screening

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Study question: To determine the factors associated with unbalanced chromosomal rearrangement in blastocysts originating from the parental inversion.

Summary answer: The risk of generating unbalanced blastocysts originating from the parental inversion is affected by the inversion segment proportion and gender of pericentric inversions carriers.

What is known already: Inversion carriers have an increased risk of having fertility problems, recurrent miscarriages or an abnormal chromosomal offspring. Previous studies in spermatozoa suggested the probability of generating unbalanced spermatozoa is affected by many factors including, e.g. the length of the inverted segment, the position of the breakpoints. Study in translocation carriers suggested sperm FISH cannot be used as a reliable predictor of blastocysts and preimplantation genetic diagnosis (PGD) outcome. However, there is few studies analyzed the PGD outcome of inversion carriers.

Study design, size, duration: A retrospective study from October 2011 to October 2017, a total of 482 blastocysts originating from 145 oocyte retrieval cycles from 128 couples with one of the partners carrying inversion were investigated. The mean maternal age was 32.08 years (20–44 years). Meanwhile, a total of 1228 blastocysts from 406 age-matched patients with normal karyotype were analyzed as control group.

Participants/materials, setting, methods: Trophectoderm biopsy of blastocysts was performed on the 5th or 6th day of development. Whole genome amplification (WGA) was performed on all samples, and the WGA was analyzed with SNP array or NGS. The inversion was classified into two categories: paracentric and pericentric inversions. Further, the blastocysts from pericentric inversions carriers were analyzed according to inversion segment proportion of the length of the inversion over the length of the whole chromosome and the gender.

Main results and the role of chance: For paracentric inversions, we detected chromosome abnormalities in 44/162 embryos (27.16%) while 117 showed a normal chromosome (72.22%). Seven embryos (5/162, 3.08%) with unbalanced rearrangement originating from the parental inversion were identified. For pericentric inversions, first, the pericentric inversions were divided into 3 groups according to the inversion segment proportion (<30% group, 30-50% group, >50% group), the normal embryo proportion was 58% (56/99), 69.23% (57/84), 7.37% (63/133). The unbalanced rearrangement originating from the parental inversion was 3/99 (3.03%), 9/84(10.71%), 44/133(33.08%) respectively in three groups, with significant difference ($p<0.01$). Second, we analyzed the unbalanced rearrangement from maternal and paternal inversion, interesting, the unbalanced rearrangement proportion originating from paternal inversion was higher than maternal inversion in >50% group (43.64%(24/55) vs 25.64%(20/78), $P<0.05$. Further, In order to determine whether an interchromosomal effect (ICE) occurred in blastocysts obtained from inversion carriers. Aneuploidies unrelated to inversion chromosome were identified in both inversion carriers and control group. In the samples from inversion carriers, a total of 20768 chromosomes were examined and 182(0.88%) aneuploidies were identified. In the control group, 54032 chromosomes were assessed and 510 (0.94%) aneuploidies were identified. There was no apparent ICE detected in blastocysts of inversion carriers.

Limitations, reasons for caution: Due to the difficulty in recruiting patients with inversion, the sample size of this study is moderate and more cases should be added.

Wider implications of the findings: Our results indicate the risk of generating unbalanced embryos is low for paracentric inversions, however, the risk is affected by inverted segment proportion and gender for pericentric inversions. There was no apparent ICE detected in blastocysts of inversion carriers. This study may provide data for genetic counseling of inversion carriers.

Trial registration number: not applicable.

P-598 Non-invasive preimplantation genetic testing – aneuploidy screening (PGT-A) using spent culture medium (SCM)

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Study question: Could the same genome profile be identified if spent culture media (SCM) instead of trophectoderm (TE) were used for aneuploidy screening (PGT-A)?

Summary answer: PGT-A using SCM was capable but not as efficient as using TE in euploid identification.

What is known already: By comprehensively screening all 24 chromosomes, PGT-A has been shown to increase implantation and ongoing pregnancy rates. However, the current practice of PGT-A requires an invasive biopsy procedure on the embryo, usually at the blastocyst stage. While the embryologists performing this delicate procedure are usually skilled, the long term effect of laser ablation at the site of biopsy and the removal of a small cell mass from a preimplantation embryo remains unknown for the babies born. It would be ideal if euploid embryos could be accurately identified via a non-invasive approach.

Study design, size, duration: This is a prospective study comparing PGT-A results of SCM samples to the routine results based on TE of the same embryos. Couples undergoing PGT-A cycles were recruited from a university hospital infertility centre. The study is still ongoing, and this interim review is consisted of 70 blastocysts from 24 PGT-A cycles between March 2017 and September 2017.

Participants/materials, setting, methods: All recruited cycles underwent intracytoplasmic sperm injection (ICSI). Normally fertilized embryos were individually cultured in sequential media, with change of simple to complex media on day 3. Blastocysts were biopsied on day 5 or day 6. The complex media in which these embryos were cultured since day 3 were collected. After whole genome amplification (WGA), TE samples were analysed by array comparative genomic hybridization (aCGH) and SCM samples were analysed by next generation sequencing (NGS).

Main results and the role of chance: All TE samples were subjected to routine PGT-A by aCGH (GenetiSure Pre-Screen 8×60 K microarray, Agilent), from which 69 (98.6%) produced an interpretable result. For SCM from the 70 corresponding embryos, 60 samples (85.7%) had cell free DNA that were successfully amplified. These WGA products were then subjected to NGS (Veriseq, Illumina), with 39 of them (65.0%) giving an interpretable result. There was an 88.2% concordance rate in sex determination. When SCM results were classified into euploid and aneuploid, the concordance rate with TE results was 71.8%. Overall, 12 euploids were identified by SCM, in which 6 were also euploids based on TE biopsy, 3 contained low level of mosaicism, 1 involved a segmental error on one chromosome, and 2 false negatives probably due to presence of cumulus cells in the SCM. Interestingly, there were 3 aneuploids identified by SCM which were euploids by TE biopsy. These three embryos were already transferred based on their euploid status as established from TE, resulting in 2 miscarriages and 1 ongoing pregnancy.

Limitations, reasons for caution: Our study was limited by the small sample size. While sperm contamination could be eliminated by using ICSI, further care might be needed in oocyte denudation to avoid maternal contamination. Several rinsing could be incorporated into the media change on day 3 of embryo development.

Wider implications of the findings: Spent culture media from embryos have the potential to become the first non-invasive method in euploid identification and gender determination. Gamete and embryo handling may be refined to allow better accuracy and consistency. Much research is needed to elucidate the origin of cell free DNA found in SCM.

Trial registration number: Research Licence R3004 by the Council on Human Reproductive Technology of Hong Kong.

P-599 Model ensembling and synthetic features in PGS cycles with single embryo transfer

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Study question: Can model ensembling and synthetic features be used to evaluate the impact of the wide range of clinical, as well as morphological and kinetic parameters of embryo development *in vitro* on the clinical pregnancy rates in autologous IVF PGS cycles with single embryo transfer.

Summary answer: Model ensembling and synthetic features can be used evaluate the impact of factors affecting clinical outcomes in IVF PGS cycles with high accuracy and superior predictive performance.

What is known already: Comprehensive chromosomal screening has been proven as the best option to increase clinical outcomes in autologous IVF cycles for advanced maternal age patients. Recent advances in statistical learning (also known as Machine learning or Artificial Intelligence) coupled with a steady increase in the number of IVF cycles with PGS has created a background for effective, non-bias and reproducible data analysis via model ensembling.

Study design, size, duration: 1029 cycles of IVF PGS treatment with single embryo transfer between January 2013 and November 2017 were included in the study (average age 35.5 ± 4.8). 175 clinical factors (age, BMI, AMH, FSH, AFC, stimulation protocol, stimulation duration, gonadotropin dosage, gravidity history, diagnosis, etc.) and 145 morphological and kinetic parameters of embryo development (number of eggs retrieved, number of 2PN, blastulation rate, embryo morphology, euploidy rate per cycle, number of embryos biopsied on day 5 and day 6, number of euploid embryos per cycle, fertilization rate, etc.) were recorded for each IVF PGS cycle. Clinical pregnancy rate (PR) was defined by the presence of a fetal heartbeat at 6-7 weeks of pregnancy. To find the optimal combination of prediction algorithms loss-based supervised learning method was employed.

Participants/materials, setting, methods: From 320 original features 3247 synthetic features were created (by Weight of Evidence for columns, Encoding of categorical levels of the feature, Cross Validation Target Encoding, Time series, etc), tested and 609 features were selected for each IVF PGS cycle. 104 statistical models were trained and ensembled in the final model. Predictive accuracy was evaluated by 5-fold cross-validation and AUC value (ROC curve).

Main results and the role of chance: Combined predictions from multiple weak learners (generalized logistic regression, random forest, gradient boosting, etc.) processed by Generalized Model Stacking produced a predictive performance of AUC = 0.8047. The probability of positive clinical outcome was calculated for each IVF PGS cycle and ranged from 0.239 to 0.850 (baseline prediction – 0.63). A number of previous biochemical pregnancies, blastocysts morphology and time when embryo became available for biopsy had highest variable importance in the ensembled model (0.82, 0.52 and 0.43, respectively). These results were confirmed by univariate analysis: the statistically significant difference in ongoing PR was detected between the groups of patients with and without a history of previous biochemical pregnancies (72.36% (479/662) and 43.88% (136/310), respectively, $\chi 2 = 73.72$, OR = 3.349, CI 2.527-4.438). Similarly, significant difference in ongoing PR was detected between embryos biopsied on day 5 versus day 6 (69.34% (405/584) and 54.28% (241/444), respectively, $\chi 2 = 24.53$, OR = 1.906, CI 1.475-2.463) and between PGS cycles where good versus fair embryos were transferred (67.95% (422/621) and 57.62% (208/361), respectively, $\chi 2 = 10.61$, OR = 1.560 CI 1.193-2.040).

Limitations, reasons for caution: Retrospective study and heterogeneity of patients included.

Wider implications of the findings: Analysis of the data proved that history of previous biochemical pregnancies, embryo morphology and timing of blastocyst biopsy have the biggest impact on clinical pregnancy rates in IVF PGS cycles with SET. Ensemble methods of statistical learning offer superior performance over their singleton counterparts and essentially help to transform medical records into medical knowledge.

Trial registration number: Not applicable.

P-600 Low progression of sperm motility kinetics related aneuploidy ratios of a fertilized embryo

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Study question: Does the sperm motion kinetic profile have an effect to aneuploidy ratios of in-vitro fertilized embryos?

Summary answer: We found that there is a specific relationship between aneuploidy ratios of in-vitro fertilized embryos and the Computer Assistant Sperm Analysis (CASA) profiling of sperm.

What is known already: Sperm motion kinetics is related to the sperm centrioles complex, which is involved in sperm directional movement. This is because a sperm's centriole complex is related to the microfilament of sperm motility. But the sperm motion kinetics profile has not reported that there is a co-relationship with aneuploidy ratios of embryos.

Study design, size, duration: We performed a retrospective study about the relationship between the sperm motion kinetics analysis data and the pre-implantation genetics screening data. We tried to set up a prediction model for aneuploidy ratios of fertilized embryos. Total number of analysis sampleanalysis' sample number is 1504 and the sample was collected from March 2017 to December 2017.

Participants/materials, setting, methods: We performed the sperm profile analysis using the CASA system before conducting in-vitro fertilization with the ICSI. Then we analyzed sperm kinetics profiles and the PGS outcome (normal and abnormal ratios). We calculated sperm speed (VSL, VCL and VAP) and its progression (LIN = VSL/VCL, STR=VSL/VAP, WOB=VAP/VCL, ALH and BCF). Then, normal and abnormal PGS ratios were compared to each of the sperm's speed and progression ratios.

Main results and the role of chance: Straight of sperm kinetics profiling showed a significantly high normal PGS ratio compared to the curved sperm kinetics profiling. This was especially true when a sperm's head balance in movement had a stronger relationship with the PGS abnormal outcome ratios. Therefore, the abnormal embryo of PGS had a different chromosomal desegregation pattern, just like having a high population of large chromosomal aneuploidy ratios, such as 1 to 10 chromosomes. The spindle of the sperm is localized at the sperm neck region. The moving direction of the sperm is associated with the sperm neck connection and the tail microfilament is associated with the rolling movement to move forward. Then after fertilization, sperm nuclear and spindle changed into a paternal nuclear formation and was distributed as chromosomal segregation. It is possible that a specific moving pattern of a has some effect to chromosomal segregation in the fertilized embryo.

Limitations, reasons for caution: This is a retrospective study and a limited number of normal IVF-ICSI cycles were used for analysis.

Wider implications of the findings: Low straightness profile of sperm kinetics for male patients may be recommended to perform PGS analysis before embryo transfer, in order to save IVF cycles and time.

Trial registration number: It is no application trial registration.

P-601 Validation of using the mitochondrial genome for clinical embryo identification

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Study question: Can analysis of PG-Seq™ low pass next generation sequencing data for PGT-A also provide genetic information for the identification of embryos in a clinical scenario?

Summary answer: RHS' PG-Seq™ and Embryo ID Single Nucleotide Variant (SNV) Panel readily generates a unique embryo signature providing a simple novel identification protocol.

What is known already: PG-Seq™ with DOPlify® WGA provides superior amplification of the mitochondrial genome. The mitochondrial genome contains single nucleotide variants (SNVs) that can be used to differentiate individuals. Additionally, mitochondrial DNA (mtDNA) is maternally inherited, providing a novel opportunity for DNA-based confirmation of maternal origin of embryo biopsies. During Preimplantation Genetic Testing for Aneuploidy (PGT-A) by Next Generation Sequencing (NGS), biopsies from different patients are often processed together. The mitochondrial genome is sequenced during PGT-A and has the potential to be used to differentiate the maternal origin of the analysed embryos.

Study design, size, duration: A large putative panel of mitochondrial SNVs was compiled from published literature and by comparative analysis of PG-Seq™ data in IGV. Any SNVs associated with disease-related markers, that were monomorphic or that were in regions of low depth of coverage using DOPlify®, were excluded. Two panels comprising 14 and 33 SNVs were assessed using multiple patient embryos and a large in silico dataset.

Participants/materials, setting, methods: Embryos from 50 PGT-A patients and a dataset of 380 publicly available human mtDNA sequences representing the global population was compiled. PG-Seq™ analyses 48 embryo biopsies in a single NGS run. Combinations of mtDNA sequences modelling 4 embryos per patient (ie 12 mtDNA genomes), 2 embryos per patient (ie 24 mtDNA genomes), or 1 embryo per patient (ie 48 patients) were tested. Ten thousand randomly selected mtDNA genome combinations were run for each scenario.

Main results and the role of chance: A total of 47 SNVs were assessed. Using the 380 mtDNA sequences, 177 SNV signatures were observed, with 125 being unique to one individual and 52 being shared by 2 or more individuals. Using the embryo biopsy data, all 47 SNVs were conserved within a patient's cohort. The 14 SNV panel differentiated 20 of 50 patients' embryos. The 34 SNV panel differentiated 28 of 50 patients' embryos. In combination, the 47 SNVs differentiated 48 patients' embryos. In the 10,000 simulated cases using the 380 mtDNA genome database and modelling embryos from 12 patients, on average, the 14 SNV panel differentiated 83.7% of embryos, and the 47 SNV panel differentiated 86.7% of embryos. In the simulated cases with embryos from 24 patients, on average, the 14 SNV panel differentiated 71.4% of embryos, and the 47 SNV panel differentiated 75.2% of embryos. In simulated cases with embryos from 48 patients, on average, the 14 SNV panel differentiated 55.9% of embryos, and the 47 SNV panel differentiated 61% of embryos. Should embryo signatures match in a clinical setting, the rest of the mtDNA genome can be used to further differentiate samples.

Limitations, reasons for caution: Maternally-related patients will share mtDNA genomes. In this case, it will not be possible to distinguish between embryos from these patients using the Embryo ID panel or the rest of the mitochondrial genome. De novo mutations within the SNVs are rare but possible, which may result in matching errors.

Wider implications of the findings: RHS' Embryo ID panel has the potential to improve PGT-A practice by providing a DNA-based confirmation of maternal origin and sibling embryo identification. This could be used for sample tracking within an IVF or genetic service provider laboratory. Embryo ID will be incorporated into a future release of PG-Seq™ software.

Trial registration number: -

P-602 The optimal time and whole genome amplification method for spent culture media in pre-implantation genetic testing

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Study question: What is optimal time to collect samples and whole genome amplification (WGA) method for spent culture media in pre-implantation genetic testing (PGT)?

Summary answer: The media from Day5-6 has the highest DNA concentration and the highest specificity and sensitivity for comprehensive chromosome screening (CCS) combined with the PicoPLEX amplification.

What is known already: Recently, in order to reduce costs associated with biopsy and minimize the possibility of damage, blastocoels fluid and spent culture media are being explored for non-invasive PGT (NI-PGT). But the extensive use of NI-PGT has been hotly debated due to the accuracy of the procedure, particularly regarding the possible of cumulus-corona radiate cells contamination. Also the degraded cell free DNA in spent culture media is difficult to amplification and may fail to test. For embryonic genetic screening of spent IVF media to become reliable, several steps must be optimized. These include improving DNA collection methods, DNA amplification, and screening techniques.

Study design, size, duration: This was an experiment study including 9 donated intracytoplasmic sperm injection (ICSI) embryos. Each embryo was cultured in a separate single 25 µl droplet of sequential media (G1 and G2, Vitrolife) to the blastocyst stage. About 20 µl spent culture media was collected at 9 am of Day3/4/5/6 separately and the blastocoel fluid was aspirated before the biopsy. In general, each embryo had four spent culture medium samples, a blastocoel fluid and a TE biopsy sample.

Participants/materials, setting, methods: All the spent culture medium and blastocoels fluid samples were used quantitative-PCR to qualify the amount of DNA. For the non-invasive PGT, all the samples were subject to three WGA methods (PicoPLEX, MALBAC, DOP-PCR) separate and sequencing in BGI-500 with about 15 Mb total reads (36 bp+10 bp barcodes). The TE biopsies underwent the conventional PGS procedure, and concordance of TE biopsies and spent culture media results was assessed.

Main results and the role of chance: The average DNA concentration was 0.58, 0.10, 0.3, 0.71, 0.43 pg/µl by q-PCR in Day1-3, 3-4, 4-5, 5-6 and BF samples. For WGA, we have modified the methods to eliminate the effects of degraded DNA, and the mean concentration after amplification was 21.16, 21.12, 25.52, 28.41, 23.31 ng/µl (PicoPLEX); 20.65, 10.41, 24.12, 43.23, 20.58 ng/µl (DOP-PCR); 6.63, 6.12, 9.02, 9.51, 10.35 ng/µl (MALBAC) in Day1-3, 3-4, 4-5, 5-6 and BF samples. For the 45 NI-PGT samples, the amplification rate for each methods were 77.78% (35/45) (PicoPLEX), 64.44% (29/45) (DOP-PCR) and 80% (36/45) (MALBAC) separately. But the results showed that PicoPLEX had the highest reproducibility than the other two methods. And the amplification rate was much higher in Day4-5 (92.59%) and Day 5-6 (96.29%) than Day 3-4 (74.07%), Day 1-3 (44.44%) and BF (62.96%) samples. Though Day 4-5 and Day 5-6 have higher amplification rate, the concordance rate of genetic screening results compare to TE samples were much higher in Day 5-6 (76.92%, 20/26) samples than Day 4-5 (52%, 13/25). And it should be noted that some media sample from Day 1-3 and Day 4-5 were euploidy female while the corresponding TE samples were male. These results indicated the cumulus-corona radiate cells contamination.

Limitations, reasons for caution: At the time of writing the sample size is relatively modest and is need recruiting more populations to the further prospective controlled study. Even though, we have explored the optimal process for NI-PGT, the sensitivity and specificity are still to improve in NI-PGT compare to conventional PGS.

Wider implications of the findings: This is the first study to compare different WGA method and DNA collection methods in different culture stage. And the results of the study might help to reduce maternal contamination and improve the accuracy of the NI-PGT in clinical application.

Trial registration number: NA.

P-603 The interval between human chorionic gonadotropin (hCG) priming and oocyte retrieval effects euploidy rate in PGS cycles

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Study question: Does the interval between hCG priming and oocyte pick up (hCG-OPU interval) affect the euploidy rate in preimplantation genetic screening (PGS) cycles?

Summary answer: The hCG-OPU interval affects euploidy rate in PGS cycles.

What is known already: The optimal time interval of hCG-OPU has been encountered controversy, whether prolonged interval (>36 h) has any influence on the euploidy rate of blastocyst. In most IVF programs, the commonly practiced interval was 32 to 36 h. However, several studies had shown that ideal IVF performance can be obtained when oocyte retrieval was done more than 36 h (up to 39 h) after hCG priming to increase more high quality oocyte or cleaving embryos.

Study design, size, duration: This is a retrospective study. The *next-generation sequencing* (*NGS*) - PGS outcome data from blastocysts biopsied was conducted to identify differences in euploidy rate. This study included 2090 biopsied embryos from 499 patients undergoing PGS between January 1, 2016 and October 31, 2017.

Participants/materials, setting, methods: All blastocysts were analyzed by the hCG-OPU interval (34-35, 35-36, 36-37, 37-38 and 38-39 hours), women age (≤38 and > 38 years) and the infertility groups (oocyte donor, normal-responders, poor-responders and hyper-responders). Logistic regression analysis was performed to identify whether the hCG-OPU interval is associated with the euploidy rate. Our primary outcome was the effect of the hCG-OPU interval in euploidy rate by women age and the infertility groups.

Main results and the role of chance: According to logistic regression analysis, the hCG-OPU interval (OR: 1.102, 95% CI: 1.010-1.202, p = 0.028) and women age (OR: 0.931, 95% CI: 0.916-0.947, p<0.001) were associated with the euploidy rate. In the patients with age ≤38 years, the euploidy rate within the group of hCG-OPU interval 34-35 hours (32.1%, 37/138) was statistically lower than that in groups of interval 35-36 (42.3%, 113/331; P = 0.046), 36-37 (43.6%, 207/594; P = 0.017) and 38-39 hours (47.2%, 42/122; p = 0.011). In the older patients (age > 38 years), the euploidy rate in group of interval 34-35 hours (9.8%, 12/44) was significantly lower than that in groups of interval 35-36 (24.0%, 36/150; p = 0.048) and 38-39 hours (27.3%, 12/44; p = 0.039). In all infertility groups, the interval 34-35 hours showed the lowest euploidy rates (oocyte donor: 37.5%, poor-responders: 18.2%, hyper-responders: 31.3% and normal-responders: 23.6%). However, the interval of 38-39 hours in all infertility groups showed higher euploidy rates (oocyte donor: 52.6%, poor-responder: 41.2%, hyper-responders: 35.8% and normal-responders: 44.2%) than that in 34-35 interval. Furthermore, in normal-responders, the euploidy rates of interval 38-39 (44.2%, 42/95) and 37-38 hours (36.7%, 94/256) were significantly higher than that in interval 34-35 hours (23.6%, 21/89; p = 0.003 and 0.024, respectively).

Limitations, reasons for caution: Retrospective study of patients included.

Wider implications of the findings: Our findings indicate that hCG-OPU interval in PGS is associated with euploidy rate regardless with different groups of women age or infertility groups. Especially 34-35 interval shows a adverse effect on euploidy rate. This is a importance finding as embryo biopsy in the forms of PGS is increasingly applied.

Trial registration number: No formal funding has been received for this study.

P-604 Is it the best choice for balanced translocation-carrier couples to get children by PGD method?

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Study question: If it is the best way that balanced translocation-carrier couples should get children with preimplantation genetic diagnosis(PGD) method.

Summary answer: IVF-ET with PGD may not the best choice for translocation-carrier couples to get healthy babies.

What is known already: In order to reduce the high miscarriage rate of balanced translocation-carrier couples, PGD is the most recommended way. But some other studies reported that the cumulative live birth rate of natural pregnancy cycles is as high as 60-80% in couples with structural chromosome rearrangements, and balanced translocation-carrier couples obtained high implantation rate per blastocyst and delivery rates in IVF-ET without PGD cycles.

Study design, size, duration: A retrospective analysis of 441 couples with balanced translocation-carrier was performed. The patients come to our genetic counseling clinic from Oct. 2002 to Oct. 2012. And we obtained their pregnancy outcomes when they made return visits or by phone and e-mail follow-up way.

Participants/materials, setting, methods: Patients were grouped according to different preferences as expectant management, intrauterine insemination, regular in-vitro fertilization and embryo transfer (IVF-ET) without preimplantation genetic diagnosis (PGD), IVF-ET with PGD, or gametes donation. Pregnancy loss rate, pregnancy success rate defined as delivery of at least one healthy child or an ongoing pregnancy in the third trimester, cumulative pregnancy rate, and average cost of per pregnancy success were calculated.

Main results and the role of chance: The couples for expectant management, IVF-ET without PGD, IVF-ET with PGD may gain a successful pregnancy out of every 1.64, 3.22, and 4.66 times per treated cycle (p<0.01). The pregnancy loss rate of couples for expectant management, IVF-ET without PGD, IVF-ET with PGD were 39.13%, 25% and 25%, while the cumulative pregnancy rate of couples were 67.74%, 39.13%, and 23.07% (p<0.01), and the average cost of per pregnancy success were about ¥3286, ¥116,000, and ¥238,000.

Limitations, reasons for caution: This study relied on retrospectively investigate and survey, which may have led to some degree of recall bias.

Wider implications of the findings: If the doctors tell the balanced translocation-carrier couples the detail information of these different ways to get pregnant, it may help them choose a suitable method to get healthy babies.

Trial registration number: No.

P-605 Filling the gap of short-read next generation sequencing in PGD by long-read approach

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Study question: Structural chromosomal abnormality is a common phenotype in PGD cases. A cost effective strategy to distinguish the disease chromosome from the wild-type is challenging.

Summary answer: The uprise of long-read next generation sequencing (NGS) provides a direct and cost effective approach to identify the precise breakpoint for non-carrier embryo selection.

What is known already: In the past, haplotype reconstruction could be the way to access the carrier status of individual embryo in PGD cases with structural chromosomal abnormality, including balanced translocation, inversion, deletion and/or duplication. However, a significant drawback is the

unpredictable recombination events. Currently, the advances in long-read NGS create the opportunity to identify the exact breakpoint at a relatively lower cost, but it has never been used in the preparation of PGD.

Study design, size, duration: Two PGD cases were studied so far using long-read NGS. The first patient was an ectrodactyly male with a 610 kb pathogenic duplication identified by aCGH. The second patient diagnosed with karyotype 46,XX,t(2;13)(q33.3;q12.1) and already went through the PGD cycle. After breakpoint identification, specific PCR primers were used to target the mutations. The breakpoint was validated by Sanger sequencing. The whole-genome-amplified biopsy samples were subjected to the breakpoint analysis. Haplotype reconstruction was performed by microsatellite markers.

Participants/materials, setting, methods: We performed PCR-free whole genome sequencing on peripheral blood DNA using MinION sequencing (Oxford Nanopore Technologies). The data was obtained within a 48-hour sequencing run and aligned. The specific genomic breakpoints have been revealed and PCR primer pairs were designed for breakpoint amplification and Sanger Sequencing. Day5/6 trophectoderm biopsies were subjected to whole genome amplification (SurePlex DNA Amplification System, Illumina) and the products were used for PGD analysis.

Main results and the role of chance: In the ectrodactyly case, we identified the genomic breakpoint by nanopore whole-genome sequencing and the duplication was found to be exactly 683.9 kb. The breakpoint was confirmed by PCR and Sanger sequencing. In family study, the breakpoint was completely linked to the ectrodactyly phenotype and concordant with haplotypes of 5 microsatellite markers flanking the duplication. Trophectoderm biopsies were collected for in vitro fertilization PGD and the patient specific breakpoint PCR assay was performed on the WGA products. Three out of seven embryos were identified to inherit the duplication, hence carrier of the disease chromosome. The carrier status of all embryos was further confirmed by haplotyping. A similar strategy was used to detect the breakpoint of the t(2;13) translocation in the trophectoderm biopsy samples. The long-read sequencing came across the aberration site on derivative chromosome 13 and gap PCR were used to define the embryos with or without balanced translocation. Overall, the result of breakpoint PCR analysis and the haplotype reconstruction were concordant.

Limitations, reasons for caution: The limitation of nanopore whole-genome sequencing is the fact that it is PCR-free and may require micrograms of DNA. Multiple MinION runs may be required to identify the target disease sites.

Wider implications of the findings: Long read next generation sequencing is an emerging technology for whole-genome structural variant detection with single base pair resolution. We demonstrated its potential to accurately determine the genomic breakpoint in the ectrodactyly and t(2;13) patients and may represents a novel strategy for PGD of unknown structural aberration.

Trial registration number: not applicable.

P-606 Excluded cells during blastocyst formation: is aneuploidy the reason?

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Study question: Is the exclusion of cells at compaction during blastocyst formation due to aneuploidy?

Summary answer: No higher aneuploidy rate is observed in excluded cells when compared to the corresponding trophectoderm biopsies. The ploidy status among them is highly concordant.

What is known already: Time-lapse technology has allowed the detection of developmental anomalies that could not be properly assessed with single observations. The exclusion of cells from compaction during the blastocyst formation is occasionally observed.

The incidence of chromosomal abnormalities decreases through embryo development. While some aneuploidies are compatible with the blastocyst stage and onwards, developmental arrest during culture is the main mechanism against severe chromosomal abnormalities. Embryo self-correction of chromosomal abnormalities has also been proposed. Aneuploidy rescue through the exclusion of the abnormal cells from the developing embryo could be one of the potential mechanisms for self-correction.

Study design, size, duration: Retrospective observational study performed from July 2016 to November 2017. Chromosome content of 17 pairs of trophectoderm biopsies and excluded cells from blastocysts of PGT-A patients were analysed.

Participants/materials, setting, methods: Cells excluded from compaction during blastocyst formation and the corresponding biopsied trophectoderm cells from 15 PGT-A patients were analysed to determine their chromosomal constitution. Only fully hatched blastocysts with left over cells in the zona pellucida were considered. Excluded cells as well as trophectoderm biopsies from hatched blastocysts were separately processed by a-CGH or NGS.

Results regarding ploidy concordance were evaluated. The specific chromosomal constitution of the sample pairs was analysed.

Main results and the role of chance: Seventeen pairs of trophectoderm biopsies and excluded cells were analysed. In 3 cases there was no DNA amplification of the excluded cells.

Ploidy concordance was observed in 13 of the 14 pairs that could be analysed. Discordant results were found in one blastocyst diagnosed as a mosaic that showed euploidy in the corresponding excluded cells.

Five of 14 trophectoderm samples were diagnosed as euploid. All their complementary excluded cells showed the same euploid chromosomal constitution.

In 8 of 9 cases diagnosed as aneuploid after trophectoderm biopsy, the excluded cells were also aneuploid. Full concordance of the chromosomal constitution was observed in 4 of them while certain differences were detected in the remaining 5. There seems to be a tendency for more severe chromosomal abnormalities in the excluded cells.

A high ploidy concordance is observed among trophectoderm cells and cells excluded from compaction in biopsied PGT-A blastocysts.

Limitations, reasons for caution: Retrospective observational study with limited sample size.

Wider implications of the findings: According to our results, exclusion of certain cells during blastocyst development does not seem to be related to aneuploidy rescue.

Trial registration number: Not applicable.

P-607 Polymorphic variants of chromosomes decrease cleaved embryos rate in vitro fertilization and embryo transfer treatment

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Study question: This retrospective study comprehensively analyzed the correlation between chromosome polymorphisms and the outcome of IVF–embryo transfer treatment for infertile couples.

Summary answer: Polymorphic variants of chromosomes affect the embryo cleaved rate of in vitro fertilization and embryo transfer treatment.

What is known already: Studies indicate that chromosome polymorphisms may cause certain clinical effects, such as infertility and spontaneous miscarriage. Little report has studied whether chromosomal variation, other than Y chromosome variation, affects the outcome of IVF–embryo transfer treatment.

Study design, size, duration: During the period from October 2014 to November 2017, 1415 infertile couples who had received their first IVF–embryo transfer treatment cycle in our hospital were selected for this retrospective study, and the frequency of chromosomal polymorphic variations was calculated.

Participants/materials, setting, methods: 1415 infertile couples were divided into four groups: 1182 couples with normal chromosomes (Group 1). 129 couples with $1qh^{+/-}$, $9qh^{+/-}$ or $16qh^{+/-}$ (Group 2) ;56 couples with ps+, pss or pstk+ of the acrocentric chromosomes (Group 3); And 48 couples with pericentric inversion of chromosomes 9 (Group 4).The fertilized rate, embryo cleaved rate, good quality embryos rate, clinical pregnancy rate(CPR), implantation rate and early stage miscarriage rate after IVF-embryo transfer treatment were compared.

Main results and the role of chance: There were no statistically significant differences among the four groups in patient's fertilization rate, good quality embryos rate, clinical pregnancy rate(CPR) and implantation rate. But the chromosomal polymorphism groups (Group 2, Group 3 and Group 4) had lower cleaved embryos rate comparing with control group (Group 1) (95.83, 95.05, 89.90 and 97.5% respectively, P<0.05). The first trimester pregnancy loss rates in Group 4 patients were higher than in control group (25% versus 4.4%, P<0.05), but not in Group 2 and Group 3.

Limitations, reasons for caution: The limitations are the number of heteromorphism carriers was insufficient. Furthermore, the chromosome analysis method in the present study had a 400-550 BPHS banding resolution; some potential variations could not be distinguished from common polymorphism variations. Therefore, a greater number of samples and more sensitive techniques are needed.

Wider implications of the findings: Chromosomal polymorphism carrier could decrease cleaved embryos rate. chromosome 9 pericentric inversion may increase first trimester pregnancy loss and impact IVF outcomes. We should afford individual genetic counseling suggestion according to the polymorphism types.

Trial registration number: not applicable.

P-608 Level of embryonic mosaicism do not seem to be influenced by extrinsic features: analysis of 298 mosaic embryos

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Study question: Is the percentage of embryo mosaicism related to culture and/or patient characteristics?

Summary answer: The rate of mosaic cells detected in an embryo does not seem to be correlated to different culture conditions nor to patient's origin.

What is known already: Embryonic mosaicism is defined as the contemporary presence of two or more genetically different cell lines in the same embryo. It is a common phenomenon in human embryos due to mitotic errors after fertilization and could have an impact on embryo developmental and implantation potentials. It has been reported that mosaic embryos hold the potential to implant, leading to the birth of healthy babies. Anyway, the extent of mosaicism can influence the success rate. Lower aneuploidy percentage has been demonstrated to maintain higher chances of resulting in the birth of healthy babies compared to embryos with higher mosaicism levels.

Study design, size, duration: In this preliminary study, only cycles where at least one mosaic embryo was found, were enrolled. In 195 IVF cycles performed in 185 patients, 298 mosaic embryos were obtained. Mean female and male ages were 35.39 ± 4.22 and 38.83 ± 5.79 years old, respectively. All biopsies were performed at blastocyst stage and diagnosed with either array-CGH or NGS-based PGS. Single embryo cultures were performed in standard or time-lapse incubators at 37°C, 6%CO_2, 5%O_2 in sequential or 1-step media.

Participants/materials, setting, methods: Embryos were divided in two groups on the basis of their mosaicism percentage: ≤40% (N = 160) and >40% (N = 138). Differences among culture systems (culture media and incubators), embryo quality (morphological grade at embryo and blastocyst stages, day of biopsy and morphokinetic development) and patient's characteristics (female body mass index [fBMI], FSH dose, female and male ages, antral follicle count [AFC] and seminal parameters) were analyzed in the two groups.

Main results and the role of chance: One-step and sequential media were employed for 20.0% and 80.0% and for 16.7% and 83.3% of the embryos in ≤40% and >40% groups, respectively. Time-lapse and standard incubators were used for culturing 58.8% and 41.2% and 63.0% and 37.0% of the embryos in ≤40% and >40% groups, respectively. The rates of day-3 excellent, good and poor morphology grades were 72.3%, 22.7% and 5.0% in ≤40% group and 73.9%, 20.1% and 6.0% in >40% group, respectively. The rates of excellent, good and poor blastocyst grades were 31.9%, 28.1% and 40.0% in ≤40% group and 32.6%, 32.6% and 34.8% in >40% group, respectively. Biopsies were performed on day-5, day-6 or day-7 in 57.5%, 38.7%, 3.8% and in 61.6%, 34.8%, 3.6% of the blastocysts in ≤40% and >40% groups, respectively. Normospermic, oligoastenoteratospermic and testicular men were 26.9% and 27.4%, 39.8% and 38.5%, 33.3% and 34.1% in ≤40% and >40% groups, respectively. None of these values reached a statistical significance. The morphokinetic development did not show statistical differences at any time-points, neither at embryo nor at blastocyst level. No differences were found between the two groups according to the distribution of the fBMI, the dose of FSH, the AFC and the female/male ages.

Limitations, reasons for caution: The number of mosaic embryos enrolled in the study need to be enlarged. It should be reasonable to test also other different mosaicism level cut-offs. A comparison with both euploid and aneuploid blastocysts obtained in the same cycles could better clarify the role of embryonic mosaicism.

Wider implications of the findings: To date, a lot of different culture systems are available and the decision about which of them should be preferred is extremely subjective. Our study suggests that the level of embryonic mosaicism is not ascribable to culture conditions or to patient's origin.

Trial registration number: Not applicable.

P-609 Cryptic complexity identified by whole-genome mate-pair sequencing in complex chromosomal rearrangements: implications on preimplantation genetic diagnosis and reproductive genetic counseling

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Study question: Two Complex Chromosomal Rearrangement (CCR) cases were characterized in detail in order to provide more precise abnormal pregnancy risk estimations and better genetic counseling.

Summary answer: Cryptic rearrangement complexity increases abnormal pregnancy risk in couples with CCRs and reproductive problems.

What is known already: CCR carriers are at high risk for chromosomally unbalanced pregnancies, which may lead to recurrent miscarriages or affected offspring. The nature of each rearrangement, including the number of chromosomes and breakpoints involved, have significant implications on the percentage of unbalanced gametes following meiotic segregation of CCRs. As a result, reproductive risk in CCR carriers may remain underestimated with the use of conventional, low-resolution methods. Detailed characterization of CCRs is therefore crucial to unravel potential higher rearrangement complexity, and subsequently, aid in better prenatal preimplantation genetic diagnosis (PGD) and reproductive genetic counseling in couples with reproductive problems.

Study design, size, duration: Non applicable.

Participants/materials, setting, methods: In the present study, whole-genome mate-pair sequencing (WG-MPS) was used to further characterize and delineate the breakpoints of a rare, paternally-transmitted t(6;7;10) CCR in a phenotypically normal father and daughter, and a de novo t(6;7;8;12) CCR in a female patient with mild intellectual disability. Results were validated with Sanger sequencing and Fluorescence In Situ Hybridization (FISH).

Main results and the role of chance: WG-MPS allowed accurate reconstruction of all derivative (der) chromosomes involved, and interestingly, cryptic complexity was revealed in both cases.

In the rare, paternally-transmitted CCR, WG-MPS detected an additional cryptic translocation breakpoint on der(6). FISH using a chromosome (chr) 6 custom-designed probe and a chr10 control probe confirmed that the interstitial chr6 segment, created by the two chr6 breakpoints, was translocated onto der(10). Previous sperm-FISH investigations in the father analyzing the possible segregation patterns revealed that only two combinations out of sixty-four (3.1%) are balanced. Identification of a cryptic breakpoint in this study and accurate delineation of the full CCR complexity, suggest that the number of possible segregation patterns is even higher, and concurrently, the percentage of balanced gametes becomes even lower.

In the de novo t(6;7;8;12) CCR, WG-MPS revealed a chromothripsis rearrangement involving thirty-eight translocation breakpoints and two heterozygous deletions. Haploinsufficiency of the SOX5 gene, disrupted by two breakpoints and a heterozygous chr12 deletion, most probably underlies intellectual disability in the patient. Since it has been generally estimated that there is an additional ~3.5% risk per breakpoint for unbalanced gamete production, it is expected that this massively complex rearrangement will have a significant impact on the patient's reproduction risk.

Limitations, reasons for caution: Even though WG-MPS can successfully delineate the full complexity of CCRs, an applicable strategy for PGD still remains very challenging.

Wider implications of the findings: In concordance with available literature, this study highlights the importance of investigating CCRs using a combination of conventional and next generation sequencing-based methods in order to delineate their full complexity, and thus provide better abnormal pregnancy risk estimations and reproductive genetic counseling in CCR couples.

Trial registration number: Non applicable.

P-610 Introducing direct CGG repeat analysis in preimplantation genetic diagnosis (PGD) for fragile X syndrome: an overview of clinical outcomes for 116 patients

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Study question: Does incorporating analysis of CGG repeat number in PGD embryos have a significant effect on the clinical outcomes for fragile X premutation carriers?

Summary answer: Performing CGG repeat analysis for embryos provides patients with an additional layer of information that can assist with the embryo transfer decision making process.

What is known already: Fragile X mental retardation gene (*FMR1*) CGG repeat testing has been the routine method of analysis on living individuals and prenatal samples. Historically, PGD for fragile X syndrome has been performed via linkage analysis alone. While differentiating between the normal allele and the expanded allele has been extremely beneficial for patients, it lacks the ability to further distinguish between intermediate, premutation and full mutation embryos. As a result, patients and physicians have been making decisions concerning embryos without potentially impactful clinical information.

Study design, size, duration: Outcome data was obtained on 116 patients, who underwent PGD for fragile X syndrome via a linkage-based technology (Karyomapping; Illumina, USA), and direct CGG repeat analysis, between 01/2014 and 12/2017. Trophectoderm biopsy samples from 852 embryos were received from 194 IVF cycles.

Participants/materials, setting, methods: This cohort of patients carried either an intermediate, premutation, or full mutation *FMR1* allele and the average maternal age was 34.03 ± 0.32 (standard error of the mean). As 33 patients banked embryos from multiple IVF cycles, a total of 160 PGD tests were performed; 148 of these included aneuploidy screening via aCGH or NGS (Illumina, USA). Following exclusion of embryos with no diagnosis, sex aneuploidy, or that were untested, results were obtained in 771 embryos.

Main results and the role of chance: CGG repeat results were obtained in 756/771 samples (98.05%). Overall, the maternal normal allele was inherited in 48.59% of embryos. Of the embryos that inherited the expanded allele, 70.97% remained in the premutation or intermediate range. The following rates of expansion from premutation to full mutation in embryos were observed: 0% (0/74) in the maternal premutation group of 55-59 CGG repeats, 7.78% (7/90) in the 60-69 group, 9.52% (4/42) in the 70-79 group, 30.30% (10/33) in the 80-89 group, 91.67% (22/24) in the 90-99 group, and 100% (47/47) in the 100-199 group. Notably, 3.02% (12/397) of embryos contracted from the maternal expanded allele size (ranging from 57 to >200). The number of embryos per PGD test increased to (6.73 ± 0.71) when patients banked embryos, compared to those who did not (4.91 ± 0.29). There was a significant increase in the average number of embryos available for transfer from 1.33 ± 0.11 (euploid and normal *FMR1* alleles) to 2.07 ± 0.17 when including premutation embryos (P<0.001). Follow up from patients revealed that 70 normal allele embryos, 8 premutation, and 2 normal or premutation embryos were transferred; resulting in 38 live births and 6 ongoing pregnancies. Subsequent confirmatory testing was pursued by 5 patients revealing concordant results with PGD.

Limitations, reasons for caution: A little over 100 patients were evaluated in this study, but clinical outcomes were not obtained on all. In addition, a large portion of patients did not pursue prenatal or postnatal confirmatory testing via outside laboratories. Continued follow up will aid in providing additional analysis.

Wider implications of the findings: This data set presents useful groundwork for understanding the clinical implications of CGG repeat testing in PGD embryos. The differentiation between premutation and full mutation PGD embryos provides more complete diagnostic information; therefore highlighting the need for counseling regarding options for embryo transfer.

Trial registration number: not applicable.

P-611 External verification of the Agilent Technologies OnePGT solution for PGT-M on blastomere and trophectoderm biopsies from UZ Leuven

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Study question: To assess the level of concordance between OnePGT (Agilent Technologies) and Gold Standard reference data of embryo biopsies for preimplantation genetic testing for monogenic disorders (PGT-M).

Summary answer: A conclusive result was obtained for 113/118 (95.8%) embryos analyzed for PGT-M, with a concordance of 100% between the results of OnePGT and the Gold Standard.

What is known already: OnePGT is an innovative next-generation sequencing (NGS)-based solution for all-in-one Preimplantation Genetic Testing (PGT) of *in vitro* fertilized (IVF) embryo biopsies. OnePGT can be used to genetically profile IVF embryos by testing a single- or few-cell biopsy to 1) identify those free of a specified Mendelian disorder or translocation and/or 2) prioritize chromosomally normal i.e. euploid embryos for transfer in a single workflow. This enriched level of information can hence improve success rate of implantation and live birth.

Study design, size, duration: A total of 118 whole genome amplified-embryo biopsies previously analyzed with SNParray and the siCHILD algorithm were submitted for analysis with the OnePGT solution. These samples, together with gDNA from the parents and reference family member(s), originated from 16 families encompassing 15 unique single gene disorders. Both affected and unaffected embryos were included. Data analysis and interpretation was kept blinded and concordance with SNParray/siCHILD results was conducted only after the OnePGT results were recorded.

Participants/materials, setting, methods: Twelve families segregated a dominant, 1 family an X-linked and 3 families an autosomal recessive disorder. In 5 families, the phasing reference was a child of the IVF-parents while for the other families the parents of the carrier IVF-parent were available as phasing references. DNA-samples were library prepped with OnePGT reagents and sequenced on a NextSeq 500 instrument (Illumina). The NGS data was analyzed with the proprietary OnePGT software tool, in particular the PGT-M pipeline.

Main results and the role of chance: The library preparation was successfully performed on a total of 189 samples (including embryo's and respective family members). A conclusive call was achieved for 113 embryonic samples from a total of 118 (95.8%), with 5 embryos receiving an inconclusive call (4.2%). The root cause of these inconclusive calls included cross-over or breakpoints in close proximity of the disease locus (4/5), and nullisomy of the

chromosome of interest (1/5). For two of the inconclusive cases, the Gold Standard result was also inconclusive indicating a biological reason for failure. In addition, one embryonic sample inconclusive in the Gold Standard protocol received a conclusive result in OnePGT. For concordance analysis, both the inconclusive results from OnePGT and Gold Standard were excluded. A 100% concordance was obtained for the 112 embryo samples analyzed with OnePGT when compared with the Gold Standard result, of which 50 unaffected embryos were correctly called as unaffected and 62 affected embryos were correctly called as affected.

Limitations, reasons for caution: This study is limited by the population size which is not sufficient to calculate % specificity, % sensitivity or positive predictive value. For Research Use Only. Not for use in diagnostic procedures.

Wider implications of the findings: The results of this study indicate that the OnePGT solution is able to accurately determine the disease status of embryo biopsies, both from blastomere and trophectoderm sample types, for a wide range of single gene disorders.

Trial registration number: not applicable.

P-612 A proposed method to minimize male gamete contribution to aneuploidy in the embryo cohort

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Study question: Can a new method to select spermatozoa enhance the genomic competence of the male gamete and yield euploid embryos in couples with all abnormal conceptuses?

Summary answer: Microfluidic selection yields spermatozoa with high progressive motility and genomic integrity, enabling the generation of chromosomally normal embryos despite history of recurrent, total aneuploid conceptuses.

What is known already: The presence of a male factor can negatively impact embryo cleavage and chromosomal status. In addition, dysfunction of the male genital tract increases sperm chromatin fragmentation that, particularly in cases of double strand breaks, can lead to aneuploidy of the male gamete. Thus, in couples with a relatively young female partner, the recurrent appearance of aneuploid embryos may reflect the contribution of the male gamete.

Study design, size, duration: In a 16-month period, 4 couples with history of high Sperm Chromatin Fragmentation (SCF) and embryo aneuploidy after several ART attempts underwent a successive cycle of ICSI where semen specimens were processed in a microfluidics chamber. Fertilization and clinical pregnancy rate were assessed and compared between different sperm preparation methods. SCF was assessed by TUNEL and sperm aneuploidy by FISH analysis. Chromosomal analysis was carried out by preimplantation genetic testing for aneuploidy (PGT-A) on conceptuses.

Participants/materials, setting, methods: Consenting men had their ejaculates screened by standard semen analysis according to WHO 2010 criteria. Ejaculate specimen were processed by density gradient and MFSS. SCF was measured by TUNEL utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted under fluorescent microscopy with an adopted threshold of 15%. FISH analysis was carried out on at least 1000 spermatozoa by 9 chromosome probes.

Main results and the role of chance: In a total of 18 cycles, the average age for the men was 44.5 ± 15 years. The average semen parameters were: concentration of 14.9 ± 23.5×10^6/mL, motility of 26.3 ± 17%, 2.0 ± 0% normal morphology. The SCF was 34 ± 12% and the sperm aneuploidy rate was 4 ± 2%. In 13 cycles, after selection by density gradient, the total motility rose to 48.7 ± 9%. The mean of oocytes injected was 7.1 ± 4 with a fertilization rate of 68.7%. The euploidy rate was 3 embryos out of 30 (10%). These euploid embryos were transferred in two patients and two clinical pregnancies were established, both resulting in miscarriages. In 5 cycles, where the sperm was processed by MFSS, the total motility increased to 98.3%±2 (P<0.0001). The sperm morphology became 4% and the SCF reduced to 1.8 ± 1 (P<0.001). The average number of injected oocytes was 5.7 ± 3 with a subsequent fertilization of 78.1%. Following MFSS processing, there were 7 euploid blastocysts out of 14 (50%) that, after transfer, yielded a pregnancy in all four patients (100%) (P<0.001).

Limitations, reasons for caution: This is a pilot study on a small number of subjects. However, this microfluidic method is capable of selecting spermatozoa with better morphology, higher chromatin integrity, and presumably lower sperm aneuploidy, which may likely yield euploid embryos in couples with subtle male factor infertility.

Wider implications of the findings: According to this study, the selection of a genomically competent male gamete may enhance the euploidy of the conceptus. Couples with a relatively young female partner and recurrent aneuploid embryos may benefit from MFSS selection to improve reproductive outcome due to an occult male factor.

Trial registration number: N/A.

P-613 Genomic profiling of spermatogenetic function in azoospermic men

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Study question: Can sequencing of the genome shed light on the genes involved in the spermatogenic function of the seminiferous tubules of azoospermic men?

Summary answer: Men with secretory azoospermia have a mutated set of genes with compromised function responsible for the impaired meiotic process.

What is known already: Azoospermia represents about 1% of all men in the fertile age and about 15% of the infertile men. Azoospermia is seldomly due to pre-testicular factors, but the most common forms are testicular and post-testicular. While the post-testicular type is due to obstruction, whether congenital or acquired, the most puzzling type remains the secretory azoospermia where scattered dysfunctional seminiferous tubules strive to proceed through the germline meiotic process toward the generation of spermatozoa.

Study design, size, duration: Over a period of 2 years, NGS assessment was carried out on surgically retrieved spermatozoa from 9 azoospermic men, who were categorized as obstructive (OA) or non-obstructive (NOA) based on their spermatogenetic profile. Specific gene mutations and ICSI pregnancy outcomes were assessed and compared between the two groups.

Participants/materials, setting, methods: Consenting men being treated at our center for infertility provided their specimens. DNA extraction was carried out from at least 500 spermatozoa followed by PCR-based random hexamer amplification (average DNA concentration 395 ± 217 ng/ul and quality of 1.7 ± 0.1 nm). Following DNA sequencing of the specimens by NGS, gene mutations, including duplications and deletions, were detected by CASAVA and VarScan2 software programs.

Main results and the role of chance: Our analysis included a total of 9 consenting men with an average age of 45.2 ± 8 yrs. Six OA patients were treated by epididymal aspiration or testicular biopsy with an average concentration of 2.0 ± 3×10^6/ml and a motility of 0.5 ± 1%. In all OA cases, the reason for the obstruction was a prior vasectomy. Also these men underwent 6 cycles with a pregnancy and delivery rate of 50%. Three NOA men underwent testicular biopsies that yielded spermatozoa in all cases. They were treated by ICSI in 3 cycles with a pregnancy and delivery of 66.7%. NGS reported no difference in aneuploidy of spermatozoa from the OA and NOA groups. The sequencing of DNA was carried out on 70,000 genes of which only 2 were mutated in the obstructive patients, while non-obstructive azoospermic men had 123 genes mutated (P<0.0001). Among the genes mutated, the most represented were related to mRNA transcription (n = 26) followed by genes involved in spermatogenesis (n = 15), DNA repair (n = 14), centrosomal function (n = 13), and apoptosis (n = 12).

Gene expression analysis by RNA sequencing reported 12 genes, of which 9 were significantly underexpressed (P<0.001). Their functions included calcium mobilization (CXCL16), acrosomal integrity (ACR), and overall regulatory genes of spermatogenesis (ZMYND15).

Limitations, reasons for caution: This is still a limited number of observations carried out on men being screened for infertility. Moreover, testicular

specimens were donated by patients with specimens that yielded spermatozoa and it should be extended to include men with spermatogenic arrest and germ cell aplasia.

Wider implications of the findings: NGS screening of infertile men will help characterize the spermatogenic function of the seminiferous tubule. This will help designate patients who benefit from testicular sampling versus those who would not. Moreover, querying the genome and the epigenome will help shed light on the etiology of this severe form of infertility.

Trial registration number: N/A.

P-614 Reassessing the prevalence of aneuploidy in sperm retrieved from different levels of the male genetic tract

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Study question: We questioned the notion that sampling epididymal and testicular tissues yields spermatozoa with higher incidence of aneuploidy than those found in the ejaculate.

Summary answer: With the adoption of advanced molecular genetic techniques, we confirmed that surgically retrieved spermatozoa have at least comparable incidence of aneuploidy compared to ejaculated spermatozoa.

What is known already: Previous studies, including our own, evidenced, in a few men and by only 4 chromosome probes on about 100 cells, that these testicular spermatozoa have a remarkably higher (13%) occurrence of aneuploidy. This notion, however, did not translate into a higher incidence of miscarriages nor a lower rate of pregnancy. Moreover, ICSI offspring generated from surgically retrieved gametes did not suffer from an increased aneuploidy than those generated from ejaculated specimen. In light of the availability of more accurate molecular genetic techniques, we have decided to challenge this dogma.

Study design, size, duration: Nine chromosome FISH was carried out on 2 donor controls, the ejaculates of 67 men, and surgical specimens of 9 azoospermic men. DNA sequencing technology was carried out on the ejaculates and surgical samples of 20 men. A combined assessment was performed on non-azoospermic (non-NOA) men with high DNA fragmentation in their ejaculate. ICSI pregnancy outcome was also recorded and compared. Additionally, an analysis was carried out to compare obstructive (OA) and non-obstructive (NOA) men.

Participants/materials, setting, methods: Consenting men treated for infertility provided their specimens. FISH was performed on at least 1000 spermatozoa with a threshold of >1.6% with 2-3% FISH error. DNA was extracted and amplified from a comparable number of spermatozoa by PCR-based random hexamer amplification (average DNA concentration 610 ± 102 ng/ul and quality of 1.7 ± 0.1 nm). By NGS, duplications and deletions by Copy Number Variants (CNVs) were then calculated for all chromosomes by CASAVA and VarScan2 software programs.

Main results and the role of chance: A total of 76 couples were included in our study (maternal age 35.8 ± 4 yrs and paternal age 39.4 ± 8 yrs). Aneuploidy by FISH yielded 0.9% for the donor control but rose in the study group to 3.6% in the ejaculated, 1.2% for the epididymal, and 1.1% for testicular spermatozoa. NGS yielded 1.2% for the control while in the study was 11.1% for the ejaculated specimen and decreased to 1.8% in the epididymal and 1.9% for the testicular ($P<0.0001$). The ICSI pregnancy rate for the ejaculated specimen was 47.2% and 55.5% for the surgically retrieved.

Paired aneuploidy assessment in the same individual (non-NOA) on the ejaculated and testicular samples evidenced a sperm chromatin fragmentation (SCF) of 20% in the ejaculate while on the testicular spermatozoa was only 8%. Aneuploidy assessment by FISH evidenced 2.8% in the ejaculated and 1.2% in testicular biopsy while with NGS became 8.4% and 1.3% in testicular biopsy ($P = 0.02$), respectively. The pregnancy rate was 0% with ejaculated while 100% with the testicular spermatozoa.

An additional aneuploidy assessment by NGS in exclusively azoospermic men evidenced, in the OA 6.7% while in the NOA 5.1%.

Limitations, reasons for caution: This is still a limited number of observations carried out on men screened for infertility. If confirmed, this study may suggest that testicular sampling is safe in terms of retrieving euploid spermatozoa and may be considered in non-azoospermic men in order to obtain cells with lower SCF.

Wider implications of the findings: This study challenges the dogma that testicular spermatozoa conceal a higher proportion of aneuploidy. This implies that testicular gametes do not contribute to chromosomally related pregnancy losses. Moreover, this may explain why offspring from testicular biopsy do not evidence higher autosomal or gonosomal aneuploidy than those resulting from ejaculated spermatozoa.

Trial registration number: N/A.

P-615 Differential expression of testicular function can shed light on why some NOA men successfully yield spermatozoa

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Study question: Can RNA-sequencing analysis of testicular biopsy samples of NOA men indicate a spermatogenic defect and identify the best candidates for surgical retrieval?

Summary answer: By sequencing transcripts of NOA men, we identified differential gene expression profiles suggesting maturation arrest in NOA men who failed to yield spermatozoa after TESE.

What is known already: Men with non-obstructive azoospermia (NOA) can typically be categorized into three varying degrees of impaired testicular function: hypospermatogenesis, maturation arrest, or germ cell aplasia (sertoli cell–only syndrome). Although it can be difficult to analyze these conditions during a targeted testicular biopsy, a detailed assessment is of great importance, as the likelihood of retrieving no spermatozoa via testicular aspiration occurs in 50% of NOA cases. To supplement histology assessment of testicular tissue, RNA-sequencing can be utilized to differentiate gene expression profiles of NOA men with spermatozoa from men without spermatozoa.

Study design, size, duration: Over 9 months, surgical samples from 5 NOA men undergoing testicular sperm extraction (TESE) were assessed for differential expression by RNA-sequencing and grouped for comparison based on the presence of spermatozoa in their respective samples. Gene expression profiles of these men were compared to a control group with acquired obstructive azoospermia (OA).

Participants/materials, setting, methods: Among the NOA study group ($n = 5$), 2 patients had spermatozoa in their surgical samples, whereas 3 patients did not possess any spermatozoa, even after an extensive search of the samples (71 ± 10 minutes) by several embryologists. Samples were processed for RNA isolation and sequenced by Illumina HiSeq at 2×150 bp configuration per lane with ~58 M reads per sample. A log fold change of >2 and a FDR $P<0.05$ were considered significant.

Main results and the role of chance: Quantitative analysis of RNA extracted from the samples averaged a concentration of 41.1 ± 29 ng/μL and an RNA integrity number of 4.6 ± 1. Upon differential expression analysis, gene ontology enrichment analysis indicated a differential expression of genes related to spermatogenesis, with 30.8% of genes related to a compromised spermatogenic function significantly under-expressed in NOA patients with no spermatozoa identified, and 16.7% of spermatogenesis-related genes significantly under-expressed in NOA patients with spermatozoa, compared to an OA control. Most interestingly, differentially expressed genes unique to NOA patients without spermatozoa were related to the regulation of meiosis (TEX11; $P = 0.0002$) and were significantly under-expressed, with 77.8% of genes involving synaptonemal complex assembly affected (HORMAD1, SYCE1, SYCE3, SYCP1, SYCP2, and TRIP13; $P<0.0001$), as well as processes related to the regulation of chromosome organization (MEIOB; $P<0.00001$) and chromosome segregation (STAG3; $P<0.01$). These gene families were normally transcribed in NOA patients from whom spermatozoa were successfully retrieved as compared to the control. These findings suggest a maturation arrest of germ cells in NOA patients who failed to yield spermatozoa via TESE.

Limitations, reasons for caution: This study would benefit from an increased sample size to confirm findings. Although it is currently difficult to query the epigenome and gain consistent and predictive information on the

infertility status of these men, this method represents a way to identify novel markers to understand the etiology of this condition.

Wider implications of the findings: While the etiologies of NOA are not fully understood, RNA-sequencing may help gauge the spermatogenetic progress of male germ cells, potentially enabling reproductive urologists to select the best candidates for successful sperm retrieval. This may also serve to better counsel severe male factor patients and provide realistic expectations for procreation.

Trial registration number: N/A.

P-616 Factors affecting the probability of finding at least one euploid embryo

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Study question: What clinical factors affect the probability of finding at least one euploid embryo?

Summary answer: The number of biopsied blastocysts and female age were found to be significantly associated with the probability of finding at least one euploid embryo.

What is known already: It is well known that the number of embryos available for transfer is significantly lower in patients undergoing preimplantation genetic screening (PGS) compared to regular IVF simply because of chromosomal selection. Thus, the clinical evaluation of a patient applying for PGS is important in order to maximize the probability of finding at least one euploid embryo for transfer. There have been various studies investigating factors which might affect the probability of finding euploid embryos, such as the number of generated embryos, the number of oocytes and AMH (Ata et al., 2012., Kahraman et al.,2016, La Marca et al., 2017).

Study design, size, duration: This retrospective cohort study was conducted in a private IVF center between September 2011 and December 2017. A total of 2001 PGS cycles were analysed and 5952 trophectoderm samples were diagnosed either by array Comparative Genomic Hybridization (aCGH) and by next generation sequencing (NGS).

Participants/materials, setting, methods: The probability of finding at least one euploid embryo per cycle was evaluated according to maternal age, body mass index (BMI), AMH, number of cumulus-oocyte complexes (COCs), and number of biopsied blastocysts using multivariate analysis as a generalized linear mixed model. Four categories were defined for COCs: <4; 4-9; 10-15; >15.

Main results and the role of chance: Maternal age, body mass index (BMI), AMH, the number of cumulus-oocyte complexes (COC), and the number of biopsied embryos were introduced in the multivariate analysis as independent factors. The probability of finding at least one euploid embryo per cycle was defined as the target variable. The analysis showed that the generated model was statistically significant (F:57.155, p<0.001). Female age and the number of biopsied blastocysts were found to be statistically significant in the multivariate analysis. With a one unit (year) increase in female age, the likelihood of finding at least one euploid embryo decreased by 18.6% [OR (95%CI): 0.814 (0.789, 0.840), p<0.001)]. With a one unit increase in the number of biopsied blastocysts, the likelihood of finding at least one euploid embryo increased by 78.9% [OR (95%CI): 1.780 (1.627, 1.948), p<0.001)]. The number of trophectoderm biopsies was found to be the most significant variable affecting the probability of finding at least one euploid embryo after adjustment for age.

Limitations, reasons for caution: The study population is limited to a single IVF setting, results may show variation with different IVF clinics. This study is based on trophectoderm biopsy samples, cleavage stage biopsies have been shown to have higher aneuploidy rates.

Wider implications of the findings: The estimated probabilities of finding at least one transferable embryo based on age and number of biopsied embryos enables more accurate counselling of patients.

Trial registration number: Not applicable.

P-617 Querying gene expression to profile human spermatogenesis in infertile men

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Study question: We question whether gene products isolated from men with different categories of infertility may shed light on their seminiferous tubule function and gamete competence.

Summary answer: Loss of expression of DNA repair and apoptotic genes provide information on the ability to successfully treat couples by assisted reproductive technology (ART).

What is known already: The limitation of evaluating human semen characteristics, even according to the most stringent criteria, is known to provide limited information on the performance of spermatozoa even when used for ART. Standard semen analysis is ill-defined in predicting reproductive outcome in men with unexplained infertility. For this purpose, attempts have been made to detect ploidy, DNA integrity, highly detailed morphology and maturational status of the spermatozoon. Epigenetic assessment by profiling RNA transcripts in the sperm cell has been proposed as an alternative method to screen the male partner for infertility and predicts pregnancy outcome.

Study design, size, duration: In an 18-month period, we assessed the expression of genes in relation to spermatogenesis and reproductive outcome. RNA-Sequencing (RNA-Seq) was carried out on the specimens of 19 consenting men. By sequencing the genome, we assessed ejaculated specimens of 5 infertile couples that were compared to 5 fertile and 3 donors acting as controls. Additionally, 5 non-obstructive azoospermia (NOA) men were compared to one obstructive azoospermia (OA) control.

Participants/materials, setting, methods: Ejaculated spermatozoa and testicular biopsy tissue was used to isolate total RNA using a spin column commercial kit. The nucleic acid quality and spermatozoal RNA concentration was assessed. The RNA samples were then made into paired-end libraries. Pilot paired-end 76 bp RNA-Seq using an Illumina platform (NextSeq 500) was carried out and expanded to 60 M reads. Expression values were calculated in Fragments Per Kilobase Of Transcript Per Million Fragments Mapped reads (FPKM) and normalized read counts.

Main results and the role of chance: The study group (N = 19) had a mean age of 35.9 ± 3 and normal sperm parameters, RNA concentration was 14.3 ± 6 ng/μL (9.1-21.3) with an RNA integrity number of 5.9 ± 1.7. The control couples (n = 3) with a female age of 38.3 ± 5 and male age of 33.6 ± 7 years evidenced normal semen parameters and delivered 3 healthy offspring. Five men underwent ICSI with their female partner (mean age of 35.6 ± 3 years) and achieved a fertilization of 79.3% (30/42), and all delivered. Additionally, 5 men underwent ICSI (female partner with a mean age of 34.8 ± 1 years) and achieved a fertilization rate of 71.4% (30/42), however, they failed to obtain a pregnancy. A total of 86 genes were differentially expressed ($P<0.001$) between the infertile and control cohorts. Of them, 24 genes were overexpressed and 62 under-expressed. Specifically, DNA repair genes *(APLF*, *CYB5R4*, *ERCC4* and *TNRFSF21)* and apoptotic modulating genes (*MORC1*, *PIWIL1* and *ZFAND6*) were remarkably under-expressed (P<0.001) in study cohort. MORC1 and PIWIL1 were also significantly under-expressed in NOA men (P<0.0001). Additional 26 genes DNA repair related, were unique to NOA men. Among those, 8 genes were linked to specimens that yielded spermatozoa while the remainder 18 were specific of men with spermatogenic arrest.

Limitations, reasons for caution: This study profiles men with various stages of spermatogenesis and must be confirmed in a larger cohort. While the contribution of the female partner cannot be excluded, gene expression profiling of infertile men may serve as an assay to measure reproductive potential of the male gamete and seminiferous tubule function.

Wider implications of the findings: Deep sequencing of sperm RNA is a reliable and reproducible technique that may aid in the diagnosis and screening of infertile men. Epigenetic analysis evidenced that DNA repair and apoptosis

genes are linked to seminiferous tubules function indicating their involvement in meiosis and apoptosis typical of germ cells.

Trial registration number: N/A.

P-618 The karyotype of the blastocoel fluid demonstrates low concordance with both trophectoderm and inner cell mass

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Study question: Is blastocoel fluid (BF) karyotype comparable to inner cell mass (ICM) and trophectoderm (TE) cells derived from the same blastocyst?

Summary answer: BF has an increased karyotype discordance rate between ICM and TE and does not adequately represent the status of the rest of the embryo.

What is known already: In assisted reproductive technology (ART) different biopsy methods are used to obtain embryonic material for genetic analysis. Recently, the discovery of amplifiable cell-free DNA in blastocoel fluid (BF) made it the object of attention by representing a potentially less invasive way of obtaining DNA for preimplantation genetic screening (PGS). However, the use of BF-DNA for PGS still remains questionable, as few of the preliminary studies showed contradictive results regarding aneuploidy detection rates and karyotypic concordance between BF and different biopsied samples. Therefore, additional studies are warranted to investigate the potential use of BF-DNA for diagnostic purposes.

Study design, size, duration: In the present study we utilized the most widely used VeriSeq™ PGS (Illumina Inc, USA) platforms for next generation sequencing (NGS)-based comparative chromosome analysis of BF-DNA and TE and ICM cell populations from 16 cryopreserved blastocysts, donated for research by patients who have undergone IVF treatment. The study was approved by the Bioethics Committee of the Biological Institute of the National Research Tomsk State University. All the patients have signed an informed consent.

Participants/materials, setting, methods: All BF, TE and ICM samples derived from a single blastocyst were whole-genome amplified and analysed following the Illumina next-generation sequencing (NGS) VeriSeq™ PGS protocol. Data analysis and genome-wide profile visualization was performed by applying standard settings on Illumina BlueFuse Multi v4.3 Software with embedded aneuploidy calling algorithm. Prior to embryo analysis, mixing experiments were performed in three replicates using aneuploid cell lines to mimic embryonic mosaicism and evaluate NGS sensitivity in mosaic aneuploidy detection.

Main results and the role of chance: Internal validation of mixing experiments revealed that Veriseq™ PGS NGS-platform is able to distinguish mosaic losses and gains that are present in at least 20% of cells. Next, after WGA of embryo samples, a sufficient amount of DNA was detected in all of ICM and TE samples (16/16), but only in 14 out of 16 BF biopsies (87.5%). Following sequencing and quality control, genomic profiles were obtained for 10 BF samples (10/16, 62.5%) and compared to corresponding TE and ICM, while the karyotypes of TE and ICM were obtained and compared in 14 embryos out of 16 (87.5%). The analysis revealed that BF-DNA was burdened with mosaic aneuploidies and the total number of affected chromosomes in BF was significantly higher compared to the TE and ICM ($P < 0.0001$), while no difference was observed between TE and ICM. Thus, only 40.0% of BF-DNA karyotypes (4/10) were fully concordant to TE or ICM, compared to 85.7% between TE and ICM (12/14), making TE more representative of embryonic chromosomal status than BF ($P<0.03$).

Limitations, reasons for caution: The major limitation was the number of embryos analyzed. Additionally, BF aspiration was performed after embryo thawing, which can potentially affect DNA quality and subsequent results. Finally, we sequenced the whole trophectoderm cell population, which is opposite to TE biopsy, when only a small number of cells are analyzed.

Wider implications of the findings: Using BF-DNA as a single source of DNA for PGS can potentially lead to an increased rate of false positive findings, at least using current approach. Blastocentesis cannot be ruled out in the future, although the improvement of current sample handling protocols and aneuploidy calling algorithm may be warranted.

Trial registration number: not applicable.

P-619 Comparison of the times to achieve an ongoing pregnancy in advanced maternal age patients with PGS and young IVF patients without PGS

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Study question: Do young IVF patients achieve an ongoing pregnancy earlier than older patients applying for PGS?

Summary answer: Kaplan-Meier survival analysis based on the number of frozen embryo transfers showed that PGS patients achieved an ongoing pregnancy with significantly less number of transfers.

What is known already: It has been shown that more than 40% of the blastocysts are aneuploid increasing with advanced maternal age (Schoolcraft et al., 2010). These chromosomal abnormalities adversely affect IVF outcomes even in younger patients (Munne, 2012). Transfer of euploid embryos have been shown to result in higher implantation rates especially when trophectoderm biopsy was used (Scott et al., 2013). Evaluation of the time between pick-up and transfer as days may be influenced by many patient and clinical factors, thus misrepresenting the interpretation of survival analysis. To overcome this bias, each embryo transfer cycle is used as a time unit.

Study design, size, duration: This retrospective cohort study includes 2400 frozen-thawed embryo transfer cycles (FET) of 1956 couples whose embryos were frozen, thawed and transferred between August 2011 and December 2017. Blastocysts of young patients with a good ovarian reserve were frozen and transferred later (freeze-all group; n = 1456). Aneuploidy screening was routinely applied for advanced maternal age (>37), patients with an history of abnormal fetal karyotype and with repeated implantation failures (>2) (PGS group; n = 914).

Participants/materials, setting, methods: The mean female age was 29.8 ± 4.2 and 35.1 ± 4.9 for the freeze-all and the PGS groups, respectively ($p<0.0001$). The mean number of cumulus oocyte complexes and metaphase II oocytes was 21.6 ± 10.5 and 17.3 ± 8.7 for the freeze-all group and 14.6 ± 9.7 and 12.1 ± 7.9 for the PGS group, respectively ($p<0.0001$). The mean number of frozen blastocysts was 6.7 ± 3.9 and 4.9 ± 3.5 for the freeze-all and the PGS groups, respectively ($p<0.0001$). PGS was done either with aCGH or NGS.

Main results and the role of chance: In the Kaplan-Meier survival analysis performed, at most four subsequent FET cycles were taken into consideration for each patient. However, once a patient reached an ongoing pregnancy, any further FET cycles were not cumulatively counted since the purpose of the study was to determine the number of FET cycles required to obtain an ongoing pregnancy. When survival curves were compared in the Kaplan-Meier analysis, it revealed that achieving an ongoing pregnancy was one cycle earlier in the PGS group than the freeze-all group ($p<0.0001$). The overall ongoing pregnancy rate of FET cycles with PGS was significantly higher when compared to transfers without PGS (49.3% vs. 44.1%, respectively ($p<0.05$)), despite a higher average age in PGS cycles (29.8 ± 4.2 and 35.1 ± 4.9, respectively) and a significantly lower AMH level in the PGS group (5.6 ± 3.9 and 3.2 ± 2.9, respectively). Additionally, FET cycles with PGS were 1.19 (1.0589 to 1.3527 95 %CI) fold more likely to result in an ongoing pregnancy when compared to non-PGS FET cycles with significantly less frozen embryos (6.7 ± 3.9 and 4.9 ± 3.5*, respectively) [*number before aneuploidy screening].

Limitations, reasons for caution: Self-evidently PGS patients received an euploid blastocyst transfer. The number of embryo transfers is directly related with the number of vitrified blastocysts and therefore with ovarian reserve.

Wider implications of the findings: PGS may be offered to young IVF patients with a good ovarian reserve to decrease the time to achieve an ongoing pregnancy.

Trial registration number: not applicable.

P-620 Alterations in global epigenetic profile (DNA methylation and histone modifications) of cumulus cells in women with endometriosis

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Study question: This study aimed to evaluate global DNA methylation and histone methylation/ acetylation levels in cumulus cells of infertile patients with endometriosis.

Summary answer: Differential epigenetic changes (DNA methylation and acetylation/ methylation of lysine 9 of histone3 (H3K9ac and H3K9me2) in cumulus cells were detected in endometriosis patients.

What is known already: Two epigenetic mechanisms, DNA methylation and post-translational modifications of histone residues (H3K9ac and H3K9me2 as the markers of gene activation and silencing, respectively), are known to regulate the gene expression and their modifications being the main reasons of human diseases.

Although the precise etiology of endometriosis is poorly understood, several evidences recommend that epigenetic factors are associated with the molecular aspect underlying endometriosis. Ovarian cells such as cumulus cell, which surrounds oocyte is one of the main cells that influence by endometriosis.

Study design, size, duration: This study was conducted on 12 infertile endometriosis patients and 12 normo-ovulatory patients with tubal factor infertility or egg donors). All women, who were diagnosed with endometriosis either by laparoscopy or pathological examination, constituted the study group. Endometriosis was categorized according to the revised American Fertility Sterility classification. Cumulus-oocyte complexes were obtained from follicles during ovarian puncture. Only cumulus cells with metaphase II oocytes were selected for this study.

Participants/materials, setting, methods: Chromatin extracts from cumulus cells were prepared following fixation with formaldehyde and then shearing into fragments by sonication. Nucleosome ELISA was performed on chromatin fractions using antibodies against H3K9ac (activating mark), H3K9me2 (repressing mark) and MeCP2 (as a marker of DNA methylation). Relative assay level for incorporation of MeCP2, H3K9me2 and H3K9ac proteins in chromatin fractions were calculated by normalizing ELISA signals (after background subtraction) to core histone H1 content.

Main results and the role of chance: Our data revealed that cumulus cells of endometriosis patients were globally DNA hypermethylated compared to the cells from controls ($P = 0.03$). Furthermore, a significant hyperacetylation as well as hypermethylation at histone H3K9 ($P = 0.04$ and $P = 0.01$, respectively) was observed in endometriosis samples compared to the control group.

Limitations, reasons for caution: For getting more comprehensive information, we need to analyze epigenetic alterations in cumulus cells that are involved in the ovarian functions in infertile endometriosis patients.

Wider implications of the findings: These results clearly show that epigenetic profile such as DNA methylation and histone acetylation/methylation were significantly altered in cumulus cells of endometriosis patients and these alterations may be responsible for infertility in such patients.

Trial registration number: not applicable.

P-621 Customising the limit of detection for PGT-A and PGT-SR using NGS

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Study question: What is the lower limit of detection for segmental copy number variations by Next Generation Sequencing using PG-Seq™?

Summary answer: Whilst aneuploidy detection can be achieved from 300,000 reads, detection of a 100 kb microdeletion requires over 13 million reads, or sequencing 1-2 samples per run.

What is known already: Next generation sequencing platforms provide a highly flexible and customizable workflow to attain a variety of levels of resolution for the detection of segmental aberrations unlike other DNA analysis platforms previously used for preimplantation genetic testing (PGT). In simple terms higher resolution for the detection of smaller genetic aberrations can be achieved by reducing the number of samples in a single sequencing run to increase the total reads for each sample available for analysis.

Study design, size, duration: Library Preparation and 48 sample multiplex NGS was performed according to standard PG-Seq™ protocol (RHS Ltd). Initially, to evaluate the minimum number of mapped reads required to detect a 7 Mb segmental loss and 31 Mb gain, PG-Seq™ NGS files were randomly downsampled to produce files containing reads ranging from approximately 500,000 to 100,000 reads. The minimum number of reads required to detect incrementally smaller chromosome aberrations was determined in silico.

Participants/materials, setting, methods: Individually sorted 5-cell aliquots from a segmental deletion and duplication cell line (GM14485, Coriell Institute) were analysed using PG-Seq™ according to manufacturers instructions.

Main results and the role of chance: Downsampling and analysis of sequencing read data showed the detection of both the 7 Mb deletion and 31 Mb duplication indicated that detection was still possible using 100,000 mapped reads. However, the PG-Seq™ quality control score increased as mapped reads declined from approximately 300,000 to 100,000 mapped reads per sample. In silico modelling indicated the detection of a 100 kb segmental copy number variation would require 6-19 million mapped sequencing reads per individual sample. To achieve >6 million reads for a single sample would require no more than 3 samples to be sequenced on a MiSeq sequencer. A higher level of confidence for reporting would be achieved for samples with mapped reads of 13-19 million, and no more than 1-2 samples could be sequenced in a single MiSeq run.

Limitations, reasons for caution: In silico data analysis does not replace the need for validation of NGS workflows using cell line or clinical samples to determine the resolution of the chosen workflow.

Wider implications of the findings: In silico analysis of sequencing data highlights the technical limitations and financial implications for the detection of small segmental aberrations by NGS.

Trial registration number: -

P-622 OnePGT proof of concept for PGT-M and PGT-SR on blastomere and trophectoderm biopsies

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Study question: Investigating the OnePGT product as a single assay for preimplantation genetic testing for monogenic disorders (PGT-M) and preimplantation genetic testing for aneuploidies (PGT-SR) for blastomere and trophectoderm embryo (TE) biopsies.

Summary answer: OnePGT accurately determined the single gene disorder status of 48/48 embryos for PGT-M, and the presence of an unbalanced translocation in 33/33 embryos for PGT-SR.

What is known already: OnePGT is a next-generation sequencing (NGS) haplarithmisis-based solution for Preimplantation Genetic Testing (PGT) of in vitro fertilized (IVF) embryo single-cell or TE biopsies. OnePGT is a wet- and dry lab solution allowing concurrent PGT-M, PGT-SR of whole-genome amplified (WGAed) products biopsied from IVF embryos in a single assay. This innovative product provides a sample-to-answer solution, from wet lab to cloud-based analysis software, which has haplarithmisis in its heart.

Study design, size, duration: To test the performance of Agilent's OnePGT solution, we performed a blinded clinical verification study on 109 embryos (32 couples) donated to Maastricht UMC+ for research purposes. For PGT-M, 67 embryos (20 couples) that are previously analyzed with conventional STR-PCR PGD as well as phasing reference (a close relative) samples (PGT-M) were processed in our facility. For PGT-SR a total of 36 embryos (11 couples), previously analyzed with FISH or arrayCGH PGD, were tested.

Participants/materials, setting, methods: In total 36 couples (109 spare embryos unsuitable for uterine transfer) were participated in this study. These embryos were affected with monogenetic disorder, unbalanced translocation or had poor morphology. All embryos were re-biopsied, WGAed and processed according to the OnePGT library preparation protocol. Subsequently, NGS libraries were sequenced and analyzed via the PGT-M or PGT-SR pipeline in the OnePGT software in a blinded fashion. The onePGT diagnoses were then compared with the conventional PGD method.

Main results and the role of chance: For the 36 embryos for which both OnePGT and the conventional PGD methods gave a conclusive result, 100% concordance was demonstrated. The main reason for inconclusive results were loss-of-heterozygosity-like regions in the reference family members, affecting 10 embryos. Another 14 samples were found to be affected by the common chromosome instability (CIN) phenomenon, such that the copy number discrepancies between OnePGT and STR-PCR were confirmed by SNP-array analysis supporting the OnePGT results. The quality control module of the OnePGT software identified 8 samples with no genomic DNA but with a high mitochondrial DNA content.

Regarding the PGT-SR analysis, a conclusive result was obtained for all samples (36/36, 100%). Of the 36 embryos, 3 were found to be with CIN, as shown by copy number discrepancies between OnePGT and FISH/arrayCGH, which were further confirmed by an orthologous method (Agilent GenetiSure PreScreen). Overall, the OnePGT diagnoses on 33 embryos were 100% concordant with the gold standard conventional PGD.

Limitations, reasons for caution: Although the sample-size (number of embryos) in this study is not sufficient to reach to the 0.9 statistical power, we have increasingly expanded the samplesize in collaboration with UZ Leuven.

Wider implications of the findings: OnePGT is a single-assay solution for both PGT-M and PGT-SR, applicable on single-cell or TE WGAed genomes derived from cleavage-stage or blastocyst-stage IVF embryos, respectively.

Trial registration number: The study was funded by Agilent Technologies.

P-623 Clinical impact of reporting embryo mosaicism for preimplantation genetic testing for aneuploidy (PGT-A)

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Study question: Reporting mosaicism allows prioritizing embryo transfer. This study examines the clinical impact of reporting mosaicism in PGT-A practice.

Summary answer: Of 813 PGT-A patients, about 5% (43) had only euploidy-mosaic embryos. Those embryos, accounted for 6.6% of analyzed embryos, are transferrable but with caution.

What is known already: Mosaicism in early preimplantation embryos is recognized as a common phenomenon and it appears to be readily detectable in D5/6 biopsied specimens with next generation sequencing platform. Transfer of mosaic embryos may result in healthy babies (Greco et al., NEJM, 2015). Although it is generally believed that the results of chromosomal ploidy and mosaicism detected in biopsied specimens are likely correlated to the healthy status of the embryos, controversy exists as to whether or not to report mosaicism due to insufficient knowledge on this biological or artefactual phenomenon.

Study design, size, duration: PGS data were collected from 813 cycles and 4423 embryos from January 1 to December 31, 2017. Patients (average age = 38.4) were divided into three groups: ≤34, 35 to 40, and ≥41. Embryo biopsies were performed on D5 or D6. Illumina's VeriSeq PGS platform was used for sequencing and data analysis.

Participants/materials, setting, methods: Euploidy was defined as no gain/loss detected in any chromosomes screened. Euploidy-mosaic (Eup-M) was defined as an absence of whole chromosome gain/loss, but with low confidence combined with a profile of one or more whole chromosome(s) or segmental region(s) that showed 25 to 80% gain or loss. Embryos with mosaic chromosomes coupled with the presence of one or more whole chromosomal gain/loss were excluded from this classification since those were defined as aneuploidy.

Main results and the role of chance: Of 813 patients, 205 (25.2%) have all aneuploidy embryos. In 569 patients at least one or more euploid embryos were available for transfer. An overall of 396 embryos were identified as Eup-M. The distribution of euploidy, euploid-mosaic and aneuploidy embryos in three age groups are tabulated below:

Ch. Status/ Age	≤34 (#)	35-40 (#)	≥41 (#)	Overall (#)
Euploidy	53.4% (639)	36.1% (812)	13.8% (135)	34.0% (1586)
EUP-M	13.1% (157)	8.8% (197)	4.3% (42)	6.6% (396)
Aneuploidy	33.5% (401)	55.1% (1240)	81.9% (800)	55.2% (2441)
Total # embryos	1197	2249	977	4423

More euploid-mosaic embryos were present in younger women than that in older women because the latter had more aneuploid embryos which also had mosaic chromosomes. Frequently, only one (32.9%, 187/569) or two (10.4%, 59/569) mosaic embryos were detected in PGT-A cases, up to 6 mosaic embryos were observed in one patient. In 43 patients (7.6%, 43/569), only mosaic embryos were available for transfer.

Limitations, reasons for caution: In patients (~8%) who have only euploid-mosaic embryos available for transfer, extensive genetic counseling should be recommended to assist in decision making. Further study is warranted to follow the pregnancy results from transfers of those embryos.

Wider implications of the findings: The frequencies of mosaicism appears to be relatively low as indicated in this study. However, mosaicism will affect embryonic development and may create real issues for PGT-A where biopsied specimens may not precisely reflect the inner cell mass and/or the whole embryos, thus may lead to false-negative or false-positive results.

Trial registration number: N/A.

P-624 Paternal factors in recurrent pregnancy loss: a proteomic insight

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Study question: Do differential protein expression lead to aberrant embryo development leading to loss of foetus in idiopathic recurrent pregnancy loss (iRPL)?

Summary answer: Differential protein expression in spermatozoa of partners may be the contributing factor towards impaired embryo development and pregnancy loss in patient with iRPL.

What is known already: Defective paternal genome has been attributed to be an important cause for spontaneous recurrent pregnancy loss (RPL). Controlled level of reactive oxygen species (ROS) is essential for normal sperm function while elevated levels results in increase in oxidative damage to sperm chromatin, induce mutations, oxidize proteins, and membrane lipids. There is enhanced ROS generation in spermatozoa of partners of iRPL resulting in augmented lipid peroxidation, protein carbonylation and thionylation.

Study design, size, duration: This prospective study consisted of male partners of iRPL patients (n = 16) with no female factor abnormality as revealed by gynaecologic investigation including karyotyping and age matched fertile healthy volunteers (n = 20). All samples were collected during 2013-2015 after getting institutional ethical approval and written consent from the participants.

Participants/materials, setting, methods: Seminal ejaculates were collected by masturbation after 2-3 days of sexual abstinence and analysed according to World Health Organization criteria. Samples from each group after separation by 2D-DIGE were subjected to mass spectrophotometric analysis to identify differentially expressed proteins (DEPs).STRING and Cytoscape softwares were used for pathway analysis.Two key proteins, HSPA2 and GPx4 of the identified pathways were validated by Western blot analysis.

Main results and the role of chance: Based on the relative abundance of the common protein spots 27 were overexpressed and 9 were underexpressed, while 18 proteins spots were identified with low abundance. A fold change >2 was considered as overexpressed or underexpressed. A total of 6 spots were picked for protein sequencing analysis owing to their abundance and position on the gel. This included GPx4, JIP4, ZN248 to be overexpressed while HSPA2,GSTM5, TF3C1, CC74A was underexpressed in RPL group. Owing to its greater coverage, HSPA2 was subjected to single nucleotide polymorphism (SNP) analysis wherein of 444 SNPs were synonymous and 56 were found to be nonsynonymous SNPs. It was found that 18 SNPs were deleterious using a combinatorial servers-SIFT, Polyphen, Provean, nsSNP analyser and SNPs and GO. Protein structural analysis with these amino acid variants was performed by using HOPE, ConSurf, Swiss PDB viewer, Chimera and NOMAD-Ref servers to check their solvent accessibility, molecular dynamics and energy minimization.

Limitations, reasons for caution: 2D-DIGE though a robust technology in terms of reproducibility, has a limitation because all the identified differential protein spots needs to be subjected to mass spectrophotometry to identify all DEPs. Secondly, increasing the sample size would augment the biological variability and statistical power of the study.

Wider implications of the findings: This pilot study showed that in case of iRPL paternal factors play a role which can be further analysed to develop biomarkers for iRPL. Additionally, our in silico analysis suggests that screening of SNP variants of HSPA2 such as V106E, D55Y, T301R, R314P,S343Pmay be useful for predicting outcome.

Trial registration number: Not applicable.

POSTER VIEWING
Reproductive endocrinology

P-625 The effect of the duration of elevated progesterone during the late-follicular phase of ovarian stimulation on live birth rates following IVF/ICSI

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Study question: Does a longer exposure to late-follicular elevated progesterone (LFEP) hinder live birth rates (LBRs) after fresh embryo transfer?

Summary answer: LBRs are only weakly affected by the duration of LFEP.

What is known already: The overproduction of progesterone (P) during ovarian stimulation has been associated with endometrial genetic/epigenetic changes in the luteal phase and, most importantly, lower LBRs after a fresh embryo transfer. Therefore, in daily clinical practice, P levels on the day of human chorionic gonadotrophin (hCG) administration are often measured and a freeze-all strategy is adopted whenever LFEP (>1.50 ng/ml) occurs. However, a recent retrospective analysis has concluded that also the duration of LFEP >1.00 ng/ml prior to hCG administration may affect clinical pregnancy rates. We assessed whether this result was reproducible, namely when using LBR as the primary outcome.

Study design, size, duration: We performed a retrospective cohort analysis including all women undergoing ovarian stimulation for IVF/ICSI in a university-affiliated tertiary referral centre between January 2010 and March 2015. A total of 4312 cycles were included. To minimize potential confounding, only patients with GnRH antagonist pituitary suppression who underwent fresh embryo transfer using autologous oocytes were included. Couples with planned embryo biopsy, managed natural cycles or in-vitro maturation were excluded from the analysis.

Participants/materials, setting, methods: The primary outcome was live birth, defined as a live born delivery after 24 weeks. LBRs were compared among different P elevation duration groups (0 days, 1 day, 2 days or ≥3 days) using multivariable regression analysis to account for potential confounders. This analysis was repeated using two cut-offs of LFEP: 1.00 and 1.50 ng/ml.

Main results and the role of chance: The average female age of the cohort was 34.0 ± 5.0 years. The mean number of oocytes retrieved was 9.3 ± 5.7 and 64.7% of the fresh embryo transfers were performed at cleavage stage (day 3), with a single embryo being transferred in 51.0% of all cases.

In the univariable analysis, the duration of LFEP >1.00 ng/ml was not associated with a significant decrease in LBR. Specifically, LBRs were 25.8%, 24.1%, 23.6% and 24.2% according to whether the patient had LFEP >1.00 ng/ml lasting for 0, 1, 2 or ≥3 days respectively (p = 0.259). However, LBR for the same LFEP durations, but using LFEP >1.50 ng/mL as the threshold, lowered significantly as the duration of LFEP increased: 25.8%, 18.4%, 15.1% and 13.8%, respectively (p<0,001).

Although the duration of LFEP >1.50 ng/mL predicted LBR independently in the univariable analysis, the relative frequency of having such LFEP levels for 2 or ≥3 days was exceedingly rare (1.2% and 0.2%, respectively). Moreover, the duration of LFEP >1.50 ng/ml was no longer a statistically significant LBR predictor in the multivariable regression analysis performed (which accounted for female age, number of preceding IVF/ICSI cycles, type/dose of exogenous gonadotrophins, endocrine profile at hCG administration, number of oocytes retrieved, embryo stage, number and quality at transfer).

Limitations, reasons for caution: Although this study includes a large sample set and adjusts for multiple potential confounding, the results are limited by its retrospective nature and the inability to include all relevant potential confounders (e.g. smoking habits). Better extrapolation could be obtained by validating these results prospectively.

Wider implications of the findings: Although LBRs were discretely lower if LFEP >1.50 ng/ml lasted for more than 1 day, such an event seemed rare and was no longer significant when accounting for potential confounding. Therefore, one may postulate that the clinical value of measuring P prior to hCG administration is limited.

Trial registration number: not applicable.

P-626 Efficacy of metformin therapy to reduce pregnancy-induced hypertensive disorders in women with silent impaired insulin-sensitivity

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Study question: Could metformin therapy, throughout pregnancy, be effective to reduce the risk of late pregnancy-induced hypertensive disorders (PIHD) in women with defective insulin-signaling system function?

Summary answer: Metformin therapy, throughout pregnancy, reduced the development of third trimester gestation-induced hypertensive disorders in women with silent defective insulin-signaling system function from 23.6% to 13.8%.

What is known already: Pregnant women with impaired insulin sensitivity are at a high risk for developing pregnancy-induced hypertensive disorders (PIHD). Previous data support that 2 h-insulinemias greater than 215.25 pM after 75 g-glucose overload could be a predictor for detecting women at high risk of developing PIHD. Indeed, compared with pregnant women at low risk (2 h-insulinemias lower than 215.25 pM), those at high risk displayed a 4-fold higher occurrence of gestational hypertension and pre-eclampsia. Additionally, it is accepted that metformin (MTF) therapy is able to prevent poor pregnancy outcome in women diagnosed for gestational diabetes mellitus (GDM).

Study design, size, duration: We performed a 2-year-prospective study in 226 multiparous women that became spontaneously pregnant either under oral MTF therapy (2 g/day; n = 29) or not (n = 198). During gestational week 25-26, women were subjected to an oral glucose tolerance test (OGTT) measuring insulinemias on both sample-times. Thereafter, women were classified at low (being the 2 h-insulinemias lower than 215.25 pM) or at high (being the 2 h-insulinemias greater than 215.25 pM) risk for developing PIHD (LR-PIHD and HR-PIHD, respectively.

Participants/materials, setting, methods: Overnight fasting pregnant women (age 29-37 years) were bled before (sample time-zero) and 2 h after 75 g-oral glucose load (OGTT). Glucose (enzymatic-colorimetric assay) and insulin (chemiluminescence) plasma concentrations were determined in samples times zero and 2 h from the OGTT. Finally, HOMA-IR score (glucose in mM x insulin in mIU/mL x 0.044) and Glucose (in mg/dL) to Insulin (in mIU/mL) ratio (G:Ir) were calculated for both time-samples. Anthropometric data were recorded throughout pregnancy.

Main results and the role of chance: Biochemical data indicate that 125 and 101 women were at LR-PIHD and HR-PIHD, respectively, MTF-treated women pertained to the HR-PIHD group. Comparing data from HR-PIHD MTF-treated (n = 29) versus MTF-untreated women (n = 72), although time-zero glycemias were similar, time-2 h glycemias were significantly ($P<0.05$) lower in MTF-treated than in MTF-untreated women. Plasma insulin and HOMA-IR values were statistically similar in both groups, regardless of the sample-time examined. Finally, G:Ir values were significantly ($P<0.05$) higher in MTF-treated than in MTF-untreated women although in sample-time zero only. While 17/72 MTF-untreated women developed any PIHD, conversely, PIHD was noticed in 4/29 MTF-treated women. Starting woman body mass index, weight-gain throughout pregnancy, gestational length, multiparity and, the appearance of polihydramnios and newborn respiratory distress were similar among groups; none stillbirth was noticed. Although mean values of weight at birth were similar among groups, macrosomic babies were born to MTF-untreated mothers only (3/72). Finally, while PIHD (hypertension/pre-eclampsia) were developed by 17/72 (23.6%) MTF-untreated women, a better pregnancy outcome was noticed in MTF-treated women because only 4/29 (13.8%) developed PIHD. Data indicate that pregnancy outcome in women at HR-PIHD, due to their impaired insulin sensitivity (2h-insulinemias >215.25 pM), could be highly benefited if treated with MTF throughout pregnancy.

Limitations, reasons for caution: Data emerging from this prospective study need to be corroborated by studying the beneficial effect of MTF therapy throughout gestation, on poor pregnancy outcome in a larger sample-population of pregnant women at high risk of developing PIHD, defined as having 2 h-insulin values greater than 215.25 pM during an OGTT.

Wider implications of the findings: We have established that by applying the simple criterion ¨2-h insulinemia > 215.25 pM during an OGTT¨, pregnant women with silent impaired insulin sensitivity are at high risk of developing PIHD, however, such increased risk could be reduced when these women are treated with MTF throughout pregnancy.

Trial registration number: Not applicable.

P-627 Expression of reproductive hormone receptors and contraction-associated genes in porcine uterus during the estrous cycle

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Study question: What is mechanism of uterus contraction activity in a porcine model?

Summary answer: hormonal receptors and contraction-associated proteins were dynamically regulated depending on the estrous cycle.

What is known already: Contraction of uterus tissue frequently occurs throughout the estrous cycle and is regulated by several endogenous factors, including estradiol, progesterone, luteinizing hormone, follicle-stimulating hormone, oxytocin (OXT) and contraction-associated proteins (CAPs).

Study design, size, duration: Experimental study.

Participants/materials, setting, methods: mRNA and protein expression levels of reproductive hormonal receptors, including estrogen receptors, progesterone receptor and luteinizing hormone/choriogonadotropin receptor in addition to CAPs including OXT, OXT receptor (OXTR), hydroxyprostaglandin dehydrogenase 15-(NAD) and gap junction α-1 protein, were examined in the porcine uterus according to the follicular and luteal phases.

Main results and the role of chance: hormonal receptors and CAPs were dynamically regulated depending on the estrous cycle. In conclusion, genes associated with uterine contraction and its regulatory hormonal receptors in the porcine uterus were differently regulated in the follicular and luteal phases.

Limitations, reasons for caution: These experiments were limited to only mRNA measurements, these result differ from those of the current study due to the distinct environmental conditions for other animals.

Wider implications of the findings: Genes associated with uterine contraction and its regulatory hormonal receptors in the porcine uterus are critically involved in the remodeling and contraction of uterine tissue and may be required to modulate the physiological status of the uterus.

Trial registration number: not applicable.

P-628 Effects on body weight and waist/hip circumference during a 1-year three-component lifestyle RCT in obese PCOS women

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Study question: Is a multidisciplinary one-year cognitive-behavioural lifestyle treatment more effective to decrease weight and waist/hip circumference than usual care?

Summary answer: A three-component lifestyle program for women with PCOS achieved a weight loss of 5%. A self-induced weight loss by publicly available services is less effective.

What is known already: Obesity in women with polycystic ovary syndrome (PCOS) negatively affects all clinical features. There is a large number of small uncontrolled (two-component) trials demonstrating that losing 5 to 10% of initial body weight has shown promising results on reproductive, metabolic and psychological level. Weight loss programs seem to be effective in the short term; however, most of the initial weight loss is regained within 1 year. The biggest challenge is to achieve a reasonable and sustainable weight loss. More intensive (three-component) trials are needed to enhance adherence and achieve a sustainable weight-loss of 5 to 10%.

Study design, size, duration: The present study is a longitudinal randomized controlled trial (RCT) to study the effectiveness of a three component 1-year

cognitive-behavioural lifestyle intervention in overweight/obese women with PCOS. A total of 209 participants are randomly assigned to three groups: 1) CBT provided by the multidisciplinary team or; 2) CBT provided by the multidisciplinary team and Short Message Service (SMS) or; 3) usual care: encourage weight loss through publicly available services (control group).

Participants/materials, setting, methods: Women with menstrual cycle disorders are systematically screened using a standardised protocol. Data of 209 women diagnosed (according this protocol) with PCOS according to the Rotterdam criteria, a Body Mass Index above 25 kg/m2 were included between September 2009 and August 2016. Outcome variables were measured at the start of the study, at three, six, nine and twelve months. Mixed modelling is applied for longitudinal analyses of the data.

Main results and the role of chance: The reduction in BMI within one year was -2.18 kg/m^2, 95% confidence interval (CI) [-2.72, -1.64] in the lifestyle intervention group (P<0.001), and -0.91 kg/m^2, 95% CI [-1.59, -0.23] in the control group (P<0.009), a difference of -1.27 95% CI [-2.14, -0.41] (P<0.004). The mean weight loss in the lifestyle intervention group was -6,39 kg, 95% confidence interval (CI) [-7.93, -4.85] (P<0.001) and -2,44 kg 95% CI [-4,37, -0,50], in the control group (P = 0.014), a difference of 3.96 kg, 95% CI [-6.43, -1.49] (P<0.002). Body Mass Index at baseline was no predictor for weight loss. The chance of a 5% BMI reduction was 7.19 times larger in the lifestyle intervention group than in the control group.

Waist circumference significantly decreased in both groups: - 4.84%, 95% CI [-6.48, -3.21] and -4.75%, 95% CI [-6.98, -2.51] respectively, but there was no difference between the lifestyle intervention and controls (P .944). Hip circumference also decreased significantly: -4.17%, 95% CI [-5.04, -3.31] versus -2.14%, 95% CI [-3.30, -0.97] and significantly more (P .006) in the lifestyle intervention group.

Limitations, reasons for caution: All PCOS patients with a BMI > 25 kg/m^2 and eligible for ovulation induction treatment are obligated to follow the lifestyle modification protocol prior to fertility treatment. After inclusion of 150 patients, we applied an interim power analysis to the complete cases.

Wider implications of the findings: These findings support that three-component multidisciplinary cognitive- lifestyle interventions should be incorporated in daily practice to support women with PCOS to achieve a healthy weight loss of at least 5%.

Trial registration number: Registered at the Netherlands National Trial Register with number NTR2450 on August 2nd, 2010.

P-629 The influence of cadmium, copper, lead, selenium, and zinc concentrations in follicular fluid on embryo developmental dynamics by time-lapse microscopy

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Study question: This study investigated the influence of cadmium (Cd), copper (Cu), lead (Pb), selenium (Se), and zinc (Zn) in follicular fluid (ff) on the dynamics of embryo development.

Summary answer: Zinc concentrations in ff affect the dynamics of embryo development from tC to t4. Pb impede the dynamics of embryo development from tF to t4

What is known already: The negative impact of the environment in which the oocyte grows affects the quality of the embryo and the time at which subsequent developmental stages are reached. The oocyte can probably be damaged by a toxic microenvironment during the primordial stage of follicle development and at the time of oocyte maturation. One suggested cause of egg-cell damage is the deleterious effects of excess amounts of heavy metals.

Study design, size, duration: The present study was conducted in medical center 'Ovum" between March 2016 and April 2017. The study involved 220 women undergoing ICSI treatment for the first time. The inclusion criteria were: age below 35 years, FSH level ≤ 10 mIU/mL, AMH level ≥ 1.5 ng/mL, and Body Mass Index (BMI) ≤ 30 kg/m^2. Women with severe endometriosis, metabolic disease, or leiomyoma were excluded from the study.

Participants/materials, setting, methods: The ovarian stimulation was carried out according to the short protocol: injections of gonadotropin-releasing hormone analogue were followed by human (group A,n = 110) and recombinant (groupB,n = 110) FSH administration. The growth of embryos was monitored with time-lapse system.The trace metals measurements were performed by the electrothermal-atomic absorption spectrometry method. After six weeks, the presence of the embryo cardiac activity were assessed via ultrasound. The relationship between concentrations of trace metals and embryo kinetic data were analyzed.

Main results and the role of chance: Pregnancy was achieved in 57 women – 29 (26,36%) of them were stimulated with hFSH and 28 (25.45%) were stimulated with rFSH.No statistically significant difference was found between the two groups with regards to the type of gonadotropins used (Chi^2 = 0.278, df = 1, p = 0.575).Comparing time of embryo development in groups A and B it was noted that almost all successive stages of embryo development (except tF) occurred earlier in women who achieved pregnancy than in women in whom pregnancy was not achieved.In both groups possitive correlations were found between mean level of Zn and the embryonic development periods: tC-t2, t4 and negative correlations occurred for the Pb levels with times tF–t4.In regards to other trace metals, we have observed no significant relationships. ROC curve analysis showed no statistically significant differences between accuracy of using FSH and trace metals levels to predict pregnancy after IVF.

Limitations, reasons for caution: The results of this study where ICSI was performed with ejaculated sperm and for male-factor infertility cannot be generalized to all ICSI offspring because the indications for ICSI have nowadays been extended and ICSI is also being performed with non-ejaculated sperm and reported differences may thus either decrease or increase.

Wider implications of the findings: It has been hypothesized that zinc supplementation may improve achivement pregnancy in patients undergoing ICSI. It raises the need for further research into this problem by larger research groups.

Trial registration number: 8/07/2016

P-630 Non-invasive detection of dominant follicle metabolite composition in PCOS women receiving rFSH, clomiphene citrate or aromatase inhibitor for ovarian stimulation

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Study question: Does metabolite composition of dominant follicle change in women with PCOS receiving rFSH, clomiphene citrate (CC) or aromatase inhibitor (AI).

Summary answer: While CC use changes the developing follicle metabolite content negatively rFSh or AI will not cause any change.

What is known already: Although conventional ovarian stimulation drugs used in assisted reproductive technology cycles allow selection and maturation of oocytes they may lead to retrieving of compromised quality oocyte that may cause fertilization failure or compromised embryo development. rFSH, CC and AI are the three most commonly used drug groups for ovulation stimulation. Although a number of studies have looked at the metabolites compostion of reproductive tissues MRS-based approach to identify the impact of drugs used for ovarian stimulation on human follicular fluid has not been carried out.

Study design, size, duration: This is a case controlled study, enrolled 26 women who undergoing IVF/ICSI due to PCOS.

Participants/materials, setting, methods: A total of 21 infertile PCOS women equally assigned to three groups consisting of women receiving rFSH or CC or aromatase inhibitor. Each group of women underwent MR spectroscopy of a dominant follicle before hCG injection as soon as the detection of a follicle with a mean diameter of at least 16-18 mm. N-acetylaspartate (NAA), lactate (Lac), creatine (Cr), and choline (Cho) content of follicle were measured as ppm.

Main results and the role of chance: Four fold decrease in Cho signal was detected in subjects receving CC compared to fertile subjects. Cho signal of subjects taking rFSH or AI were significantly higher than in the CC group.

Significantly increased Lac signal was detected in the dominant follicle of subjects taking CC. Three-fold higher Cr signals was detected in fertile subjects compared to CC group. Likewise, Cr peak of subjects taking rFSH or AI were significantly higher than in Cr signal of CC group.

Limitations, reasons for caution: The low number of patients being studied is the basic limitation of our study.

Wider implications of the findings: Prediction of the developmental capacity of egg before OPU with spectroscopy may increase the accuracy of best quality embryo selection. Thus, it may be possible to see the developmental defects of the growing oocyte before OPU and to take treatment steps for improving oocyte developmental potential.

Trial registration number: NA.

P-631 Experimental study of rat bone marrow stem cell on polycystic ovary syndrome model of rattus norvegicus decreasing fas ligand expression and apoptotic index

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Study question: This study aims to compare Fas ligand expression and apoptotic index in rat ovaries with or without the administration of Rat Bone Marrow Stem Cell.

Summary answer: There was a decrease of Fas ligand expression and apoptotic index in the administration of rat bone marrow stem cell in rat with PCOS model.

What is known already: Hiroyuki et al. conducted further research by comparing ovarian expression and localize of FAS and Fas Ligan (FasL), caspace-9. This study found TdT-mediated dUTP-Biotin nick-end-labeling (TUNEL) which indicates positive result, highly increase in Polycystic Ovary Syndrome (PCOS) ovaries compared with healthy ovaries (p<0,005), FasL level and caspace-8 increase.

Study design, size, duration: This study used experimental design with post-test only control group design conducted on rats with PCOS model. The large sample of the study, based on Federer's formula with the amount of 27 rats. The study was conducted on January to May 2016.

Participants/materials, setting, methods: The large sample of the study, based on Federer's formula with the amount of 9 rats/group, was then divided into three groups which are rats without treatment, PCOS model rats, and PCOS model rats with stem cell. The dependent variables in this study were Fas ligand expression and apoptotic index. If the distribution is normal, the data analysis used ANOVA test. If it is not normal, the data analysis used non-parametric Kruskal Wallis test.

Main results and the role of chance: The characteristics of the research subjects (age and weight) of rats were in normal distribution. It was found that there was no significant difference in the weight of rats (P = 0.924) and the age of rats (p = 0.556). There was no significant difference on Fas ligand expression in the three groups (p = 0.002). There was no significant difference on apoptotic index for three groups (p = 0.002). The comparison of Fas ligand expression and apoptotic index between the untreated rats and PCOS model rats was 1.6000 + 1.126 vs 3.377 + 1.065, p = 0,001; 2.533 + 1.322 vs 5.822 + 2.892, P = 0.001. There was significant difference on Fas ligand expression and apoptotic index in PCOS model rats and PCOS model rats with stem cell (3.377 + 1.065 vs 0.933 + 0.529, p<0,0001; 5.822 + 2.892 vs 1.400 + 0.812, p<0,0001). There was no significant difference between the untreated rats and PCOS model rats with stem cell (1.6000 + 1.126 vs 0.9333 + 0.529, p = 0.148; 2.533 + 1.322, p = 0.148 vs 1.400 + 0.812, p = 0.217). There was strong correlation between the decreased Fas ligand expression and apoptotic index (R = 0.94286).

Limitations, reasons for caution: The use of rats as samples could not represent women with PCOS as a whole.

Wider implications of the findings: The result of the study is in line with the previous literature regarding benefit of stem cell in PCOS. Stem cells have the effect on Fas ligand and apoptotic index at PCOS cases.

Trial registration number: Not applicable.

P-632 Cumulative live birth rate after in vitro maturation (IVM) in patients with polycystic ovary syndrome (PCOS): retrospective analysis of 1,110 cycles

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Study question: What is the cumulative live birth rate after IVM in patients diagnosed with PCOS?

Summary answer: The cumulative live birth rate after IVM is satisfactory, and similar to that achieved after IVF.

What is known already: IVM is an emerging assisted reproductive technology technique that eliminates risk of ovarian hyper-stimulation syndrome in patients with PCOS. However, there is a lack of data on the effectiveness and safety of IVM cycles.

Study design, size, duration: This retrospective cohort study included 1,110 PCOS patients (1,110 IVM cycles, 1,206 transfers). The most common indication for IVM was ovulation induction failure (93.5%). Mean age, body mass index (BMI), and anti-Müllerian hormone levels were 28.8 ± 3.5 years, 21.9 ± 3.2 kg/m^2, and 12.5 ± 3.6 ng/mL, respectively. The majority of patients had primary infertility (81%); mean infertility duration was 3.6 ± 2.5 years.

Participants/materials, setting, methods: Women who were diagnosed with PCOS (Rotterdam criteria) underwent IVM at a large center in Ho Chi Minh City, Vietnam between 2014 and 2016. Human chorionic gonadotropin (hCG) priming was used in 921 patients. Exclusion criteria included uterine abnormalities, donor or pre-implantation genetic screening/diagnosis, loss to follow-up cycles and cycles without embryo transfer. Data on the number of live births 12 months after initiation of IVM were used to calculate the cumulative live birth.

Main results and the role of chance: Mean number of immature oocytes retrieved per patient was 15.9 ± 10.2. Maturation and fertilization rates were 69.0 ± 23.9% and 68.7 ± 22.5%, respectively. First embryo transfer was fresh in 76.5% of patients and frozen in 20.4%. No case of ovarian hyperstimulation syndrome (OHSS) was reported. The ongoing pregnancy rate and live birth rate per embryo transfer were 33.6% and 26.8%, respectively, and the 12-month cumulative live birth rate was 29.1% (66% in singleton and 34% in twins). Median time to ongoing pregnancy and live birth were 2.4 and 8.8 months, respectively. The cumulative live birth rate was the same with or without hCG-priming (30.1% vs 24.3%, p = 0.13), but median time to live birth was shorter in hCG-priming vs non hCG-priming cycles (8.7 vs 9.6 months, p<0.001) since a freeze-only strategy was used when hCG-priming was not given. Multivariate logistic regression analysis revealed that BMI, number of IVF attempts and number of good embryos were significant independent predictors of live birth after IVM.

Limitations, reasons for caution: The retrospective design of this study was the most important limitation. In addition, pregnancy-related complications (e.g. pre-eclampsia and diabetes mellitus) were not documented.

Wider implications of the findings: Cumulative live birth rate after IVM in PCOS women was acceptable and similar to that after IVF. IVM is a convenient, low cost option in these patients and has been shown to eliminate the risk of ovarian hyper-stimulation; therefore, IVM may be an alternative to IVF in PCOS women.

Trial registration number: not applicable.

P-633 Natural cycle cryopreserved embryo transfer: is a washout period needed after a failure of fresh embryo transfer?

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Study question: Does delaying modified natural cycle (mNC) frozen embryo transfer (FET) after a failed fresh embryo transfer (ET) improve the live birth rate (LBR)?

Summary answer: In mNC FET cycles, delaying FET after a failed fresh cycle does not improve the LBR.

What is known already: The optimal timing of FET after a failed fresh IVF-ET cycle is controversial. While some studies suggest that a "washout" period is unnecessary, others support the postponement of FET after a failed fresh cycle for at least one menstrual cycle, out of concern that there may be residual effects of the controlled ovarian stimulation, which may interfere with the subsequent cycle. Until now, published studies included only artificial FET cycles, and the use of the natural cycle, which is a popular and effective method for FET, has not been investigated.

Study design, size, duration: A retrospective cohort study reviewing all first FET cycles performed after failed fresh ET attempts, between 2009 and 2016 (n = 212).

Participants/materials, setting, methods: The study included patients aged 18-45, who underwent FET in a single university-affiliated IVF unit. Only mNC FET cycles were included, where hCG was used for ovulation triggering, and vaginal progesterone was given as luteal support. Cycles were divided according to the time interval between oocytes retrieval and FET into immediate FET group (<50 days) and delayed FET group (≥50 days). Clinical pregnancy was defined as fetal heart activity on ultrasound.

Main results and the role of chance: Overall, 127 patients had an immediate FET and 85 patients had a delayed FET. Mean age was 32.6 ± 5.1 and 34.0 ± 5.6 respectively (p = 0.06), and mean number of previous failed cycles was 3.3 ± 2.6 and 4.0 ± 3.0, respectively (p = 0.09). The immediate and the delayed FET groups did not differ with regard to LBR (22.8% vs. 14.1%, respectively, p = 0.11) or clinical pregnancy rate (27.6% vs. 20.0%, respectively, p = 0.21). On multivariate regression analysis, delaying mNC FET was not associated with live birth (aOR 0.56, 95%CI 0.25-1.22) or clinical pregnancy (aOR 0.61, 95%CI 0.30-1.24), after controlling for woman's age, infertility diagnosis, fresh ET protocol, and the number of embryos transferred. The study was powered to detect a 2-fold increase in the LBR, with alpha of 0.05 and power of 80%.

Limitations, reasons for caution: The study limitations include its retrospective design and small sample size. It was not sufficiently powered to detect a small difference in LBR.

Wider implications of the findings: These data should reassure patients and clinicians who desire to proceed with FET immediately after fresh IVF failure.

Trial registration number: IRB reference number 0220-17-WOMC

P-634 iTRAQ-based proteomic profile of ovarian granulosa cells from women with PCOS undergoing IVF/ICSI

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Study question: To investigate the differences of protein expression in ovarian granulosa cells between women with PCOS and the controls patients receiving IVF/ICSI.

Summary answer: This study reveals the differences of protein expression in granulosa cells from PCOS women and the controls undergoing IVF/ICSI.

What is known already: The pathogenesis of PCOS, the most common cause of ovulatory disturbance leading to infertility, is not clear, particularly with regard to proteins expressed in the ovarian granulosa cells from PCOS women. Proteomic analysis is a powerful technological tool for investigations of human diseases. iTRAQ is a quantitative method that has frequently been used in proteomic studies and demonstrated to be effective and accurate in characterizing various diseases. However, iTRAQ has not been applied in the proteomics profile of granulosa cells proteins from PCOS women undergoing IVF.

Study design, size, duration: Eight women undergoing IVF/ICSI with tube factor or male factor infertility were enrolled in this study. Four patients with PCOS and insulin resistance were included in PCOS group. The other four with regular ovulatory cycles, normal basal FSH and LH levels, and with 15 or more antral follicles were included as the control. Standard long GnRH agonist protocol was used in both groups. The dose of gonadotropin for ovarin stimulation is FSH 100-150IU/d.

Participants/materials, setting, methods: The granulose cells were collected soon after oocyte retrieval. The protein expression profile of each sample was detected by isobaric tags for relative and absolute quantitation (iTRAQ) technique coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The up- and down-regulated proteins were analyzed based on Gene Ontology and KEGG enrichment pathway. The proteins were analyzed in terms of molecular function, cell location and biological processes for finding the key protein molecules.

Main results and the role of chance: The estradiol levels in serum on hCG administration day were comparable in PCOS group and the controls. There was no difference in the number of ooccytes retrieved between the two groups. A total of 3556 protein were identified, among which 486 differentially expressed, including 406 up-regulated and 80 down-regulated protein in granulosa cells from women with PCOS. Gene ontology analysis showed that most differentially expressed proteins were located in the extracellular region.The differential expression proteins in PCOS groups and the controls were mainly related to the protein binding and catalytic activity of some enzymes, and play important role in the cellular process, metabolic process, biological regulation and regulation of biological process. KEGG enrichment analysis linked 373 differentially expressed protein to 27 different metabolic pathways, with most proteins involved in the metabolic pathways, biosynthesis of secondary metabolites, ribosome, and microbial metabolism in diverse environments.

Limitations, reasons for caution: The differentially expressed protein need to be verified by Western blot.

Study funding:This study was supported by grants from the National Natural Science Foundation of China (81200453), Science & technology department of Sichuan Province (2014KJT062-2014SZ0001), and Chengdu science and Technology Bureau (2015-HM01-00494).

Wider implications of the findings: These iTRAQ results provides new insights into the pathogenesis of PCOS, which may facilitate the discovery of novel therapeutic targets to treat female infertility associated with PCOS.

Trial registration number: None.

P-635 To compare the effect of metformin plus myoinositol vs metformin alone in terms of clinical pregnancy rate in infertile PCOS women

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Study question: Whether combination of metformin and myoinositol is better as compared to metformin alone in improving clinical pregnancy rate in infertile PCOS women undergoing ovulation induction.

Summary answer: Clinical pregnancy was significantly higher due to synergistic effect of metformin and mho-inositol as compared to metformin alone in infertile PCOS women undergoing ovulation induction.

What is known already: Polycystic ovary syndrome is the most common cause of anovulatory infertility. Metformin has been used for decades as either primary therapy or after failed ovulation induction in infertile PCOS women. Mechanism of action of metformin is through improving peripheral insulin resistance. Recently there is literature on use and function of inositols in PCOS women which have been shown to act at the ovarian level where it improves insulin resistance and improves oocyte quality. Myo-Inositol is the main type required for improving oocyte quality. But there is no consensus on dose, duration and any benefit of combining the two drugs.

Study design, size, duration: A randomised controlled trial was conducted with 120 infertile PCOS women (January 2016 to May 2017). Group I was the intervention group and received Metfromin 500 mg+ Myoinositol 600 mg TDS and Group II (n = 60) received Tab Metformin 500 mg TDS. The therapy was continued for total 6 months and couples were advised to try for natural

conception. Those who didn't conceive after 3 months, were given ovulation induction and intrauterine insemination.

Participants/materials, setting, methods: Baseline clinical and hormonal profile and infertility work up was done and patients were recruited according to inclusion criteria. Clinical and hormonal parameters were repeated at 3 months. Improvement in HOMA-IR was calculated after 3 months of therapy. Primary outcome was clinical pregnancy rate at the end of 6 months. Secondary objective was ovulation rate, ongoing pregnancy rate, OHSS, abortion rate, multiple pregnancy rate and improvement in clinical / metabolic profile of patients.

Main results and the role of chance: Baseline characteristics were comparable in two groups.After 3 months therapy, improvement in clinical and hormonal parameters were comparable in two groups except for improvement in menstrual cycle length and HOMA-IR index, which were significantly better in group I. more patients (14/60; 23.3%) conceived spontaneously in group I as compared to group II (8/60;13.3%), though the difference was not statistically significant($p = 0.15$). Overall conception rate was significantly higher in group I (38/60; 63.3%)) as compared group II (20/60; 33.3%) in Group II with $p = 0.001$. Five patients in group I and 1 patient in group II had multiple pregnancy. one patient in group I had early onset ovarian hyper stimulation syndrome (OHSS) and 4 had late onset OHSS. All late onset OHSS were due to multiple pregnancies. Group II had no case of OHSS. The ongoing pregnancy rate (POG>20 weeks) was 55% (33/60) in Group I and 26.67% (16/60) in Group II which was significantly higher in Group I ($p = 0.002$).

Limitations, reasons for caution: The main limitation was small sample size. Serum progesterone were not done on day 21 of cycles to document ovulation. More data on large sample size is required to document the synergistic effect of metformin and myo-inositol in terms of live birth rate in infertile PCOS women.

Wider implications of the findings: This was the first study to document better efficacy of combined use of metformin and my-inositol over metformin alone in infertile PCOS women. The two can be used to improve clinical pregnancy and ongoing pregnancy rate in infertile PCOS women undergoing ovulation induction cycles.

Trial registration number: CTRI/2017/07/009021

P-636 Convenience and efficacy of treatments for endometrial preparation prior to frozen embryo transfer (FET): a randomised-controlled trial comparing stimulated cycle (SC) vs natural cycle (NC)

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Study question: Can ovarian stimulation with rec-FSH used for endometrial preparation for women undergoing FET be more convenient (number of visits reduced) than natural cycle?

Summary answer: Endometrial preparation with rec-FSH decreases significantly the number of visits needed to schedule the FET, without impairing the ongoing pregnancy rate.

What is known already: Before frozen embryo transfer, it is necessary to ensure that the transfer is carried out at a time when the endometrium is receptive, defined as the "implantation window". Endometrial preparation can be performed by hormone replacement therapy, FSH stimulation, or close monitoring of a natural cycle. The natural cycle may appear more physiological but more burdensome as it needs more monitoring, and reduces flexibility for patients and the organization of centers. To date, no study compares the convenience of FSH stimulation compared to natural cycle for FET.

Study design, size, duration: Prospective open-labeled randomised controlled study in a public IVF center from may 2015 to oct 2017. To demonstrate a decrease of one visit with SC compare to NC, the sample size was calculated as 48 patients per group (two-sided alpha-error 0.05, power 90%), increased by 30% for cancellation rate, ie 62 patients per group and 124 patients in total.

Participants/materials, setting, methods: Women aged 18 to 38 y undergoing their first or second FET (day 2/3 embryos) were randomized to either NC with close monitoring by ultrasound and hormonal measurements beginning at day 12, or stimulation with rec-FSH 75 IU/day from day 6 to 11 and HCG triggering when leading follicle > 17 mm. The study compares number of visits (ultrasound and blood tests), progesterone levels before FET, HCG positive and ongoing pregnancy rates and spontaneous miscarriages.

Main results and the role of chance: 124 women were included, 62 in both groups. Mean age (+SD) 32.8+4.0 vs 33.9+3.3 (p 0.11) for SC and NC respectively, and other baseline characteristics, were comparable. The primary outcome, number of visits, was significantly lower in the SC group (3.7+0.9 vs 4.5+1.1, $p<0.05$). The number of blood tests (2,7+0.9 vs 3.5+1.1, $p<0.05$) as well as the number of ultrasound performed (1.1+0.4 vs 1.4+0.6, $p<0.05$) were also significantly lower in the SC group compared to NC. The number of FET during "non-opening" hours was lower in SC compared to NC but not statistically different (21.1% vs 28.3%, $p = 0.38$). The cancellation rate was also lower in SC compared to NC but not statistically different (8.1% vs 14.5%, p = 0,26). The mean progesterone level was significantly higher in SC 4.2+1.7 vs NC 3.2+1.2 ng/ml ($p<0.05$), measured 72 h before FET. The number of embryo transferred was comparable in SC and NC (1.30+0.5 vs 1.38+0.5, $p = 0.40$). The HCG positive rate and ongoing pregnancy rate per transfer were higher in SC compared to NC but not statistically different (31.6% vs 24.5%, $p = 0.41$ and 22.8% vs 20.7%, $p = 0.79$, respectively). The miscarriage rate was higher in the SC vs NC but not statistically different (21.7% vs 13.3%, $p = 0.51$).

Limitations, reasons for caution: Monitoring cycles with strict criteria in both groups limits the potential bias. The power does not allow us to conclude on pregnancy and cancellation rates. A medico-economic study will be provided as the higher cost with rec-FSH is balanced by the cost and constraints of more visits and cancellation.

Wider implications of the findings: Burden of treatment is a major issue for infertile patients. For women reluctant to have injections, NC is a good option but women must be aware of increased monitoring. SC enables to reduce monitoring and cancellation rates, and offers more flexibility for patients and IVF centers.

Trial registration number: N° EUDRACT: 2015-A00088-41 ClinicalTrials.gov ID: NCT02834117

P-638 Function of Lin28a/Akt/mTOR in regulating premature ovarian failure

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Study question: Does Lin28a play a role in regulating premature ovarian failure?

Summary answer: Overexpression of lin28a in oocyte can enhanced the activity of Akt/mTOR pathway, which may play a role in regulating premature follicle activation.

What is known already: Infertility is a prevalence of disease that besets 15% of couples in childbearing age around the world. Premature ovarian failure (POF), defined as primary ovarian defection and characterized as cessation of menstruation or absent function of ovarian follicles in women under the age of 40, accounts for approximately 1% of infertility disease in women at childbearing age. The study on the specific mechanism of how premature ovarian failure occurs remained further exploration. Lin28a is mRNA binding protein and highly expressed in embryogenesis. Akt/mTOR pathway is also widely reported to be involved in regulating follicologenesis.

Study design, size, duration: Litter size, oocyte number and follicle number of 2-month, 3-month, 4-month and 5-month old Lin28a and wild type mice were counted (n = 6). 100 Oocyte that can be visualized under the microscope was isolated from 3-month (n = 4) and 4-month (n = 4) Lin28a transgenic and wild type mice, and the expression of proteins downstream of the Akt/mTOR pathway including phosphorylated AKT (Ser 473), phosphorylated ribosomal s6 and phosphorylated S6K1 were analyzed by Western blotting and normalized by beta-actin.

Participants/materials, setting, methods: Mice: wild type (ICR), lin28a transgenic mice, female, the age is from 2-month old to 5-month old.

The superovulation result was determined by pregnant mare's serum gonadotropin (PMSG)

Mouse Vasa homologue (Mvh) was used as marker when performing immunohistochemistry to identify oocyte during follicle counting.

western blot was conducted to detect the protein level.

Main results and the role of chance: Lin28a transcripts were found to be highly expressed in the Lin28a mice ovaries. The immunofluorescent staining showed that Lin28a was mainly expressed in the cytoplasm of oocyte. Consistent with the decrease of oocyte number and litter size at 4-month old Lin28a transgenic mice, there was a decrease of primordial follicle and increased primary follicle number of Lin28a transgenic mice from 4-month old compared with wild type control. Expression of downstream molecule of Akt/mTOR pathway-phosphorylated S6 and p-Akt (Ser473) in the oocyte was significantly increased in 3-month and 5-month old Lin28a transgenic mice, which indicated that there may be an interplay of Lin28a/Akt/mTOR in regulating POF.

Limitations, reasons for caution: The technique for in vitro culture of primordial follicle is not available.

The human sample is not easy to be achieved.

Wider implications of the findings: Premature ovarian failure is an important factor that causes women infertility. However, current therapy for POF patient is estrogen treatment. In this study, we explore the functional roles of Lin28a and Akt/mTOR pathway to restore the ovarian reserve and open new treatment strategy to patient who suffered from POF.

Trial registration number: Not applicable.

P-639 Hyperglycemia creates an optimal environment for endometrial cells to exhibit resistance to metformin effects

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Study question: Evaluate the antiproliferative effect of therapeutic metformin doses on endometrial cells when exposed to normal and hyperglycemic environment, imitating PCOS and diabetes.

Summary answer: Endometrial cells exposed to high glucose levels exhibit resistance to anti-proliferative metformin effects, an effect potentially related to estrogen receptors expression.

What is known already: Endometrial hyperplasia predisposes for the development of endometrial cancer (EC). Women with PCOS are prone to endometrial hyperplasia due to anovulatory cycles with chronic elevated estrogen serum levels and an increased incidence of adipositas, knowns as an additional risk factor for EC development. Metformin, an insulin-sensitizing agent commonly used in the treatment of type II diabetes, is also used off-label in women with PCOS for ovulation induction. Recently, metformin treatment has been suggested as therapy to inhibit cellular overgrowth and hyperplasia in several tissues, however detail analysis of endometrial tissues under various conditions remain to be examined.

Study design, size, duration: This study is an *in vitro* experimental study using endometrial cell lines treated with metformin under different conditions for 7 days.

Participants/materials, setting, methods: Two different endometrial adenocarcinoma type I human cell lines (Ishikawa and HEC-1A cells) with different estrogen receptors expression were treated long-term (7 days) with low metformin doses (0 mM, 0.01 mM, 0.1 mM, 0.5 mM, 1 mM and 5 mM) cultured in an environment with normal or high glucose concentrations. Moreover, all groups were treated in the presence of 10^{-9} mM estradiol. The proliferation rate and colony formation were performed using crystal violet assay.

Main results and the role of chance: Metformin treatment shows dose-dependent manner effects in the colony formation of both cell lines, decreasing the number and size of colonies independent of the glucose levels present in the medium. Nevertheless, 5 mM metformin treatment showed expressive inhibition effect (demonstrative data). Additionally, in a normal glucose environment 0.5 mM, 1 mM and 5 mM metformin doses decreased the proliferation rate of HEC-1A cells 32% ($P = 0.05$), 38% ($P = 0.02$) and 55% ($P<0.01$), respectively. However, in the presence of high glucose levels, only the highest metformin treatment dose (5 mM) was able to decreased the proliferation rate of this cells (40 %; $P<0.01$), showing significant 15% ($P<0.01$) lower effect when compared to the cells treated in an environment with normal glucose levels. Moreover, this resistance was not observed in Ishikawa cells, where the range between 0.5 mM to 5 mM metformin dose decreased significantly the proliferation rate of this cells in normal (12%; $P<0.01$, 25%; $P = 0.04$ and 55%; $P = 0.02$ respectively) and high (18%; $P<0.01$, 37%; $P<0.01$ and 62%; $P<0.01$) glucose levels. In addition Ishikawa cells did not show different response to metformin effect when compared to cell cultured in normal glucose levels, suggesting that this resistance may be driven by estrogen receptors expression.

Limitations, reasons for caution: These results are regarding to *in vitro* cell line culture, therefore patient viability response are not measured.

Wider implications of the findings: Our data suggest that metformin may be a long-term treatment strategy for anovulatory patients at risk, such as PCOS women, preventing EC development. Furthermore, our results showed the importance of glucose metabolism for the EC treatment response.

Trial registration number: Not applicable.

P-640 The axis and location of male and female pronuclei affect both the determination of first cleavage plane and further development in human embryos

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Study question: Are the axis and location of male and female pronuclei involved in both forming the first cleavage plane in human embryos and their further development?

Summary answer: Both the axis and location of male and female pronuclei are involved in determining the first embryonic cleavage plane and affecting further development.

What is known already: Several studies have investigated how the site of the second polar body (2^{nd} PB) might be involved in plane formation in the first cleavage, with suggestions that the 2^{nd} PB position defines the first cleavage plane in human embryos. Thus far, these studies remain controversial. Recently, time-lapse monitoring has been used to monitor and analyze early-stage embryonic development in a clinical setting.

Study design, size, duration: From January to July 2015, we conducted time-lapse imaging (EmbryoScope®) for 40 hours under culture after intracytoplasmic sperm injection (ICSI) of 672 oocytes from couples with severe male factor or fertilization failure. Of the oocytes, 485 were normally fertilized, showing extrusion of the second polar body (2^{nd}PB) with male and female pronuclei (2PN); 361 of these that formed the first cleavage plane and developed to the 2-cell stage were analyzed for this study.

Participants/materials, setting, methods: Oocytes after ICSI were cultured in an EmbryoScope® and images were acquired every 15 minutes for 40 hours to analyze the relationship between axis and location of the cytoplasmic pronuclei and the first cleavage plane. A straight line connecting the pronuclei was defined as the '2PN axis". Based on the direction of first cleavage relative to the 2PN axis, embryos were further classified into two groups, parallel and perpendicular, for evaluation of further development.

Main results and the role of chance: First, we analyzed the relationship between the cytoplasmic 2PN location and the first cleavage plane. Of the 361 fertilized embryos, 208 produced suitable imaging, with 198 (95.2%) forming a cleavage furrow through or close to the 2PN location at first cleavage.

Second, we investigated the relationship between 2PN axis and the first cleavage plane in 315 of the 361 embryos showing suitable images; 275 (87.3%) formed a cleavage furrow parallel to the 2PN axis at the first cleavage, while the remainder ($n = 40$) formed a cleavage furrow perpendicular to the axis.

Third, we analyzed the relationship between 2PN axis and the first cleavage plane in the 24 (6.7%) of 361 embryos with pronuclei located at the cytoplasmic margin. Of those, 20 image sets were suitable for analysis, with 18 (90.0%) forming a cleavage furrow parallel to the 2PN axis at first cleavage.

Finally, the quality of embryos was determined based on the modified Istanbul Consensus. There were significantly more poor-quality embryos in the perpendicular group than in the parallel group (50.0% vs. 25.5%, $P < 0.01$). Furthermore, clinical utility rate was significantly lower in the perpendicular group than in the parallel group (42.5% vs. 69.1%, $P < 0.001$).

Limitations, reasons for caution: Since only ICSI zygotes were analyzed in this study, we could not investigate the influence of fertilization methods on subsequent development. Further studies including molecular biological analyses would help to define the mechanisms underlying determination of the first cleavage plane in human embryos.

Wider implications of the findings: We suggest that both the axis and the location of pronuclei are essential for determining the first cleavage plane and ongoing embryonic development because the 2PN axis might be involved in positioning the mitotic spindle poles.

Trial registration number: Not applicable.

P-641 Comparison of the effect of two combinations of myo-inositol and D-chiro-inositol in women with polycystic ovary syndrome who undergo ICSI

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Study question: Do high doses of D-chiro-inositol (DCI) in combination with myo-inositol (MIO) have any effect on pregnacy rate (PR) in women with PCOS who undergo ICSI?

Summary answer: The combination of MIO with high dose of DCI achieved a higher PR in women with PCOS who undergo ICSI.

What is known already: A recent meta-analysis indicates that MIO supplementation is not sufficient to improve oocyte or embryonic quality or the percentage of pregnancies in women with PCOS who undergo ICSI, opening the possibilities to the combination with DCI. On the other hand, the evidence with the use of DCI is scarce and heterogeneous, especially in relation to the dose.

Study design, size, duration: A multicenter controlled, randomized, double-blind, parallel-group study with two MIO-DCI formulations for 12 weeks. Study group (SG): 550 mg MIO + 150 mg DCI (Caronositol®) twice daily; Control group (CG): 550 mg MIO + 13.8 mg DCI 2 times daily.

Participants/materials, setting, methods: Inclusion criteria were: women meeting Rotterdam criteria for PCOS with BMI <30 who undergo ICSI. Sixty women with PCOS were recruited, four women were excluded after randomization and fifty six started the treatment. Three of them did not complete the intervention, two due to personal reasons (one in each group), and one woman in the CG did not complete the study due to an accident. No baseline differences were observed between the two groups.

Main results and the role of chance: At the end of the 12-week study, number of MII oocytes and percentage of good-quality embryos were also similar in both groups. However, the pregnancy and live birth rates were significantly higher in the SG than in the CG (65.5 vs 25.9, $p = 0.003$ and 55.2 vs 14.8, $p = 0.002$). Respect testosterone and insulin sensitivity, a significant improvement was found from baseline to end of the study in both groups.

Limitations, reasons for caution: Results should be taken with caution due to the short sample size.

Wider implications of the findings: A combination of 550 mg MIO + 150 mg DCI twice daily could improve pregnancy and live birth rates in women with PCOS who undergoing ICSI.

Trial registration number: NCT03201601

P-642 All you need to you know about resistant ovary syndrome: a systematic review and foundation for future research

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Study question: What is the current standpoint in research and clinical management of resistant ovary syndrome (ROS)?

Summary answer: ROS presents a heterogeneity of etiologies (mutations and antibodies) and phenotypes. Hormonal therapies and in-vitro maturation (IVM) have led to pregnancies resulting in live-births.

What is known already: ROS is a rare disorder characterized by a normal amount of follicles that are arrested in development despite a high level of FSH. Patients thus have a resistance to FSH. This leads to infertility. According to Grynberg (2013), ROS is frequently misdiagnosed as premature menopause. This is because ROS is largely unknown to physicians. Indeed, there are over a hundred papers reporting ROS cases but, remarkably, there is no review on the presentation of ROS and its treatment options. Multiple hypotheses on the etiology of ROS have been made. But an overview of these hypotheses and the evidence found lacks.

Study design, size, duration: We conducted a systematic review of all published articles discussing ROS. We searched for papers on PubMed, Cochrane and Embase. The final search was performed on 26 January 2018. We also identified articles through cross-referencing. We then critically reviewed all the publications found.

Participants/materials, setting, methods: We included all articles ever published concerning women with the characterizing features of ROS, namely oligo- or amenorrhea, post-menopausal FSH level, a normal amount of follicles and an arrest in folliculogenesis. All papers regarding FSH receptor (FSH-R) mutations in women were also included. We excluded cases of subjects with a karyotype other than 46-XX. We also excluded all articles in which neither the abstract nor the text was in English, French, Dutch, Spanish or German.

Main results and the role of chance: From the articles that we found, we drew the typical clinical picture of ROS. Along with the characterizing features of ROS, patients have normal internal and external genitalia. Besides, most patients have normal secondary sex characteristics that developed on time. At the time of diagnosis, patients mostly have either primary or secondary amenorrhea, which is often preceded by oligomenorrhea. Accompanying elevated FSH, LH is often increased and estradiol is usually decreased. Multiple papers reported treatment attempts. Outcomes varied from absence of ovarian response to pregnancies resulting in at-term births of healthy babies. The most promising treatments used were clomiphene, estrogen, hMG with or without hCG, combinations of estrogen and progesterone, and, recently, IVM. ROS could be explained by a mutation in the FSH-R gene or in a molecule regulating the FSH-R or involved in the pathway downstream of FSH-R activation. Another possible cause is antibodies against FSH, against FSH-R or against a molecule involved in its regulation or the downstream pathway. The absence or presence of several of these causative factors, along with the associated cellular disturbances, has been shown in multiple ROS patients. The heterogeneity in causes and phenotypes and in particular the relationship between these two is fascinating.

Limitations, reasons for caution: It is likely that many cases were not published, especially those who did not respond to treatment (Vaitukiatis 1986). Additionally, the search and analyzing work of this review were performed by only one person. Finally, a few articles could not be taken into account due to our language restrictions.

Wider implications of the findings: This study is the only existing review on ROS. It provides a clear view on the current standpoint in clinical management of ROS. It also reports what is known on the etiology of ROS and sets the path for future research, which must aim for treatment improvement.

Trial registration number: not applicable.

P-643 Higher menopausal age but no differences in parity in women with polycystic ovary syndrome as compared to controls

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Study question: To study the menopausal age in women with polycystic ovary syndrome (PCOS) as compared to women without PCOS.

Summary answer: Menopause is clearly delayed in women with PCOS and that is also reflected in lower FSH levels.

What is known already: Models with anti-Müllerian hormone predict a later menopause in women with PCOS. A cross-sectional study using questionaires showed a later menopause in women stating that they have PCOS diagnosis.

Study design, size, duration: A prospective cohort study of women with PCOS compared to age-matched, randomly selected controls. Twenty-seven women, diagnosed with PCOS in 1992 were re-examined in 2016. Of the initial cohort of thirty-three, five had died and one declined participation. Controls were recruited from another study, these women were initially examined 1995 and re-examined in 2008. 94 controls were included.

Participants/materials, setting, methods: The PCOS women (mean age 52.4 years, range 42-60 years) were diagnosed 24 years ago. They all met Rotterdam criteria. Age-matching was used to derive a control group from women randomly selected from the same geographic area. PCOS n = 27. Controls n = 94. The women were examined using structured interviews, anthropometric measures, blood pressure, and blood tests.

Main results and the role of chance: Among women with PCOS, 37% stated that they were postmenopausal, compared to 57% of the controls (ns). The mean menopausal age in women with PCOS (53.3 years, n = 7) was significantly ($p<0.01$) higher than in controls (49.3 years n = 52). Serum-FSH levels were significantly ($p = 0.02$) lower in PCOS (31.7 IU/L) than in controls (50.1 IU/L). There was no difference in parity between women with PCOS (1.9 ± 1.3 children) compared to controls (1.7 ± 1.0 children).

Limitations, reasons for caution: The numbers of women that could state their menopausal age were lower in the PCOS group, mainly because a bigger proportion of them had not yet become postmenopausal as compared to controls despite the age-matchning.

Wider implications of the findings: The results supports previous prediction models. The prolonged ovarian hormonal activity might be a protective factor for cardiovascular events later in life in women with PCOS.

Trial registration number: not applicable.

P-645 Comparative analysis of structural differences of Ovaleap and GONAL-f: site-specific glycosylation mapping and cell signaling activation

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Study question: Could differences in site-specific glycosylation between biosimilar (Ovaleap®) and originator (GONAL-f®) recombinant-human follicle-stimulating hormone (r-hFSH) impact calcium (Ca^{2+})-mediated cell signalling activation with these treatments?

Summary answer: Site-specific glycosylation differences between Ovaleap and GONAL-f, including greater N-glycolylneuraminic acid content with Ovaleap, may impact FSH-mediated intracellular Ca^{2+} increase and the potential for immunogenicity.

What is known already: It is well known that the glycosylation profile of FSH can affect the molecule's receptor-binding efficacy, half-life and clearance, all of which have an effect on the overall biological activity of FSH. In particular, the glycosylation profile at asparagine 52 of the alfa chain (αAsn-52) is known to affect FSH receptor binding and signal transduction. Differences in manufacturing processes can lead to differences in the glycosylation patterns between biosimilar and originator recombinant FSH preparations, including the amount of non-human N-glycolylneuraminic acid that is incorporated, which may have safety implications because N-glycolylneuraminic acid can increase the risk of immunogenicity in humans.

Study design, size, duration: Glycopeptide mapping and Ca^{2+}-mediated cell-signaling activation were assessed in three batches of Ovaleap (S06622, S27266, R38915) and three batches of GONAL-f (199F005, 197F049, 197F051). The Ovaleap batches had 4–11 months until expiry and the GONAL-f batches had 5–7 months until expiry.

Participants/materials, setting, methods: Glycopeptide mapping was performed after chymotryptic digestion and separation with 0.1% TFA in acetonitrile/water on a hydrophilic interaction liquid chromatography column using Acquity UPLC coupled to a Xevo G2-S QTof Mass Spectrometer (Waters, Milford, MA, USA). Intracellular Ca^{2+} increase with 10 nM or 100 nM of the three batches of Ovaleap was compared with the increase with equimolar doses of GONAL-f in FSH receptor-transfected HEK293 cells using a bioluminescence resonance energy transfer assay.

Main results and the role of chance: Site-specific glycosylation mapping revealed differences in glycosylation patterns between Ovaleap and GONAL-f. In particular, the batches of Ovaleap had greater sialylation at all N-glycosylation sites than GONAL-f (Z-number range (Ovaleap vs. GONAL-f): αAsn-52: 178.1–183.8 vs. 173.9–176.0; αAsn-78: 183.7–188.5 vs. 175.6–178.6; βAsn-7: 305.6–311.0 vs. 284.7–290.9; βAsn-24: 211.6–219.5 vs. 184.9–189.2). N-glycolylneuraminic acid content was also higher in batches of Ovaleap, with only trace amounts of N-glycolylneuraminic acid present on GONAL-f (ranges Oveleap vs. GONAL-f: 2.6–4.7% vs. 0.0–0.4%). Differences in the potential for Ca^{2+}-mediated cell-signaling activation were observed between Ovaleap and GONAL-f. Although 10 nM and 100 nM GONAL-F, as well as the positive control thapsigargin, induced a 200–300-fold increase of intracellular Ca^{2+} compared with baseline, equimolar doses of all the three batches of Ovaleap failed to induce an increase in Ca^{2+}. It can be speculated that the differences in Ca^{2+}-mediated cell-signaling activation may be the result of the differences in the glycosylation profiles observed between the FSH preparations.

Limitations, reasons for caution: These analyses were performed *in-vitro*. The potential impact and clinical relevance of the glycosylation differences on efficacy and risk of adverse events cannot be confirmed from these findings.

Wider implications of the findings: Differences in glycosylation among FSH preparations have the potential for profound effects on the efficacy and safety of this treatment and these require further investigation using *in vitro* models and clinical settings.

Trial registration number: Not applicable.

P-646 Further characterization of a newly described hypo-androgenic PCOS-like phenotype based on adrenal and ovarian hormone profiles

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Study question: How do hormone profiles in women with the recently described hypo-androgenic PCOS-like phenotype (H-PCOS) differ from controls?

Summary answer: They differ significantly in almost all hormones of adrenal and ovarian origins.

What is known already: Women with H-PCOS are lean, characterized by excessively high AMH values for age and/or in comparison to FSH levels, by low testosterone (T), low dehydroepiandrosterone-sulfate (DHEAS) and high prevalence of autoimmunity (Gleicher et al; J Steroid Biochem Mol Biol 2017;167:144-152). They at young ages are hyper-androgenic and, likely, demonstrate mild hyper-cortisolism but in late 20 s to early 30 s, suddenly, become hypo-androgenic and, often, show mild hypo-cortisolism (Gleicher et al., Endocrine 2018; doi:10.1007/s12020-017-1498-8).

Study design, size, duration: This was a prospective study involving 7 H-PCOS patients and 9 controls.

Participants/materials, setting, methods: We prospectively investigated 3 young oocyte donors defined as H-PCOS, 4 control donors, 4 older H-PCOS infertility patients undergoing IVF, and 5 control patients in early follicular phase for cortisone, cortisol, corticosterone, 11-deoxycortisol, androstenedione, T, 17-OH-progesterone, progesterone, DHEA and Cyp17(17-0 P/P). Bloods were obtained and frozen at CHR, and transported de-identified and coded to the

NICHD for analyses by ultra-filtration LCMS/MS (Stolze et al., J Steroid Biochem Mol Biol 2016;162:110-116). Statistical analyses were performed at CHR.

Main results and the role of chance: H-PCOS donors and patients were of similar ages as controls (24.0 ± 2.6 vs. 26.8 ± 7.6 and 39.5 ± 7.6 vs.37.8 ± 6.1 years), had significantly higher AMH than controls (8.2 ± 1.9 vs.2.6 ± 07 and 8.1 ± 3.3 vs.1.2 ± 0.9 ng/mL). In non-parametric statistical analyses, H-PCOS donors did not differ from control donors in any of the other hormones. H-PCOS patients, however, demonstrated lower cortisol (P = 0.02), corticosterone (P = 0.04) and 11-deoxicortisol P = 0.04). Comparing H-PCOS donors to older H-PCOS patients no hormonal differences were apparent, while comparing normal control donors and patients, androstenedione was lower in patients than donors (P = 0.02), as was testosterone (P = 0.04) and DHEA (P = 0.04). Adjusting for AMH values, H-PCOS donors had higher corticosterone (P = 0.007), 11-deoxicortisol (P = 0.003), progesterone (P = 0.007 and Cyp17 (P = 0.021), while older H-PCOS patients demonstrated lower cortisol (P = 0.045), corticosterone (P = 0.046) but higher androstenedione (P<0.0001), higher testosterone levels (P = 0.0002) and higher Cyp17 (P = 0.04). Comparing young H-PCOS donors with older H-PCOS patients, donors had higher cortisone (P = 0.03), cortisol (P<0.0001), testosterone (P = 0.01), 17-OHP (P = 0.04) and DHEA (P = 0.02), while among controls, donors had higher cortisone than patients (P = 0.03), cortisol (P<0.0001), androstenedione (P = 0.001), testosterone (P = 0.03) but lower corticosterone (P = 0.03), 11-deoxicortisol (P = 0.04).

Limitations, reasons for caution: Considering the small number of study subjects, the data presented here have to be viewed as preliminary.

Wider implications of the findings: These data reaffirm at all ages significant differences in adrenal and ovarian function between H-PCOS-like phenotypes and controls, supporting a previously reported strong interdependence between adrenal and ovarian steroid hormone secretion (Gleicher et al; J Steroid Biochem Mol Biol 2017;167:144-152; Gleicher et al., Endocrine 2018; doi:10.1007/s12020-017-1498-8).

Trial registration number: not applicable.

P-647 Serum AMH concentration has limited prognostic value for follicle density of primordial and primary follicles questioning AMH as a parameter for the real ovarian reserve

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Study question: What is the prognostic value of Anti mullerian hormone (AMH) to estimate the ovarian density of ovarian primordial and primary follicles?

Summary answer: The prognostic value of AMH to estimate ovarian follicle density is low due to poor correlation of both parameters.

What is known already: AMH is a predictor for ovarian response in IVF therapies. AMH is also used as a marker of ovarian reserve to predict the risk of premature ovarian insufficiency (POI) in gonadotoxic therapies and to estimate the amount of cryopreserved ovarian tissue needed for transplantation.

However, the accuracy of AMH to estimate the ovarian response is limited. Furthermore, the concentration of AMH poorly correlates with the risk of developing POI after gonadotoxic therapies. The reasons for the limited use of AMH as a prognostic factor are poorly understood.

Study design, size, duration: 823 females (29.5 y [23.3; 33.1]) underwent ovarian tissue freezing before gonadotoxic therapy within the network *Ferti*PROTEKT 09/2011 – 08/2016. Based on the analysis and correlation of histologically determined follicle density (three standardized biopsies per ovary, analysed after tissue digestion) and serum AMH concentrations (analysed by the same ELISA assay) the prognostic accuracy of AMH to estimate ovarian follicle density was evaluated.

Participants/materials, setting, methods: Women with ovarian surgery and genetic causes of low ovarian reserve were excluded. First, the crude and adjusted associations between AMH and follicle density were assessed using regression analysis, adjusting for age and disease category. Second, regression models were used to predict follicle density as a function of (i) AMH concentration, (ii) AMH concentration and women age. In order to discuss the accuracy of the derived predictions the 50 and 95% prediction intervals were calculated.

Main results and the role of chance: AMH concentrations and follicular density are significantly correlated (P<0.001). However, the correlation coefficient of 0.26 reveals an only moderate relationship between the two variables. Accordingly, the prediction intervals of follicle density are rather wide.

Regression analysis did not reveal a significant effect of disease category on follicle density whereas a relationship was found between age and follicle density in a quadratic regression analysis. Modelling the follicle density as a function of both AMH and age the predicted follicle density of women aged 10, 20, 30 and 40 y and the associated 50 and 95% prediction intervals were derived:

$$\log10(\text{follicle density}) \sim 1.62 + 0.247 \times \log10(\text{AMH}) - 0.049 \times \text{age}/10 - 0.067 \times (\text{age}/10)^2$$

For instance, in women with an AMH concentration of 2.0 ng/ml, the predicted density was 38.2 per mm^3 at the age of 10 y (50% prediction interval (PI): 18.7;78.1). The density decreased to 21.4 follicles per mm^3 at the age of 20 y (50% PI: 10.6;43.4), to 8.8 at the age of 30 y (50% PI: 4.4;17.9) and to 2.7 at the age of 40 y (50% PI: 1.3;5.4). The large prediction intervals highlight the fact that knowing the patient's age and AMH value is not sufficient to reliably predict the follicle density.

Limitations, reasons for caution: Even though three biopsies were taken from each ovary to increase the accuracy of the analysis, some inaccuracy cannot be excluded.

Wider implications of the findings: As the data provide evidence for the limited prognostic value of AMH for the density of primordial and primary follicles, AMH as a parameter for the real ovarian reserve has to be questioned. Ovarian biopsies might be a better tool to estimate the amount of ovarian tissue needed for transplantation.

Trial registration number: Not applicable.

P-648 Centrosome and spindle organization variations in mouse oocytes matured in vitro under different conditions do not correlate with developmental competence after fertilization

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Study question: Is maturation, fertilization and development of mouse oocytes differentially affected by addition of follicular fluid from adult (AFF) or prepubertal (PFF) goats to maturation medium?

Summary answer: In vitro maturation conditions resulted in significant variations in centrosome-spindle organization in metaphase-II oocytes, but embryonic development after fertilization was not correlated with these variations.

What is known already: Addition of intraspecific follicular fluid to maturation medium improves both nuclear and cytoplasmic maturation of oocytes in most species, but the effect of interspecific follicular fluid has not been investigated in mouse oocytes. In this species, centrosome and spindle organization has been shown to differ between in vivo and in vitro matured oocytes and among oocytes matured under different in vitro conditions. These variations have been suggested to be related to differences in developmental competence among these oocyte populations. This led to the proposal that centrosome-spindle organization characteristics could be considered as potential biomarkers for oocyte quality.

Study design, size, duration: We analyzed spindle and centrosome organization in metaphase-II mouse oocytes matured in vitro (IVM) for 16-18 h in the presence of 10% fetal bovine serum (FBS, n = 97), AFF (n = 106) or PFF (n =

96), using in vivo ovulated oocytes (IVO) as controls (n = 70). Fertilization and development to the blastocyst stage were also recorded after in vitro fertilization for 3 h and culture for 120 h (IVO n = 123, IVM-FBS n = 114, IVM-AFF n = 96, IVM-PFF n = 114).

Participants/materials, setting, methods: Mouse B6CBAF1 cumulus-oocyte complexes matured in the presence of FBS, AFF or PFF and control IVO oocytes were fixed, immunostained for alpha-tubulin, pericentrin and chromatin, and observed under an epifluorescence microscope to evaluate nuclear maturation status, spindle dimensions and morphology, and number and configuration of cytoplasmic and spindle-pole centrosomes. In a second set of experiments, IVM and IVO oocytes were fertilized in vitro and fertilization and blastocyst rates were determined.

Main results and the role of chance: Nuclear maturation rates were equivalent among IVM groups (70-82%). In contrast to fusiform spindles with pointed poles and solitary pericentrin foci found in IVO oocytes, IVM-FBS and IVM-AFF oocytes exhibited barrel-shaped spindles with wider poles (p<0,0001) and multiple pericentrin foci in 91-96% of them (p<0,0001). IVM-PFF oocytes showed an intermediate phenotype between IVO and the other two groups of IVM oocytes, displaying rhomboid-shaped spindles with an intermediate pole width and multiple pericentrin foci in 70% of them (p<0,0001). Overall, a significant decrease in spindle length (except for IVM-FBS), and a significant increase in spindle width and area (except for IVM-PFF) was evident as a result of IVM. Regarding cytoplasmic centrosomes, IVM oocytes consistently exhibited lower numbers of cytoplasmic pericentrin foci (9-10) than IVO oocytes (15; p<0,001) and a more frequent presence of pericentrin-positive foci in the polar body (22-25% vs 3%; p = 0,0001). Despite the differences in centrosome-spindle organization among the IVM groups and between IVO and IVM oocytes, fertilization rates were equivalent among all of them (90-93%) and blastocyst rates were consistently lower in IVM (41-47%) than in IVO oocytes (90%; p<0,0001). Thus, the differential effect of AFF and PFF in centrosome-spindle organization was not correlated with developmental potential.

Limitations, reasons for caution: Although morphological and morphometrical characteristics of the spindle and spindle-pole centrosomes in IVM-PFF oocytes more closely resembled those of IVO oocytes, compared with IVM-AFF or IVM-FBS oocytes, the spindle-centrosome organization of IVO oocytes was not fully recapitulated. Moreover, other maturation deficiencies may account for their lower developmental potential after fertilization.

Wider implications of the findings: Our data support previous results showing that IVM conditions affect centrosome-spindle organization in mouse oocytes. However, contrary to some of these reports, partial recapitulation of this organization did not translate into an improved development after fertilization. Thus, centrosome-spindle organization characteristics alone may not constitute suitable biomarkers for oocyte quality.

Trial registration number: Not applicable.

P-649 Comparison of oocyte morphology and embryo quality among women with different polycystic ovary syndrome phenotypes

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Study question: Does polycystic ovary syndrome (PCOS) phenotype influence oocyte morphology and embryo quality?

Summary answer: Women with different PCOS phenotypes exhibited similar oocyte and embryo quality following controlled ovarian stimulation.

What is known already: In PCOS, phenotype A (or full phenotype combining hyperandrogenism (HA), oligo-anovulation (OA) and polycystic ovarian morphology (PCOM)) and phenotype B (HA+ OA) are considered as the more severe phenotypes. Indeed, these phenotypes are associated with higher risk of comorbidities. Additionally, it has recently been demonstrated that PCOS phenotype A and B are associated with lower clinical pregnancy rate (CPR) as compared to control patients in IVF/ICSI cycles.

Consequently, oocyte quality is suspected to be impaired in cases of combination of OA and HA. However, little is known about oocyte quality and embryo quality among women with different PCOS phenotypes.

Study design, size, duration: In this retrospective study, we have analyzed data collected from women undergoing ICSI cycles between 2006 and 2015. We identified 109 PCOS patients. According to their phenotype, patients were split into 4 subgroups: PCOS A (PCOM + HA + OA, n = 41); PCOS C (PCOM + HA, n = 31); PCOS D (PCOM + OA, n = 37). As only one patient was diagnosed with PCOS phenotype B, therefore the patient was excluded.

Participants/materials, setting, methods: Oocyte quality was the primary outcome and was assessed using morphological criteria. The following abnormalities (fragmented first polar body, abnormal zona pellucida, large perivitelline space, material in perivitelline space, abnormal shape of oocyte, granular cytoplasm and intracytoplasmic vacuoles) were recorded. A total of 602 metaphase II (MII) oocytes for the phenotype A, 462 MII for the phenotype C and 432 MII for the phenotype D were compared. Secondary outcomes included embryo quality and reproductive outcomes.

Main results and the role of chance: After controlling for confounders in logistic regression analysis, our results show that the mean numbers of total and MII oocytes rates did not differ significantly between the three phenotypes. No significant difference was found in the oocyte quality between the three groups. Fertilization rates were similar between PCOS women with phenotype A, C and D (61.8%, 59.1% and 59.2% respectively, p = 0.93). In addition, the percentage of top quality embryos obtained on day 2-3 did not significantly differ regardless of the PCOS phenotype (49.8%, 50.9% and 51.7% respectively, p = 0.86). No difference was found in the implantation rate (31.5%, 28.8% and 32.1% respectively, p = 0.84), CPR (49.1%, 51.3% and 45.5% respectively, p = 0.73) and live birth rate (35.8%, 41.0 and 39.4% respectively, p = 0.51) between the 3 groups. Altogether, our findings suggest that PCOS phenotypes have no impact on the oocyte and embryo quality or the reproductive outcomes following ICSI attempts.

Limitations, reasons for caution: This is a retrospective study with inherent limitations. The main bias is represented by the phenotypic distribution in our study population with only one patient diagnosed with PCOS phenotype B. Indeed, an excessive serum anti-Müllerian hormone level is used as a surrogate for PCOM criterion in our centre.

Wider implications of the findings: In this novel retrospective study, we compared oocyte morphological quality and embryo quality in three different phenotypes of PCOS patients. These findings suggest for the first time that different PCOS phenotypes have no impact on oocyte and embryo quality.

Trial registration number: not applicable.

P-650 Women's perceptions regarding early menopause eHealth resources to facilitate self-care

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Study question: What are women's needs and perspectives regarding early menopause (EM) eHealth resources?

Summary answer: Most women with EM have access to multiple electronic devices and are supportive of a comprehensive co-designed EM eHealth website or App with multiple features.

What is known already: Provision of evidence based information and resources are integral to high quality collaborative patient centred care, improved patient experience, promotion of best practice and optimal health outcomes. Consumer and health professional knowledge gaps regarding EM exist and potentially contribute to the observed delayed diagnosis, variation in management, dissatisfaction with care, non-compliance with cancer treatment, poor risk perception and poorer outcomes. With continuing increases in digital device ownership, women are seeking electronic resources to

facilitate knowledge access and enhance self-health management, yet current EM related eHealth resources are either lacking or are inadequate for women's needs.

Study design, size, duration: A cross-sectional study of 386 Australian women with EM recruited between May and November 2017. The study was approved by the Monash Health Human Research Ethics Committee.

Participants/materials, setting, methods: Women with a self-reported diagnosis of EM, recruited from hospital clinics or the community, completed an online or paper survey. Exclusion criteria were no formal EM diagnosis or non-Australian residence (n = 123). Data collection included: demographics, medical history, current use of electronic resources to manage health/EM, support for an App, desired features of the ideal App and EM information topics. Data analysis included descriptive statistics (mean ±standard deviation) and logistic regression.

Main results and the role of chance: Of 263 women, the mean age was 53.81(±10.68) with EM diagnosed at age 38.54 (±5.27). Most participants were diagnosed with EM ≥5 years ago (78%), lived in metropolitan areas (55%) and had post-school qualifications (71%). Reported cause of EM was surgical removal of ovaries (30%), unknown (29%), cancer therapy (26%), or autoimmune/genetic/metabolic (14%). Most women had a smartphone (84%) and 98% of those owned multiple electronic devices. Women reported they would prefer a website (59%) to an App (38%) to manage EM with only 37% reporting current App use to manage health and 2% to manage EM. However, 67% women thought an App would be helpful and 45% would be likely to use it. Future menopause App use was less likely with increasing age (OR, 0.96; CI, 0.93-0.99, p = 0.008) but there were no significant associations with residential location, education, time since diagnosis, or cause of EM. Features considered very important/essential to include in an EM App were: evidence-based information (81%), question prompt list (78%), opportunities to ask an expert (76%) and ability to record symptoms/ health measures (67%). EM topics rated very important/essential to include (>80% respondents) were: diagnosis, symptoms, physical/psychological effects, long term implications, hormonal/non-hormonal management and lifestyle changes.

Limitations, reasons for caution: Potential response bias in relation to age/self-reported diagnosis of EM. These findings may be less relevant to non-English speakers, women with lower literacy/ educational attainment and those without internet access.

Wider implications of the findings: The results of this study will help development of high quality eHealth resources aiming to improve self-care and health outcomes. Further research is required to address limitations, and co-develop resources, including user testing and evaluation.

Trial registration number: Not applicable.

P-651 AMH variability and its implications for the number of oocytes retrieved following individualized dosing with follitropin delta

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Study question: Does AMH intra- and inter-menstrual cycle variability influence the number of oocytes retrieved after individualised follitropin delta dosing based on AMH and body weight?

Summary answer: For 95% of women, the implication of AMH variability on ovarian response using individualized follitropin delta dosing is limited to ±1 oocyte.

What is known already: The intra-class correlation coefficient for AMH (age adjusted ICC 0.89, 95%CI 0.84-0.94) was higher when compared to antral follicle count (ICC 0.71, 95%CI 0.63-0.77) when measured across four cycles (Van Disseldorp et al 2010). Within the menstrual cycle AMH exhibits minimal variation. The dose of follitropin delta (Rekovelle®) is based on AMH and body weight where AMH is measured on a random day of the menstrual cycle. Whether variability in AMH across and between menstrual cycles has a clinically relevant impact on the number of oocytes retrieved is unknown.

Study design, size, duration: Cohort study of 1,326 women (aged 18-40 years) participating in a prospective randomised controlled trial undertaken in 11 countries and 37 participating IVF centres, with central AMH measurements (Nyboe Andersen and Nelson et al, 2017).

Participants/materials, setting, methods: Women had serum AMH measured twice: at a random day at screening and at cycle day 2-3 prior to stimulation by centralised laboratory using the Elecsys AMH Plus immunoassay. The variability across the cycle was assessed by analysis of variance with log-transformed AMH ratio. The model underlying the dosing algorithm was used to calculate the predicted number of oocytes based on the doses determined by the AMH values at screening and start of stimulation.

Main results and the role of chance: There was a strong correlation between AMH measured on a random day of the cycle up to 3 months previously (screening AMH) and at the start of stimulation (cycle day 2-3) (r = 0.92). The variation in AMH within-subjects between screening and start of stimulation was limited (coefficient of variation 0.23). This variability was not attributable to measurement on a random day of the menstrual cycle. There was no evidence that AMH varied across the menstrual cycle (p = 0.08). If the dose of follitropin delta had been based on the AMH at start of stimulation instead of AMH at screening this would have had a minor impact on the predicted number of oocytes retrieved. For those women with an anticipated lower response (AMH<15pmol/l) the number of oocytes retrieved would be identical in 97% of women, and for those with an anticipated higher response (AMH≥15pmol/l) the number of oocytes would be at most ± 1 oocyte in 90% of women, with a further 9% attaining ± 2 oocytes. Overall for 95% of women, the implications of AMH variability on ovarian response using individualized follitropin delta dosing is limited to + 1 oocyte.

Limitations, reasons for caution: A centralised AMH measuring laboratory was used which may give rise to reduced AMH variability. AMH measurements were compared with samples taken within 3 months prior to ovarian stimulation, longer time intervals may have shown different variability due to the age-related decline in AMH.

Wider implications of the findings: An AMH measured on a random day of a menstrual cycle prior to commencing ovarian stimulation can be used to individualise the dose of follitropin delta. The variability in AMH within and between menstrual cycles does not have a clinically important impact on the number of oocytes retrieved.

Trial registration number: NCT01956110

P-652 The Bologna criteria patient and dual stimulation for IVF using long-acting FSH - a cohort study

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Study question: What is the clinical outcome of a patient friendly double stimulation with corifollitropin alfa in a cohort of poor responders, fulfilling the Bologna criteria.

Summary answer: Significantly more oocytes were retrieved during luteal phase stimulation (DuoStim 2) compared to follicular phase stimulation (DuoStim 1).

What is known already: Bologna criteria poor responder patients have a reported live birth rates ranging from 5.5% to 11.7% per cycle started and the crucial parameter is the number of oocytes available. Different combinations of follicular and luteal phase stimulation have been suggested and the overall advantage of the double stimulation is the possibility of retrieving more oocytes within a short period of time having a lower risk of no oocytes retrieved or no embryos available for a consecutive frozen embryo transfer.

Study design, size, duration: In this retrospective cohort study 81 patients were identified in the prospective clinical database from August 2015 to December 2017 planned for DuoStim. Fifty-four patients completed both DuoStim 1 and DuoStim 2 fulfilling the Bologna criteria.

Participants/materials, setting, methods: Patients were treated in a public fertility clinic. Both stimulations were fixed GnRH-antagonist protocol starting on the second or third cycle day using 150 mg corifollipin-α. GnRH-antagonist 0.25 mg daily was introduced on the 5th stimulation day and continued until the day of ovulation trigger. Additional exogenous gonadotropin was added from the 5th or 6th stimulation day and ovulation was triggered with GnRH-agonist followed by "freeze-all". Between the two stimulations there was a four-day pause.

Main results and the role of chance: The average age of the cohort was 36.7 years (SD 3.6; range 24-42) with a mean antral follicle count of 4.4 (SD 1.9). A total of 52 (96%) patients had previously performed at least one oocyte retrieval with less than four oocytes retrieved. Prior to DuoStim 1, patients had a mean number of 2.8 (SD 1.6) failed cycles. Besides the diagnosis of POR, a total of 45 patients (83%) also had a secondary fertility diagnosis.

The mean number of oocytes retrieved in DuoStim 1 and DuoStim 2 was 2.4 (SD 2.1) and 3.7 (SD 2.6) respectively, hence, a total of 1.2 (95% CI 0.46 – 1.96) more eggs were retrieved in DuoStim 2 compared to DuoStim 1, P = 0.002. Neither the number of embryos frozen nor the cancellation rate was significantly different comparing DuoStim 1 to DuoStim 2.

Only 6% (3/54) patients did not have any oocytes retrieved at all. The overall cancellation rate after retrieval was 41% (22/54) meaning that no top-quality embryo was frozen after both stimulations.

A total of 32 patients went through at least one embryo transfer. The overall ongoing pregnancy rate of the cohort was 20 % (11/54). Four patients still have spare embryos in the freezer.

Limitations, reasons for caution: The drawbacks of this study are the retrospective nature, the small number of patients and differences in the additional gonadotrophins and dose. Whether the results can be related to older Bologna criteria patients is uncertain.

Wider implications of the findings: The results of this study corroborate the results of previous studies, showing that dual stimulation might be an alternative treatment concept with more oocytes retrieved within a shorter time; for the first time long-acting FSH was used for stimulation. Collectively this protocol reduces the treatment burden of the Bologna criteria poor responder patient.

Trial registration number: The study was approved by the Regional Ethical Committee. The study carries no Trial registration number due to its retrospective nature.

P-653 Critical role of HOTAIR long non coding RNA as an epigenetically master regulator of HOX loci genes in Cumulus Cells of patients with PCOS

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Study question: To assess relationship between *HOTAIR* expression associated with *HOXC & HOXD* clusters and determine functional epigenetic marks in women with PCOS.

Summary answer: Aberrant correlative expression of *HOXC & HOXD* clusters and *HOTAIR* gene in alliance with regulatory epigenetic marks of PCOS patients vs. healthy women was observed.

What is known already: Poly Cystic Ovary Syndrome (PCOS) is the most common endocrinal disorder affecting anovulatory infertility. *HOX* family are genes that have altered expression in human reproductive system disorder. Previous researches have shown that long non coding RNAs do epigenetic regulation of gene expression and play important roles in proliferation, apoptosis and metastatic capacity of cancers. Recent studies revealed *HOX* Transcript Antisense Intergenic RNA (HOTAIR) as lncRNA which has estradiol-dependent activation and epigenetically regulatory functions. *HOTAIR* which is located in *HOXC* genes cluster, could bind with histone modifier complexes and direct to specific *HOXD* cluster that prone to cause their expression switching.

Study design, size, duration: Our study consist of 20 patients with PCOS as a case and 20 fertile women with male infertility problems referred to the Royan Institute to get ICSI under GnRH antagonist protocol as a control population. Cumulus cells (CC) were collected from PCOS patients and fertile. Informed consents were obtained from the participants. Thirty six hours after hCG injection, ovaries were punctured and cumulus oocyte complexes were dissected.

Participants/materials, setting, methods: Total RNA was extracted from CCs and cDNA was synthetized by whole transcriptome amplification kit. Quantitative real-time PCR was performed by use of specific primer for target genes. For the epigenetic evaluation, soluble chromatin was extracted from CCs and chromatin immune precipitation (ChIP) coupled with real-time PCR was performed to quantify both activator (H3K27 methylation) and suppressor (H3K4 demethylation) epigenetic histone modification marks on regulatory regions of *HOXD10* gene as a main target of HOTAIR.

Main results and the role of chance: Obtained data showed significant increase of *HOTAIR* ($P<0.05$), *HOXC6* ($P<0.05$), *HOXC9* ($P<0.05$), *HOXC10* ($P<0.05$), *HOXC11* ($P\leq0.01$), *HOXC12* ($P<0.05$), gene expression in PCOS vs. control group but there were no significant changes in PCOS vs. PCOD group. On the other side, there was a significant decrease in expression of *HOXC8* ($P<0.05$), *HOXC13* ($P<0.05$), *HOXD8* ($P<0.05$), *HOXD9* ($P<0.05$), *HOXD10* ($P<0.05$), *HOXD11* ($P<0.05$), *HOXD12* ($P<0.05$). Interestingly there was no significant changes in *HOXD13* ($P<0.05$) contrary to HOTAIR impression on *HOXD* cluster. All above expression profiles was confirmed by epigenetic evaluation in subsequent.

Limitations, reasons for caution: Due to the small number of participant limitation, larger studies should be needed for the confirmation.

Wider implications of the findings: Current study suggest that there is significant correlation between altered expression of HOX family genes, HOTAIR lncRNA as an epigenetic regulator in PCOS disorder. This project provides new insights to understand the pathogenesis of PCOS.

Trial registration number: Not Applicable.

P-654 Role of measuring serum LH levels 12 hours post agonist trigger and administration of rescue HCG trigger

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Study question: Does measurement of serum LH levels 12 hours after agonist trigger and administration of rescue HCG trigger(if LH level <50 IU/L) improve oocyte recovery rate and maturity rate?

Summary answer: This kind of approach does not significantly improve oocyte recovery rate and maturity rate.

What is known already: With advent of antagonist protocol in IVF stimulation it has become common to administer agonist trigger for final oocyte maturation to decrease the risk of OHSS. Even though pregnancy rates are lower and miscarriage rates are higher with fresh embryo transfer in agonist triggered cycles, not much has been published about efficacy of agonist trigger in terms of oocyte recovery rate(eggs retrieved/mature size follicles on day of trigger administration) and maturity rate(metaphase II/eggs retrieved). There are few studies reporting lower oocyte recovery rate with agonist trigger if serum LH level < 15IU/L 12 hours post trigger administration.

Study design, size, duration: Retrospective study of 100 patients undergoing IVF-ICSI freeze all cycles with own eggs on flexible antagonist protocol at our centre between April – May 2016 and April- May 2017

Participants/materials, setting, methods: Group 1–50 patients triggered with agonist and serum LH levels not performed (April – May 2016)

Group 2–50 patients triggered with agonist and serum LH levels performed approximately 12 hours after trigger. If levels were below 50 IU/L, injection HCG 2000 units was administered immediately, and ovum pick up was performed 36 hours post agonist trigger. (April- May 2017)

Group 2 A (LH < 15IU/L)
Group 2B (LH 15-30IU/L)
Group 2 C (LH 30-50IU/L)
Group 2D (LH >50IU/L)

Main results and the role of chance: On an average 20 eggs were retrieved per patient in group 1 and 21 eggs were retrieved per patient in group 2(p value – 0.15). There was no significant difference in oocyte recovery rate (oocytes retrieved/follicles > 14 mm on day of trigger administration) between the two groups. (87% in group 1 and 83.9% in group 2, p value- 0.26). There was only one patient with empty follicle syndrome in group 1 and none in group 2. Oocyte maturity rate (metaphase II / eggs retrieved) was also similar between both groups. (Group 1 – 71%, Group 2 – 72.3%, p value- 0.46). No patient had LH levels <15IU/L in our study, 2 patients(4%) had LH level measuring 15-30 IU/L and 10 patients(20%) had LH level measuring 30-50 IU/L. In group 2B(LH <30IU/L), oocyte retrieval rate was lower than remaining 2 groups (Group 2B-59.5%, Group 2C- 90.3%, Group 2D- 90.6%). We had no incidence of OHSS in any of the patients.

Limitations, reasons for caution: Retrospective nature of the study and participants selected over 2 different time spans. Administration of HCG may pose a patient at risk of OHSS. Majority of the participants were hyper-responders undergoing all freeze cycles to avoid risk of OHSS(results cannot be extrapolated to all).

Wider implications of the findings: Measuring LH levels 12 hours post agonist trigger and administration of rescue HCG trigger does not improve oocyte recovery rates even if LH level was<30 IU/L(Incidence 4%). This kind of approach will just add to patient inconvenience, cost and pose them at risk of OHSS.

Trial registration number: not applicable.

P-655 FSH exposure between day 8 and day of hCG administration is an independent predictor of serum progesterone rise

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Study question: What is the effect of additional recFSH administration after one dose of corifollitropin alfa (CFA) on the progesterone rise on the day of final oocyte maturation?

Summary answer: While little increase in progesterone levels is observed after seven days of CFA stimulation, post-CFA recFSH dosing is the strongest determinant of subsequent progesterone rise.

What is known already: Late-follicular progesterone (LFP) increase is related to both ovarian response and the intensity of FSH exposure during controlled ovarian stimulation (COS). CFA-treated patients who reach the hCG triggering criteria without need for additional daily recFSH (<8days) have a lower incidence of elevated progesterone >1.5 ng/ml when compared to daily recFSH treated controls. This suggests that the pharmacokinetic profile of CFA, with its progressively declining FSH activity, also may reduce the progesterone rise. However, it remains unclear why favourable progesterone levels on the day of hCG administration are not observed in patients who need additional recFSH after CFA (>8days).

Study design, size, duration: A post-hoc subgroup data-analysis of three previously published trials; ENGAGE, ENSURE and PURSUE was performed. All were randomized double-blind trials comparing the efficacy of ovarian stimulation with 150 µg (ENGAGE/PURSUE) or 100 µg (ENSURE) CFA versus a recFSH control group. In both arms, recFSH doses were adjusted according to ovarian response. Cycles with a duration of ovarian stimulation >8 days, which includes all CFA cycles requiring additional recFSH (n = 1234) and their daily recFSH controls (n = 1026), were included.

Participants/materials, setting, methods: The studies included women aged 18–42 years with a regular menstrual cycle (24–35 days), and an indication for ovarian stimulation for IVF/ICSI. All trials were multicentre and multinational involving centres in Europe, North America and Asia. Treatment cycles <8 days were excluded. Progesterone levels were obtained on days 1, 8 and the day of hCG administration. Linear regression analysis was performed to assess the factors contributing to the serum progesterone levels.

Main results and the role of chance: Patient's demographics and baseline characteristics were comparable between groups. Mean progesterone levels on day 8 of stimulation were significantly lower in the CFA group when compared to the recFSH arm (0.6 ± 0.3 ng/ml vs. 0.9 ± 0.4 ng/ml; p<0.001), with a four-fold lower incidence of progesterone levels >1.5 ng/ml (2.2% vs. 8.7%; p<0.001). However, progesterone levels on day of hCG were comparable between groups (1.1 ± 0.7 ng/ml vs. 1.2 ± 0.6 ng/ml; p = 0.18), owing to a significantly sharper increase in progesterone levels between day 8 and the day of hCG administration in CFA-stimulated cycles (0.5 ± 0.5 ng/ml vs. 0.3 ± 0.3 ng/ml; p<0.001). Multivariable linear regression analysis, controlling for age and progesterone on Day 8, showed that the post-CFA progesterone increase was independently associated with the number of days of recFSH stimulation from day 8 onwards (0.10 ng/ml per day; 95% CI 0.08-0.12; p<0.001), the daily dose of post-CFA recFSH (0.08 ng/ml per 50 IU; 95% CI 0.05-0.11; p<0.001) and the number of oocytes retrieved (0.02 ng/ml per oocyte; 95% CI 0.01-0.02; p<0.001).

Limitations, reasons for caution: Although this post-hoc analysis is of importance to generate hypotheses on strategies to minimize progesterone rise, prospective randomized trials are needed to confirm whether reduced post-CFA recFSH dosing could prolong the favourable effect of CFA on progesterone levels without a clinically relevant impact on the ovarian response.

Wider implications of the findings: This study found that CFA treatment has a lower incidence of elevated progesterone during the first week of treatment compared to a daily recFSH regimen. Moreover, it suggests that lower daily recFSH doses following CFA could significantly contribute to maintaining these favourable progesterone levels until the day of hCG.

Trial registration number: Engage NCT00696800; Ensure NCT00702845; Pursue NCT01144416

P-656 The Effect of Alpha Lipoic Acid on expression of IRS-1, GLUT-4, and folliculogenesis in Polycystic Ovary Syndrome with insulin resistance

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Study question: This study aimed to prove the effect of Alpha Lipoic Acid on expression of IRS-1, GLUT-4 and folliculogenesis of PCOS model rats with insulin resistance.

Summary answer: After administering ALA, expression of IRS-1 and GLUT-4 were higher. There was no difference in folliculogenesis but there was significant difference in de Graff follicles.

What is known already: Polycystic Ovary Syndrome (PCOS) is a frequent endocrine disorder in women of reproductive age where insulin resistance plays an important role. Insulin resistance complicates optimal treatment of infertility. Administration of Alpha Lipoic Acid (ALA) is expected to be an alternative therapy in clomiphene citrate resistant PCOS and in PCOS which has side effects on metformin.

Study design, size, duration: This study used randomized post-test only control group design. The sample size was 30 female rats (rattus norvegicus). This study was conducted in February until May 2017 in Faculty of Veterinary, Universitas Airlangga, Surabaya.

Participants/materials, setting, methods: 30 female rats were given 100 mg/kg B testosterone propionate injection for 28 days to form PCOS model with insulin resistance. Then, they were divided into 3 groups randomly. Group I was negative control, group II got placebo, and group III got ALA. The expression of IRS-1 and GLUT-4 were assessed by Immuno Reactive Score (IRS) on immunohistochemical examination. Folliculogenesis was assessed by counting the number of follicles at each stage through Hematoxylin Eosin (HE).

Main results and the role of chance: The mean expression of IRS-1 in muscle of treatment group (4.28 + 1.05) was significantly higher than placebo (3.02 + 1.03) (p = 0.01). The mean expression of IRS-1 in ovary of treatment group (7.78 + 1.10) was significantly higher than placebo (3.06 + 1.56) (p <0.01). The mean expression of GLUT-4 in muscle of treatment group (4.28 + 0.91) was significantly higher than placebo (3.20 + 1.14) (p = 0.01). The average number of primary follicles in treatment group (58,00 + 13,11) was significantly lower than placebo (66,00 + 19,81) (p = 0,55). The average number of secondary follicles of the treatment group (18.00 + 4.62) was significantly lower than placebo (27.00 + 10.82) (p = 0.10). The average number of tertiary follicles of the treatment group (2.00 + 1.56) was significantly lower than placebo (4.00 + 1.89) (p = 0.13). The average number of de Graff follicles of the treatment group (5.00 + 3.37) was significantly higher than placebo (2.00 + 0.82) (p <0.01). The average number of corpus luteum of the treatment group (9.00 + 3.37) was significantly higher than placebo (6.80 + 3.58) (p = 0.13).

Limitations, reasons for caution: This study has limitation that it did not use normal rats control.

Wider implications of the findings: ALA can be used as an alternative therapy in PCOS patients with clomiphene citrate resistance, especially for women who cannot tolerate the side effects of metformin.

Trial registration number: Not applicable.

P-657 Among Bologna poor responders, does the type of GnRH analogue play any role when corifollitropin alfa is used?

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Study question: Does the type of GnRH analogue in different stimulation protocols affect cumulative live birth rate (CLBR) in Bologna poor responders treated with corifollitropin alfa (CFA)?

Summary answer: CLBR remains similar in different stimulation protocols using corifollitropin alfa in poor responders. However, the GnRH antagonist protocol results in more supernumerary embryos cryopreserved.

What is known already: In normal responders, CFA may increase the number of oocytes retrieved compared to recombinant FSH (rFSH). Preliminary studies using CFA in poor responders have also shown promising results in this poor prognosis population. However, it remains unclear which protocol (GnRH-antagonist, long GnRH-agonist or short GnRH-agonist) represents the ideal therapeutic approach when using CFA in poor responders.

Study design, size, duration: This is a large retrospective analysis including poor responder patients fulfilling the Bologna criteria and undergoing their first ICSI cycle with CFA, between 2011 and 2017. The study population was divided according to the ovarian stimulation protocol into three categories: long GnRH-agonist, short GnRH-agonist and GnRH-antagonist. The starting dose of CFA was 150 µg. CLBR was defined as the delivery of a live born resulting from fresh and all subsequent frozen embryo transfers.

Participants/materials, setting, methods: In the GnRH-antagonist fixed protocol, CFA was started on day2 (D2) of the cycle and GnRH-antagonist was initiated on D6. In the long GnRH-agonist, patients started the agonist from D21 of the previous cycle and CFA was initiated following confirmation of ovarian suppression. For the short protocol, GnRH-agonist was administered from D2 of the cycle and CFA from D3. If necessary, daily injections of hp-HMG (300 IU) were given from D8, until hCG-trigger.

Main results and the role of chance: A total of 757 cycles were divided into 3 groups: group A (antagonist protocol, n = 437), group B (long-agonist protocol, n = 231) and group C (short-agonist protocol, n = 89). There were no differences in baseline characteristics among the groups, including age and ovarian reserve markers. Days of additional hp-HMG following CFA were significantly different between group A, B, and C (3 vs 5 vs 3; *p*=<0.001). There were no differences among the groups in terms of number of mature oocytes, fertilization rate, embryo quality on day 3 and day of embryo transfer. CLBR were 21.0%, 20.8% and 16.9% (*p* = 0.68), respectively, for the three study groups.

Limitations, reasons for caution: The main limitation of our large study is its retrospective design which is inherent to risk of bias.

Wider implications of the findings: This is the largest analysis including Bologna poor responders stimulated with CFA. Based on our results, different CFA protocols do not increase CLBR. However, the antagonist protocol may result in more supplementary frozen embryos.

Trial registration number: not applicable.

P-658 Effect of simvastatin on improvement of polycystic ovary syndrome (PCOS) induced by Dehydroepiandrosterone (DHEA) in rats

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Study question: What is the role of simvastatin to protect polycystic ovary syndrome (PCOS) induced with Dehydroepiandrosterone (DHEA)

Summary answer: Its seems simvastatin decrease the damaging influence of Dehydroepiandrosterone (DHEA) on rat polycystic ovary syndrome model.

What is known already: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorders in women and the most common diagnostic criteria are hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. Also Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), have been confirmed to be elevated in at least a subcategory of women with PCOS. On the other hand, statins (including simvastatin) not only improve lipid profiles, but also decrease inflammatory markers, ovarian size, and oxidative stress. Moreover, the aim of this study was to investigate the protective effect of simvastatin against polycystic ovarian syndrome (PCOS) induced by Dehydroepiandrosterone (DHEA) in the female rats.

Study design, size, duration: A total of 60 Wistar female rats were randomly distributed into control, DHEA and DHEA + simvastatin (n = 20 per group) groups. The PCOS rat model was developed by the injection of DHEA (6 mg/100 g body weight diluted in 0.4 ml/rat sesame oil) for each animal during 20 days subcutaneously. Control group received only oil. After then each animal daily received simvastatin (10 mg/100 g orally) during 4 weeks or saline solution as control.

Participants/materials, setting, methods: In all groups, ovarian tissue was evaluated by morphological observation and the follicular theca fibrosis was recorded using trichrome Masson staining and morphometric technique. Serum level of anti-Mullerian hormone (AMH) was analyzed by ELISA. Furthermore, tissues were analyzed by immunohistochemistry to detect TNF-alpha and apoptosis. Data were analyzed by one-way ANOVA followed by Tukey's test using SPSS version 16 software. The p<0.05 was considered significant.

Main results and the role of chance: Our result indicated that PCOS group showed a significant increase in serum levels of anti-Mullerian hormone (AMH) compare to control groups (p <0/05). It also increases the follicular theca fibrosis and thickness of theca and decrease body loss in PCOS group significantly (p <0/05). In comparison with the control and PCOS groups, there was a significant increase in the apoptosis index and TNF-α expression (p <0/05). Also Simvastatin (10 mg/100gr) decreases serum anti-Mullerian hormone

(AMH) in the experimental group compare PCOS group significantly (p <0/001). Moreover, compared with the PCOS group, both follicular theca fibrosis and thickness of theca decreased but it's not significant. The apoptosis index was lower (p<0.05) and TNF-α expression was lower (p<0.05) in the simvastatin-treated groups.

Limitations, reasons for caution: It's in only one species and further data are required to better define the polycystic ovarian syndrome.

Wider implications of the findings: We concluded simvastatin could be useful to prevent the harmful effects of PCOS in polycystic ovarian syndrome (PCOS) induced by Dehydroepiandrosterone (DHEA) in the female rats.

Trial registration number: The results of this study indicated that Master of Science thesis in Guilan University of medical sciences with No: 102 and.

P-659 Occurrence and characteristics of recombinant human follicle-stimulating hormone (r-hFSH) dose adjustments during ovarian stimulation in a real-world US database study of 33,962 IVF patient cycles

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Study question: What is the real-world occurrence of r-hFSH dose adjustments during IVF cycles and what are the demographics and baseline characteristics of patients with dose adjustments?

Summary answer: Dose adjustments were observed during 40.7% of cycles analysed and there were clinical differences in patients whose dosing was constant versus those with dosing adjustments.

What is known already: Standard practice during individualized ovarian stimulation with r-hFSH is to tailor the starting dose according to a variety of biomarkers (age; BMI; previous ovarian response; ovarian reserve markers like antral follicle count [AFC], anti-Müllerian hormone [AMH], FSH level). In addition, many physicians adjust FSH dose depending on individual ovarian response during ovarian stimulation. Although this is considered common practice, it has been proposed that it is sufficient to maintain the starting dose of FSH without dose adaptation. There is a lack of real-world data documenting the prevalence of dose adjustments and the characteristics and demographics of patients receiving dose alterations.

Study design, size, duration: Non-randomized, observational, retrospective analysis of a large, US, real-world, electronic de-identified medical records database (IntegraMed America, Inc.), including data from >15 ART clinics serving patients from all 50 states. Data for patients receiving any GONAL-f presentation (r-hFSH; EMD Serono, Inc., Rockland, MA, USA) during IVF cycles between Jul 2009 and Dec 2016 were analysed.

Participants/materials, setting, methods: Outcomes evaluated included: overall number of cycles with a constant dose; number with at least one dose adjustment; number with a dose increase; number with a dose decrease; and the subset with both decreases and increases; the mean (standard deviation [SD]) start, end and total r-hFSH (GONAL-f) doses; and patient baseline characteristics according to dosing pattern. Dosing data were evaluated per cycle. The first cycle per patient was evaluated for patient demographics and baseline characteristics.

Main results and the role of chance: A total of 23,582 patients undergoing 33,962 IVF cycles included 13,823 cycles (40.7%) with r-hFSH dose adjustments; 40.0% (5524) of these involved more than one dose change. Among cycles with dose changes, ≥1dose increase was observed in 7939 (57.4%) cycles, ≥1 dose decrease in 8639 (62.5%) cycles and both increases and decreases in 2755 (19.9%) cycles. The mean (SD) starting dose (IU) was higher in cycles with constant dose (292[133.2]) compared with those with dose adjustments (238[122.6], 258[133.3] and 231[149.9] for cycles with ≥1 dose increase, ≥1 decrease and both increases/decreases, respectively; all p<0.0001). The median dose adjustment in all three dose adjustment groups was 75 IU. Patients treated with a constant dose were significantly older (35.9 [4.60] years), had a lower AFC (12.8[7.83]), had a lower AMH (2.2[2.90] ng/mL) and a higher day 3 FSH (8.2[4.81] mIU/mL) compared with cycles with dose adjustments (increases, decreases and both increases/decreases). Patients with constant dosing were more likely to have a diagnosis of diminished ovarian reserve (25.2%) versus those with dose adjustments (20.1%, 15.9% and 14.0%) and were less likely to have ovulatory disorders (10.6% versus 15.2%, 18.3% and 19.2%) for dose increases, decreases and both increases and decreases.

Limitations, reasons for caution: This was a retrospective, observational study of US real-world data, not specifically collected for research purposes, and may not reflect clinical practices in other parts of the world. In addition, diagnostic and drug use information may not always be validated or complete.

Wider implications of the findings: Individualized r-hFSH dose adaptation, depending on ovarian response, is highly prevalent during ovarian stimulation for ART in real world practice in the USA. These dose adjustments are used for all patient types, and more frequently in younger patients with higher ovarian reserve and diagnosis of ovulation disorders/PCOS.

Trial registration number: N/A.

P-660 Long-term health related quality of life (HRQoL), life satisfaction and health status in women with PCOS–a population-based follow-up analysis at ages 31 and 46

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Study question: Do the health-related quality of life (HRQoL), life satisfaction and self-estimated health status in PCOS change over time between ages 31 and 46 years?

Summary answer: Women with PCOS presented with decreased HRQoL and poor life satisfaction and health status up till age 46 independent of hyperandrogenism and BMI.

What is known already: PCOS is a common syndrome affecting 5-18% of women at reproductive age. The syndrome is mainly characterized as reproductive and metabolic disorders, however, it has become evident that the women also often have psychological problems. Obesity, diabetes, metabolic syndrome, irregular cycles, infertility, psychological distress and aesthetic concerns like acne and hirsutism burden these women, decreasing their HRQoL. As some of the syndrome-related issues and symptoms become untimely (infertility) or they resolve (menstrual irregularities) over time, it would be of interest to assess how HRQoL, life satisfaction and self-estimated health status change over time in these women.

Study design, size, duration: In a prospective, general population-based follow-up birth cohort (n = 5889 females), postal questionnaires were sent at ages 14, 31 and 46 yrs with respective answer rates of 95, 81 and 72%. Questionnaires on PCOS symptoms (31 yrs), 15D HRQoL, life satisfaction and self-estimated health status (31 and 46 yrs) and BMI (14, 31 and 46 yrs) were analyzed. Clinical examination and testosterone measurement were performed at age 31 (3115 women) and 46 (3280 women).

Participants/materials, setting, methods: According to questionnaires, 331 women (11.2%) presented isolated oligoamennorrhea (OA) and 323 (10.9%) isolated hirsutism (H) and 125 (4.2%) presented both symptoms (PCOS), whereas 2188 asymptomatic women where considered as controls. The 15D scores were analysed with Kruskal-Wallis H-test, life satisfaction and

self-estimated health status with chi-square analysis. The risks related to PCOS symptoms were estimated using a logistic regression model.

Main results and the role of chance: In all four study groups HRQoL decreased over time. In women with PCOS and in women with isolated H the HRQoL was decreased both at ages 31 and 46 compared with controls whereas there was no difference in the sum score in women with isolated OA. The individual HRQoL that differed between PCOS and controls were at age 31: vitality, mobility, breathing, sleeping, excretion, discomfort and complaints and at age 46: vitality, mobility, vision, hearing, sleeping, excretion, usual activities, discomfort and complaints and depression. Women with PCOS presented 3.8-fold risk for being in the lowest HRQoL quartile at age 31 and 2.5 –fold risk at age 46. The risk remained significant after adjusting for BMI, testosterone, infertility and marital and socioeconomic status. Women with isolated H or PCOS also associated with risk of low life satisfaction at age 31 (H: OR1.57 95% CI [1.06, 2.34], PCOS: 2.64 [1.59, 4.39]) and PCOS group at age 46 (2.11 [1.59, 3.84]) compared with controls. Moreover, PCOS also associated with risk of poor self-reported health status age 31 (3.60 [1.84, 7.05]) and 46 (3.85 [1.89,7.85]).

Limitations, reasons for caution: The symptoms of PCOS were self-reported and no ultrasound examinations were available. The study population was relatively young for assessing some items included in the 15D tool.

Wider implications of the findings: PCOS and isolated H associates with low HRQoL from fertile age until late reproductive years. As women with PCOS are at risk for reporting poor life satisfaction and health status at age 31 and 46, more studies aiming to improve HRQoL in these women are warranted.

Trial registration number: not applicable.

P-662 Co-administration of GnRH-agonist and a standard dose of hCG (dual trigger) for improving IVF outcome in patients with low proportion of mature oocyte

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Study question: Whether the Co-administration of GnRH-agonist and standard hCG (dual trigger) can be used for improving IVF outcome in patients with low proportion of mature oocyte?

Summary answer: For patients who have normal ovarian response but low proportion of mature oocytes, dual trigger can help improving their IVF outcome.

What is known already: Oocyte maturation occurs after the luteinizing hormone(LH) surge during the menstrual cycle. As a surrogate LH surge, GnRH-agonist and Human chorionic gonadotropin(hCG) are usually used at the end of controlled ovarian hyperstimulation(COH) to induce final follicular maturation. Patients who have low oocyte maturity or the inability to retrieve mature oocytes have poorer IVF outcomes compared with those who have more mature oocyte obtained. Now it is more frequent to see that normal responding patients with an apparently normal follicular development and E2 levels on the day of HCG injection result in low proportion of mature oocyte.

Study design, size, duration: Retrospective study was preformed to compare the stimulation characteristics of 366 IVF cycles from March 2016 to August 2017. Patients were given combination of Group A (dual trigger group, n = 123), Group B(Group A's patients' previous IVF attempt, triggered with hCG-only, n = 123), Group C (different patients form Group A, triggered with hCG-only, n = 120).

Participants/materials, setting, methods: Patients who were eligible for this study must be normal response and have a prior IVF cycle with low proportion of MII oocytes(<66%) per number oocytes retrieved. During the previous cycle, patients underwent various IVF stimulation protocols and were triggered with hCG only (5,000IU or 10,000IU). For the dual trigger cycle, patients underwent a GnRH antagonist COH protocol and dual trigger(0.2 mg GnRH-agonist plus 250ug rhCG) was preformed 36 hours prior to OPU.

Main results and the role of chance: The basic characteristic date including age, ART indications, duration of infertility, based hormonal level between the three groups were not statistically significant. The number of metaphase-II (MII) oocyte in the dual trigger group is significantly higher than Group B and Group C (6.9vs 3.4vs 4.0, p<0.05). In addition, patients who received dual trigger (group A) had a significantly higher number of top quality embryos (TQE) (1.6vs 0.7vs 0.6, p<0.05), number of embryos transferred(1.1vs 0.5vs 0.5, p<0.05), rate of clinical pregnancy (0%vs 18%vs 2%, p<0.01) while lower rate of cycle cancellation (10.2%vs 28.1%vs 20.3%, p<0.05) than the other two groups.

Limitations, reasons for caution: Our study suggested a higher pregnancy rate in the study group(dual trigger). However, this is biased due to our study design which offered this protocol to patients who had failed their previous IVF attempt. Further multicenter prospective studies are needed to strengthen our current findings.

Wider implications of the findings: This study is based on a large sample of data which may provide some new insights to treat patients who appear to have normal ovarian response and follicular development but low proportion of mature oocyte.

Trial registration number: not applicable.

P-663 Relevance of prednisolone treatment in patients with multipronuclear embryos caused by anticentromere antibodies: improvement of fertilization, blastocyst formation and pregnancy rates

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Study question: To investigate whether prednisolone treatment may improve fertilization, blastocyst formation and pregnancy rate in patients with multipronuclear embryos caused by anticentromere antibodies.

Summary answer: We retrospectively confirmed prednisolone significantly improved fertilization, blastocyst formation and pregnancy rate in patients with multipronuclear embryos caused by anticentromere antibodies compared to no prednisolone.

What is known already: Women with anticentromere antibodies have been reported to show impaired oocyte maturation, fertilization, embryo cleavage and pregnancy rates. Sometimes their embryos showed increasing amount of multipronuclear embryos (3 pronuclei or more). Based on mouse studies, injection of anticentromere antibodies interfered with meiosis and mitosis by attachment of anticentromere antibodies to the centromeric region of a chromosome. Those embryos showed impaired oocyte maturation, fertilization, embryo cleavage and blastocyst formation rates as shown in human observations. However, the precise mechanism caused by anticentromere antibodies needs further investigation. To the best of our knowledge, no therapeutic studies have been reported.

Study design, size, duration: A single-center retrospective study was performed. All patients gave written informed consent for this study. Patients who performed in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) between May 2014 and December 2017 were included in the study. Serum anticentromere antibodies were measured using CLEIA method by a clinical laboratory company, and positive anticentromere antibodies were defined as more than 10 IU/mL. Of 4651 women, 45 women had anticentromere antibodies (0.97%), performing 94 IVF/ICSI cycles.

Participants/materials, setting, methods: The average age of the positive anticentromere antibodies was 38.5 ± 3.6 years. We divided 94 cycles into two groups with or without prednisolone (64 and 30 cycles), and compared fertilization, blastocyst formation and pregnancy rates between two groups. Prednisolone (15 mg/day) was administered orally from the first day of ovarian stimulation to the day before oocyte retrieval. Ovarian stimulation protocols were similar between two groups, because those were set according to patients' age and AMH levels.

Main results and the role of chance: In the ICSI group, two pronuclear (2PN), multipronuclear, cleavage, and blastocyst formation rates with prednisolone group were significantly (P<0.05) better than those without prednisolone (41.2% vs. 20.2%, 28.1% vs. 39.4%, 62.4% vs. 41.4%, 24.1% vs.

0%), respectively. In the IVF group, however, no significant differences were observed between the two groups (20.0% vs. 19.8%, 45.3% vs. 42.3%, 40.0% vs. 29.2%, 31.3% vs. 15.4%), respectively. The titer of the anticentromere antibodies was not related to 2PN or multipronuclear rate. Clinical pregnancies were confirmed in 6 patients from prednisolone group only; 3 patients had live births and 2 patients are ongoing pregnancies. Implantation rate with prednisolone group was significantly (P<0.05) higher than that without prednisolone (7/39 vs. 0/32). Seven patients experienced both cycles with or without prednisolone. In the ICSI group of these patients, 2PN rate with prednisolone (44.3%, 47/106) significantly increased compared with that without prednisolone (20.6%, 13/63). The titer of the anticentromere antibodies in patients who improved fertilization rate with prednisolone was significantly lower than that in patients who did not (P<0.001). Patients who may benefit from prednisolone might be low titer of the anticentromere antibodies.

Limitations, reasons for caution: Since this study is a single center retrospective observational study, multi-center prospective randomized studies are needed to conclude the relevance of prednisolone treatment in patients with multipronuclear embryos caused by anticentromere antibodies. To the best of our knowledge, however, there are no studies for treatment strategies in these patients.

Wider implications of the findings: We, for the first time, revealed relevance of prednisolone treatment in patients with multipronuclear embryos caused by anticentromere antibodies. Oral prednisolone (15 mg/day) successfully improved fertilization, blastocyst formation rates and may improve pregnancy rate in patients with positive anticentromere antibodies in our limited study population.

Trial registration number: N/A.

P-664 The serum ProAMH/totalAMH ratio is linked to metabolic status but not to Polycystic Ovary Syndrome (PCOS) per se

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Study question: Is the ProAMH/totalAMH ratio decreased in Polycystic Ovary Syndrome or obese women?

Summary answer: Serum ProAMH/totalAMH ratio is not significantly different between PCOS and control women but is decreased in obese women versus controls.

What is known already: Anti Mullerian Hormone (AMH) is known to inhibit initial and cyclic follicular recruitment. Compared to controls, serum AMH level is higher in PCOS women and is supposed to act in the follicular arrest. Different molecular forms of AMH exist: one inactive (proAMH) and two active which can bind the AMHRII receptor and induce AMH specific actions. The active forms are obtained after proAMH cleavage: AMH_C (C-term domain) and $AMH_{N,C}$ (non-covalent association of N-term and C-term domains). In blood, proAMH and $AMH_{N,C}$ are found, whereas the presence of AMH_C is debated. Different AMH molecular forms ratio are described in different populations.

Study design, size, duration: Cross sectional study using 72 biobanked serum samples collected between 2014 and 2017 in one Academic Hospital.

Participants/materials, setting, methods: In a database prospectively constituted from 2014 to 2017, 39 PCOS women according to Rotterdam criteria (21 obese and 18 lean) and 33 controls (14 obese and 19 lean) were selected. Their biobanked serum were tested for total AMH and pro AMH (after desoxycholate treatment) using a Beckman Coulter Dxi automatic analyser. Relative levels of pro AMH were expressed as a proAMH ratio: [proAMH]/[totalAMH]x100.

Main results and the role of chance: PCOS and control women had similar BMI. Total AMH and Pro AMH levels were higher in women with PCOS compared to controls (p<0.001). They were not significantly different between obese and lean women (p = 0.427 and p = 0.96, respectively). ProAMH/totalAMH ratio was not significantly different between control and PCOS women (38.2 % vs 35.9%; p = 0.38). However, it was significantly decreased in obese (PCOS and control) women compared to lean ones (34.7% vs 39.4%; p<0.001). In the subgroups analysis, ProAMH/totalAMH was different between lean and obese women with PCOS (p = 0.001) but not between lean and obese controls (p = 0.15). With univariate analysis, ProAMH/totalAMH was negatively correlated to weight, BMI, waist circumference and fasting insulinemia. No correlation was found with androgens, LH or ovulation disorders.

Limitations, reasons for caution: Small sample size, limiting statistical power in subgroup analysis.

Wider implications of the findings: Metabolic status may have an impact on proAMH cleavage enzymes leading to an increased rate of circulating active AMH forms in obese women (with or without PCOS). Further studies on larger populations are required to study whether they may contribute to ovulation disorders in PCOS women and control obese women.

Trial registration number: Not Applicable.

P-665 Long-Antagonist protocol; single luteal use of long-acting GnRH-antagonist Degarelix can efficiently downregulate LH during ovarian stimulation for IVF. A proof of concept

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Study question: Does a single luteal dose of long-acting GnRH-antagonist efficiently downregulate hypophysis during ovarian stimulation for IVF?

Summary answer: A single dose of long-acting antagonist Degarelix, during luteal phase, down-regulates LH resulting in no LH rise and production of mature oocytes and implantable embryos.

What is known already: Contrary to GnRH-agonist treatment, GnRH-antagonists block GnRH receptors by competitive binding. Therefore, GnRH-antagonist eliminates various disadvantages observed when using GnRH-agonists especially reducing Ovarian Hyperstimulation Syndrome risk (OHSS), without decreasing the likelihood of live birth. Nevertheless, limited flexibility in cycle programming and asynchrony of the follicular cohort are considered drawbacks. Consequently, despite the benefits of antagonist protocols, long-agonist protocol is still the preferred treatment in the majority of IVF clinics. We hypothesized whether a single luteal dose of long-acting antagonist causes prolonged suppression of LH rise allowing thus flexibility in the starting day of gonadotropins later in the follicular phase.

Study design, size, duration: This proof of concept randomized control trial studied the efficacy of a single dose of long-acting antagonist, Degarelix. The study was performed during January-October 2017. Two groups of patients were compared. Group A (control group) consisted of 10 women, who followed a classic fixed day-6 GnRH-antagonist protocol whereas, Group B (study group) involved 10 women undergoing the new long-antagonist protocol. Both groups involved infertile women prepared to undergo IVF treatment in Assisting Nature Centre.

Participants/materials, setting, methods: According to the protocol, in late luteal phase (day-24) a bolus injection of 0,5 ml Degarelix was administrated subcutaneously to control LH surge in the follicular phase. After menses, ovarian stimulation with gonadotropins was initiated from cycle-day-2 to 10. Different gonadotropin starting days were tested to observe LH level and whether ovarian response is affected by the proximity of stimulation initiation to the day of degarelix injection.

Main results and the role of chance: The mean age of participants was not different (32,1 vs. 32,6) among groups. Stimulation ranged from 9-10 days in control group A, whether in the Long Antagonist group ranged from 10-11 days. Similar number of blastocysts produced in both groups (5.8 vs. 7.0). No LH rise was noticed in any groups. Especially, in the Long Antagonist group one patient was triggered with HCG on cycle-day-22. After fresh embryo transfer, pregnancy efficacy and possible negative effect on endometrium receptivity were assessed. All patients (except one who followed Freeze-all strategy) underwent blastocyst transfer. Clinical pregnancy was 40% in classic antagonist (group A) and 50% in the new Long Antagonist (group B).

Limitations, reasons for caution: The limitation of this study is the small size of the groups, however this is a proof of concept trial. Additionally, other doses of Degarelix could be tested to investigate differences in ovarian stimulation.

Wider implications of the findings: This is a pure novel concept for IVF couples combining security and flexibility. This new Long-Antagonist protocol addresses cycle programming that was missing, with antagonist protocols and almost zeros OHSS as it allows also the triggering with GnRH-agonist. However, larger studies are required to confirm the success of this protocol.

Trial registration number: NCT03240159

P-666 Real-life experience with follitropin delta (FD): dosing inconsistencies based on short AMH changes and treatment outcome in patients of the higher AMH spectrum

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Study question: How often will short-term changes of AMH imply a dosage change? What is the ovarian response to an individualized dose in hyper-response risk patients?

Summary answer: Short term AMH (and body weight) changes occur and affect dose choice. The utility of FD in higher AMH level patients needs further study.

What is known already: Dosing of follitropin delta (FD), which was introduced to the market in 2017, is based on body weight and serum AMH levels (measured in one distinct ELISA within 12 months before treatment). In the phase-III trial (ESTHER-1), excessive ovarian response was less frequent in patients receiving individualized FD doses. There is short-term intra-individual variation in body weight and AMH levels, which may lead to inconsistencies between the dose being prescribed at treatment planning (and drug prescription) and the later time point of actual FD utilization. Furthermore, in the ESTHER-1 trial patients with cycle irregularities and BMI >32.0 kg/m^2 were excluded.

Study design, size, duration: Retrospective analysis of all patients undergoing controlled ovarian stimulation with FD for who, at the outset of FD utilization in our center, it had prospectively been decided to repetitively measure body weight and AMH. The target ovarian response was defined as 8-14 COCs (in line with the primary outcome parameter in the ESTHER-1 trial). FD was used according to the posology. Weight and AMH is expressed ±standard deviation (SD). This study is still ongoing.

Participants/materials, setting, methods: For the initial dosing of FD, recent AMH levels and ad-hoc body weight was used. On day of the first follitropin delta injection, AMH and weight was re-assessed and compared. In two cases, AMH was re-assessed later in the follicular phase under ongoing FD administration. The number of COCs in follitropin delta and previous and/or following stimulation cycles using conventional FSH were analysed. AMH assessment was conducted using the Roche Elecsys ELISA AMH assay.

Main results and the role of chance: To date, 20 patients have been included in this analysis. The mean body weight was 71 ± 12 kg on first assessment and 72.6 ± 14.4 kg on second assessment. Mean AMH was 7.1 ± 3.8 ng/ml and 6.0 ± 2.4 ng/ml on first and second assessment, respectively. This change resulted in a dose adjustment in five patients (25%, 95%CI: 11-47%). In the two patients with another AMH value generated during FD administration, the AMH was approximately halved as compared to pre-treatment values. Individual dosing with follitropin delta achieved a target response in COC number in seven patients (35%, 95%CI: 18-57%), whereas 13 (65%, 95%CI: 43-82%) patients responded off-target. Of the off-target patients, two patients with pre-treatment AMH of 8.4 and 7.4 ng/ml showed an aresponse which led to cycle cancellation, the patient with AMH 8.4 having had produced more than 20 COCs in two previous cycles with conventional FSH dosing. A hyperresponse with GNRH-agonist and freeze-all strategy was observed in six patients (30%, 95%CI: 14-52%). A clinical pregnancy was achieved in six patients out of a total of 11 patients undergoing fresh embryo transfer after using follitropin delta for ovarian stimulation.

Limitations, reasons for caution: Short term AMH changes will not necessarily affect dosing in high AMH women, since the minimum dose still remains to be prescribed. Of note, the patient numbers presented herein are still small.

Wider implications of the findings: The implications of short term AMH and body weight changes on the performance of the individualized dosing algorithm needs to be studied further. Furthermore, the performance characteristics of FD may be modulated by AMH, with a risk of underexposure with the minimal dose.

Trial registration number: n.a.

P-667 Rescue protocol and fresh embryo transfer after trigger with analogs of GnRH

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Study question: Do GnRH agonists (GnRHa) trigger and fresh embryo transfer preserve live birth rate in high responder (HR) patients during in vitro fertilization (IVF) cycles?

Summary answer: In HR patients trigger with GnRHa and the rescue protocol seems to reduce the risk of ovarian hyperstimulation syndrome (OHSS) since ensuring excellent pregnancy outcomes.

What is known already: GnRHa trigger is effective in the induction of oocyte maturation and prevention of OHSS during IVF treatment. The trigger with analogue seems to increase the Abortion Rate (AR) and reduce the Live Birth Rate (LBR), due to the supposed luteal phase dysfunction and impaired endometrial receptivity. Some authors affirm that the solution could be the intensive support of the luteal phase; other authors suggest that the best strategy is the "freeze only" policy.

Study design, size, duration: This is a single center retrospective observational study performed in a tertiary care academic Fertility Center. We analyzed a total of 732 antagonist IVF protocols with GnRHa trigger in the period between 1/03/2013 and 31/12/2016. Among these cohort, 271 patients underwent "rescue protocols" and fresh embryo transfer. The rest underwent a freeze only policy.

Participants/materials, setting, methods: In HR patients (AMH> 3.5 ng / ml and more than 18 follicles with diam. ≥ 12 mm) the trigger was obtained with triptorelin 0.2 mg / sc. In patients considered to be at intermediate risk of OHSS (< 18 oocytes retrieved) a rescue protocol has been applied: HCG 1500 IU / sc. at retrieval + estradiol 4 mg + vaginal progesterone 400 mg a day) and fresh embryo transfer on day 3 or 5.

Main results and the role of chance: Patients included in the study were good prognosis women with a mean age of 34.13 ± 4.42 years and good ovarian reserve (FSH mean baseline of 6.29 ± 1.85 and AMH of 4.85 ± 3.34 ng / mL). The retrieved oocytes were 13.71 ± 4.26 with a recovery rate of 75.6 ± 18.84% and a percentage of M2 oocytes of 79.41 ± 15.30%. The percentage of patients with a recovery rate less than 75% was 30.33% and less than 50% of was 6.15%. A total of 548 embryos were transferred, with an implantation rate of 26% and a pregnancy rate of 39.11%. We stratified patients for transfer: 239 women performed the transfer at cleavage stage and 31 on blastocyst stage on day 5. The pregnancy rate were respectively 38% and 48%. Analyzing the evolution of the 106 pregnancies obtained, our results show: 9 abortions in the first trimester and 2 in the second trimester (10.38% abortion rate), 2 therapeutic abortions (1.8%) and 1 ectopic pregnancy (0.9 %). We had 92 deliveries (LBR

39.49%). We observed 8 cases of OHSS (2.95%): 4 cases (1.48%) were admitted to hospital for observation and medical therapy.

Limitations, reasons for caution: Although this is a single center study with an homogenous population, the retrospective nature of the analysis has wide range of biases, and although limited a risk condition for the patients.

Wider implications of the findings: In HR patients with intermediate risk of OHSS, a GnRHa trigger and rescue protocol allows fresh ET with a relative low risk of OHSS, excellent results in terms of LBR and a reduced time to pregnancy compared to a freeze only policy.

Trial registration number: NA.

P-668 The resting metabolic rate in women with polycystic ovary syndrome and its relation to the hormonal milieu, insulin metabolism and body fat distribution

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Study question: Is resting metabolic rate (RMR), a major determinant of energy expenditure, altered in women with polycystic ovary syndrome (PCOS) compared with age- and BMI-matched controls?

Summary answer: PCOS women do not seem to have an abnormal basal metabolism, as their RMR resulted comparable to control women.

What is known already: PCOS women have a higher prevalence of obesity compared to the general population, moreover women with PCOS struggle in their attempt to obtain weight loss and encounter more difficulties in weight control compared with the age and BMI-matched general population. Both genetic and environmental factors seem to be involved in this predisposition. Some authors explored the hypothesis that the energy expenditure capacity could be intrinsically reduced in PCOS, but results deriving from studies with indirect calorimetry and Doubly Labeled Water are still inconsistent.

Study design, size, duration: This is a monocentric observational prospective cohort study. It was conducted for two consecutive years from January 2013 to January 2015 and included 138 subjects.

Participants/materials, setting, methods: 107 Caucasian subjects diagnosed with PCOS, according to Rotterdam Consensus diagnostic criteria and 31 healthy, age-BMI matched control women were enrolled in the study. Energy expenditure evaluation, expressed as RMR, was obtained through the SenseWear Armband (SWA), a reliable and validated metabolic holter, never previously used in the PCOS population to this purpose. Hormonal assessment, insulin metabolism evaluated by HOMA-IR and OGTT, anthropometric features (BMI and WHR) were also assessed.

Main results and the role of chance: In accordance with the study design, age and BMI were similar in the two cohorts: median age was 26.0 ± 9.2 years in PCOS women and 25.5 ± 8.5 years in controls (p = 0.524); median BMI 26.4 ± 9.4 kg/m2 in PCOS women and 27.2 ± 12.8 kg/m2 in controls (p = 0.330). Nonetheless, the body fat distribution resulted significantly different between the two groups, suggesting a tendency towards central body fat accumulation in the PCOS population (WHR 0.79 ± 0.10 vs 0.75 ± 0.10; p = 0.020).

Median RMR resulted similar in PCOS and control women: 1520.0 ± 248.00 kcal/day vs 1464.0 ± 332.70 kcal/day (p = 0.472), even after adjusting for obesity, fat distribution, hyperinsulinemia and insulin resistance (respectively p = 0.164, p = 0.317, p = 0.377, p = 0.232). RMR resulted directly correlated with BMI, WHR, estradiol, total cholesterol, triglycerides, basal blood glucose, basal insulin, AUC insulin 240' and HOMA, while inversely with SHBG. In the subgroup of patients with WHR>0,85, PCOS women showed a significantly lower RMR compared with controls.

Limitations, reasons for caution: The relatively small number of controls might unbalance the sample size, even if an appropriate statistic was carried out. WHR is not considered an accurate estimation of body fat distribution.

Wider implications of the findings: Our results strengthen the scientific evidence regarding the normal capacity of consuming energy of women with PCOS. The finding of a significantly lower RMR rate among PCOS women with WHR>0.85 compared with controls requires further investigation about the role of body fat distribution in the metabolic homeostasis of PCOS population.

Trial registration number: clinicaltrials.gov Identifier NCT03132545

P-669 Childhood and adolescence body mass index associates with impaired reproductive function - a prospective, population-based cohort study

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Study question: What is the contribution of the prenatal environment, birth weight, as well as childhood, adolescence and adulthood body mass index (BMI) for reproductive capacity?

Summary answer: Children and adolescent girls with obesity have increased risk of infertility and childlessness in adulthood. The association seems to be partly independent of adult BMI.

What is known already: Previous studies have shown inverted J-shaped relationship between BMI during adolescence and the number of children later in life. Furthermore, obese girls (BMI95thpercentile) display more menstrual irregularities and can be more affected with infertility problems and inability to become pregnant compared to their normal weight (NW, BMI:5-85thpercentile) counterparts. Timing of adiposity peak (AP, maximum infant BMI at around 9 months) and of adiposity rebound (AR, second rise of BMI at 5-7years) is associated with obesity and unfavorable metabolic profile in adulthood. However, follow-up studies through the whole reproductive life span on the relationship between growth patterns and fertility/parity are limited.

Study design, size, duration: We study data from 5889 women born in 1966 and followed-up from gestational week 24 to age 46.

Postal questionnaires at ages 31 and 46 (answer rates 81% and 72%) addressed questions on infertility problems and treatment. The fecundability ratio (FR) was defined as the probability of pregnancy within 12 months of pregnancy attempt. Self-reported infertility at age 46 ('Have you ever had infertility problems?") and/or self-reported infertility treatments at both ages were asked.

Participants/materials, setting, methods: Prenatal and BMI data were collected by trained personnel at all stages. AR and AP were assessed using REED1 model (infancy, N = 2858, and childhood, N = 3471, separately). BMI Z-scores (for 17 time-windows from ages 2 to 31) were used to model developmental trajectories. Four latent BMI categories were identified. Deliveries were collected from the Finnish birth register. All models were adjusted for education level, marital and socioeconomic status, and smoking.

Main results and the role of chance: Mother's BMI or weight gain during pregnancy as well as birthweight, age at AP, AR or menarche were not associated to problems of infertility or number of deliveries. Compared with NW, obesity at adrenarche and menarche was associated with decreased FRs at age 31 (12.5% vs. 20.6%, OR:1.77, 95% CI [1.04-3.02] and 12.8% vs. 20.4%, OR:1.74 [1.07-2.82], respectively). In comparison to NW group, obesity at menarche, but not at adrenarche, positively associated with more self-reported

infertility (15.4% vs. 28.4%, OR:1.48[1.05-2.08]) and nulliparity (18.3% vs. 23.9%, OR:1.47[1.01-2.13]), but not with self-reported infertility treatments. Women with obesity at menarche and at age 31, but not at age 46, had less deliveries than their NW counterparts (1.80 vs. 2.07, p = 0.023; 1.87 vs. 2.06, p = 0.048, respectively). All those results remained statistically significant after adjusting.

The women in the two highest BMI trajectory groups (Group 3: 'slow weight gainers'; 14.0%) Group 4: 'constant high'; 2.3%) exhibited higher risk for low FRs compared to NW control group (Group 4: OR:3.03[1.22-7.56]); Group 3: OR:1.49[0.97-2.31]). They also tend to have increased risk of self-reported infertility (Group 4 OR:1.64[0.79-3.41]; Group 3: OR:1.49[1.02-2.05]) and nulliparity (Group 4:OR:1.90[1.13-3.19]; Group 3:OR:1.40[1.08-1.81]) and had fewer children (Group 4:1.76 (p = 0.056); Group 3:1.84 (p = 0.002) vs. NW:2.09).

Limitations, reasons for caution: Despite high participation rates throughout all collection points, children's growth data was not available for all participants due to delay in digitalization of hand written healthy records.

Wider implications of the findings: This study reveals that girls affected by obesity during childhood and at adolescence might display reduced fertility capacity in adulthood, partly independently of adulthood obesity. These results support the importance of an active prevention and treatment of obesity already in early childhood.

Trial registration number: not applicable.

P-670 Evaluating the impact of ovarian stimulation on endometrial receptivity and reproductive outcome - A sibling oocyte study

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Study question: Does " freeze all " policy improve pregnancy rate?

Summary answer: No, fresh transfer has equal or even better results than frozen transfer.

What is known already: Clinical observations that frozen embryo transfer (FET) reduces the risk of ovarian hyper stimulation syndrome and improves endometrial receptivity has been supported by literature. Evidence which has tilted the balance strongly in favor of deferring fresh transfer. Before we make 'freeze all" a norm, it is important to evaluate its effectiveness.

Study design, size, duration: A sibling oocyte study provides a unique window of opportunity to test this hypothesis by comparing outcomes in ESP (Egg Sharing patient) & ERP (Egg Receiving Patient). This is a prospective study conducted at a tertiary level infertility centre during June 2015 - June 2017.the study include 203 ESP and 276 ERP.

Participants/materials, setting, methods: In this study, the data from oocyte sharing of 203 patients (ESP), all stimulated by conventional long protocol (GnRH agonist) and followed by controlled ovarian stimulation with HMG (150IU-300IU) were included. The oocytes were shared among ESP and 276 anonymous recipients (ERP) after endometrial preparation with HRT.

Main results and the role of chance: This study revealed that pregnancy rate was statistically higher (p = 0.0003) in fresh transfer in ESP than fresh transfer in ERP (70.9% vs 53.2%). The pregnancy rate was statistically higher (p = 0.028) in fresh transfer in ESP than frozen transfer in ESP (70.9% vs 55.7%) with no significant difference (p = 0.588) in mean E2 between positive and negative pregnancy rate. It was observed that pregnancy rate was statistically higher (p = 0.03) in frozen ERP than fresh ERP (67.6% vs 53.2%). The pregnancy rate between fresh ESP and fresh transfer deferred ESP (70.9% vs 78.6%) was similar (p = 0.540).The incidence of OHSS in ESP was mild (4.4%), moderate (1.47%) and severe (0.98%).

Limitations, reasons for caution: The study group was small in no. We require larger groups for better comparison. Also other confounding factors in ERP were not taken into account during the study.

Wider implications of the findings: The statistical analysis of this study showed a better pregnancy rate among fresh transfer in ESP than in ERP or with frozen transfers ESP there by disproving the concept of endometrial affliction in cases of stimulated cycles and alleviating the need to freeze all.

Trial registration number: not applicable.

P-671 Random-start Ovarian Stimulation in Egg donors (ROSE trial). A self-controlled randomized pilot study

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Study question: Can we expect a similar oocyte yield after randomly initiating ovarian stimulation during the follicular phase when compared to conventional protocols performed in the same oocyte donors?

Summary answer: The number of total and MII oocytes from random-start protocols initiated during the follicular phase appears similar to conventional ovarian stimulation start protocols in egg-donors.

What is known already: Random-start ovarian stimulation protocols has been well studied in women with cancer for fertility preservation and, more recently, in women who desire 'elective" cryopreservation of oocytes, resulting in optimal outcomes. In the context of an egg donation program, if ovarian stimulation can be initiated irrespective of the phase of the menstrual cycle without adversely impacting oocyte yield or quality, the approach may facilitate schedules.

Study design, size, duration: Single center, prospective, randomized, clinical trial performed in egg donors during year 2017. The study group (15 participants) started ovarian stimulation randomly at different moments throughout the follicular phase, according to a list of random allocation of treatments starting at day 5,7,9,11 or 13 of the menstrual cycle (three participants allocated to each day). Controls: each patient served as her own control comparing previous cycle within 6 months under conventional initiation of stimulation.

Participants/materials, setting, methods: The study group started ovarian stimulation randomly at days 5,7,9,11 or 13 of the cycle. Participants received urinary FSH (Fostipur®) 150-225 IU/d, cetrorelix 0.25 mg/d (Cetrotide®) started five days after ovarian stimulation and triptorelin 0.2 mg (Decapeptyl®) induced the final follicular maturation. Controls: previous cycle by the same egg donor under conventional initiation of stimulation (cycle day 2/3), using the same gonadotrophin and dose. 28 cycles in total were included in the analysis. There was 01 drop-out.

Main results and the role of chance: As a whole, among young egg donors (mean age: 24.8 ± 4.5 y.o.); the results were comparable between study group vs controls with regards to: number of days of stimulation (9.2 ± 1.3 vs 10 ± 1.5 p = 0.2); gonadotrophin (IU) consumption (1898 ± 457 vs 2065 ± 808 p = 0.5); number of collected eggs (19 ± 9.2 vs 21 ± 12.2 p = 0.6) and proportion (%) of mature eggs (82 ± 10 vs 85.8 ± 5.5; p = 0.2) respectively. Additionally, the fertilization rate (ICSI%) was also similar (71 ± 7.8 vs 69.6 ± 8.7 p = 0.3).

Limitations, reasons for caution: Small number of donors included per specific day of started stimulation. These results might be extrapolated to other group of IVF patients (e.g. young women with tubal or male factor infertility) although caution is warranted since laboratory outcomes beyond fertilization were not analyzed. Future trials should address this point.

Wider implications of the findings: As ovarian stimulation can be initiated regardless of the day during the follicular phase of the menstrual cycle without adversely impacting oocyte yield or fertilization rate in egg donors, the approach may facilitate schedules in egg donation programs.

Trial registration number: NCT02821819.

P-672 Validation of a novel ELISA assay for serum AMH determination

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Study question: Validation of the novel Euroimmun MRH/AMH ELISA using the Gen II ELISA by Beckmann Coulter (GenII-BC) as a standard.

Summary answer: The novel EU AMH ELISA achieves adequate performance as compared to established GenII-BC ELISA and is suitable for broader utilization in clinical and research settings.

What is known already: Serum Anti-Mullerian Hormone (AMH) levels correspond to ovarian function and reserve. Clinically, AMH levels are routinely assessed in infertility treatment to estimate the prognosis and risk profile of a given woman.

Different assays have been developed for serum AMH assessments. Several studies demonstrated significant variations of AMH values depending on the ELISA kit used, casting doubts on the validity of AMH measurements in a clinical scenario.

Recently, the company Euroimmun (Luebeck, Germany) developed a novel MRH/AMH ELISA, based on a combination of two highly specific antibodies, different from the antibodies utilized for the GenII-BC

Study design, size, duration: This was a prospective, single-center study. AMH values were generated with/without buffer pre-mixing. Furthermore, different lots of the GenII-BC were tested as reference. The novel Mullerian-duct repression hormone (MRH/AMH) ELISA from Euroimmun (referred to as EU AMH ELISA herein) is based on the combination of two highly specific antibodies. Quantitative measurement is performed at 450 nm wavelength. Results are expressed as nanogrammes per milliliter (ng/ml).

Participants/materials, setting, methods: Frozen serum samples from 121 infertile female patients were analysed by the Euroimmun AMH ELISA and GenII-BC assay, respectively. To study assay agreement, a Passing-Bablok regression model and a Bland-Altman analysis were performed.

Main results and the role of chance: There is no difference between the AMH values generated with or without pre-mixing of probes with assay buffer in either the GenII-BC (Passing-Bablok equation: $y = 1.13 + 0.03\times$, 95% confidence intervals (CI): 1.10. - 1.18, intra class correlation coefficient (ICC) = 0.98, 95% CI: 0.88–0.99, Rc = 0.97, 95% CI: 0.96–0.99; n = 24) or in the EU-AMH ELISA (Passing-Bablok equation: $y = 1.06 + 0.04\times$, 95% CI: 1.03. - 1.10, ICC = 0.99, 95% CI: 0.97–0.99, Rc = 0.99, 95% CI: 0.98–0.99; n = 19).

The Passing-Bablok equation shows adequate performance of the EU-AMH ELISA ($y = 1.17–0.05\times$, 95% CI: 1.12–1.23; n = 121) compared to GenII-BC ELISA. The analysis confirms a linear association (p = 0.51 for non-linear association) and prove adequate correlation of the assays (ICC = 0.96, 95% CI: 0.92–0.98 and Rc = 0.93, 95% CI: 0.90–0.95; n = 121).

Limitations, reasons for caution: It is in the nature of a comparison of two different ELISA assays that an influence of the GenII-BC ELISA on assumed outliers measured by the EU-AMH ELISA cannot be excluded without comparison of these values with those of further ELISA assays.

Wider implications of the findings: The EU-AMH ELISA shows good performance in measuring serum AMH levels. Assessing the intra-assay variation the ELISA revealed a high reproducibility between different standard- and kit-lots. The present study could not reveal an influence of pre-mixing on the reproducibility of measured AMH values which is in contrast to previous investigations.

Trial registration number: not applicable.

P-673 Is there any predictive factor of resistance to clomiphene citrate (CC) for ovulation induction in polycystic ovarian syndrome (PCOS)?

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Study question: To study whether clinical, biological or ultrasonic characteristics of PCOS patients with ovulation disorder can predict total or partial resistance to ovulation induction by CC.

Summary answer: Only BMI was weakly predictive. No other factor could discriminate patients with total or partial resistance to CC from sensitive patients.

What is known already: PCOS is the most common endocrine disorder in women of childbearing age. It can be responsible for ovulation disorder and infertility. The Thessaloniki consensus defines CC as the first-line treatment for ovulation induction in PCOS patients seeking a pregnancy. It is an efficient, easy and low cost treatment. The cumulative pregnancy rate was 70 % after 6 cycles. 20 % of the patients are resistant to CC, either at the starting dose (50 mg) or 100 mg (partial resistance) or at the maximum dose of 150 mg (total resistance). Literature is still conflicting about factor(s) predicting total/partial resistance to CC.

Study design, size, duration: This large retrospective study was conducted between March 1999 and November 2017 in a single academic department of Reproductive Medicine. Patients included were diagnosed with PCOS, as defined by the Rotterdam consensus. Only patients with ovulation disorder (phenotypes A, B, D) and undergoing CC treatment for ovulation induction were included.

Participants/materials, setting, methods: 312 patients were included (1,012 cycles). To determine if the treatment was effective, an ultrasound was performed on D12, looking for a dominant follicle. A progesterone assay was conducted on D24 and ovulation was attested if progesterone was $\geq$ 5 ng/mL. Resistance to CC was defined by absence of ovulation. Four groups were formed according to CC resistance profil: no resistance, resistance to 50 mg, resistance to 100 mg, resistance to 150 mg (total resistance).

Main results and the role of chance: Compared to the group "no resistance" (n = 153), partially and totally CC-resistant patients (n = 249) were more likely to be amenorrheic (p<0.02), and had ranks of higher BMI (p<0.005) and waist circumference (p<0.005). Biologically, in the latter group, baseline serum AMH levels were significantly higher (p<0.02), as well as D4-androstenedione (p<0.02) and fasting insulin levels (p<0.01). SHBG levels were significantly lower (p<0.001). All those parameters increasingly varied between the 3 groups of resistance, although not significantly. By discriminant analysis, only one model including only BMI could significantly (p<0.0001) distinguish between sensitive and resistant patients. By ROC analysis including either BMI, AMH or D4-androstenedione, no discriminating threshold could be obtained to predict CC resistance with satisfactory sensitivity and specificity.

Limitations, reasons for caution: The main limitation of our study was its retrospective design, responsible for bias and missing data. But to our knowledge, this is the largest cohort of PCOS patients monitored for CC in the same hospital, making the evaluation more reliable.

Wider implications of the findings: Our data confirm that no parameter can be used to decide whether skipping or not the step of CC treatment or to choose the starting dose in anovulatory patients with PCOS. However, the association between BMI and CC resistance should encourage the management of obesity before and during treatment.

Trial registration number: Not applicable.

P-674 The ovarian response after follicular versus luteal phase stimulation with a double stimulation strategy

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Study question: Is the ovarian response after stimulation performed during the follicular phase (FP) versus the luteal phase (LP) with a double stimulation strategy equally efficient?

Summary answer: Stimulation during the LP in a double-successive stimulation strategy results in a lower ovarian response as compared to the FP equivalent daily dose stimulation.

What is known already: Double ovarian stimulation is an option for enhancing oocyte recruitment when a single stimulation is not sufficient, or when there is limited time for assisted reproductive technology, such as with fertility preservation. It is also a viable option for dealing with an insufficient ovarian response that can sometimes be encountered with ART, notably in poor responders. Nonetheless, there is still controversy regarding the equivalence and effectiveness of luteal phase controlled ovarian stimulation (COS) as compared to the previous follicular phase stimulation with a double stimulation strategy.

Study design, size, duration: This was an observational cohort study using data collected in a continuous series of patients scheduled for a double stimulation protocol between March 2014 and June 2017 at our institution.

Participants/materials, setting, methods: Women starting a FP (COS1) and a LP stimulation (COS2) in a double-stimulation protocol were enrolled. Both the COS1 and the COS2 used equivalent daily doses of gonadotropins in combination with GnRH-antagonist. Ovulation was triggered using GnRH-agonist. The number of oocytes retrieved, the oocyte maturation rate, the duration of stimulation, the total dose of injected gonadotropin, and the estradiol level at triggering were collected so as to compare the extent of the ovarian response.

Main results and the role of chance: A total of 77 patients were included in the analysis. The number of oocytes retrieved (5.25 ± 3.38 for COS 1 versus 3.83 ± 3.14 for COS 2; p = 0.001), as well as the number of MII oocytes (4.49 ± 3.05 for COS 1 versus 3.25 ± 2.86 for COS 2, p = 0.003) after COS 1 were significantly higher than after the COS 2. There were no significant differences in regard to the oocyte maturation rate between the two stimulations. The duration of the stimulation was significantly shorter (9.71 ± 1.46 days for COS 1 versus 11.25 ± 1.82 days for COS2; p < 0.001), the total dose of injected gonadotropins was significantly lower (2,916.23 ± 439.68 for COS 1 versus 3,374.03 ± 546.62 for COS 2; p < 0.001,) and the estradiol level on the trigger day was significantly higher (1,135.99 ± 824.28 pg/mL for COS 1 versus 661.42 ± 477.74 pg/mL, for COS 2; p < 0.001) with COS 1 as compared to COS 2.

Limitations, reasons for caution: The present study suffers from the limitation of its design involving a retrospective analysis of a prospective cohort. However, in this cohort study, numerous epidemiological variables were collected prospectively through face-to-face interviews before stimulation.

Wider implications of the findings: The two phases (FP and LP) were not equally efficient with a double stimulation strategy. Aside from in an emergency situation, a double stimulation strategy does not appear to be more effective than two separate conventional follicular phase stimulations. Further investigations are required, however, to confirm these findings.

Trial registration number: None.

P-675 A randomized, controlled trial (RCT) of AMH-based individualized ovarian stimulation for in vitro fertilization

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Study question: Does an individualized Anti-Müllerian hormone (AMH) based follicle stimulating hormone (FSH) dosing in an antagonist protocol increase the proportion of patients with an optimal number of oocytes?

Summary answer: The AMH-based individualized dosing algorithm did not optimize the number of oocytes mainly due to insufficiency of 100 IU/day of FSH in predicted hyper-responders.

What is known already: Individualizing FSH dosing for ovarian stimulation can theoretically improve the balance between benefits and risks. Current data suggest a reduced risk of ovarian hyperstimulation syndrome (OHSS), but no evidence of increased pregnancy or live birth rates. AMH is known as a good marker of ovarian response, but data are limited on how to implement the use of this predictive factor into clinical practice. Only a few RCTs have used AMH-based algorithms to optimize the FSH dosing in ovarian stimulation.

Study design, size, duration: A dual-center open-label RCT conducted January 2013 – November 2016. Eligibility was assessed in 269 women and 221 were randomized 2:1 between study and control in an antagonist cycle. Women with high AMH (≥25 pmol/L) had minimal stimulation (rFSH 100 IU/day); women with medium AMH (12 – 24 pmol/L) standard stimulation (rFSH 150 IU/day), and low AMH (<12 pmol/L) had maximum stimulation (corifollitropin). The control group had 150 IU/day of FSH irrespective of pretreatment AMH.

Participants/materials, setting, methods: All women had a presumed normal ovulatory menstrual cycle, were aged 25–38 years, weighed <75 kg, had 1st IVF or ICSI with pretreatment AMH ranging from 4–40 pmol/L and had two ovaries accessible to oocyte retrieval. Recruitment was conducted from both participating sites. Women with anovulatory PCOS, endometriosis grade III/IV, hydrosalpings on ultrasound, recurrent miscarriages (≥3) and FSH > 12 IE/L were excluded. All transfers were single blastocyst transfers.

Main results and the role of chance: After randomization 149 women comprised the study group and 72 the control group. The groups had a similar distribution of basal AMH levels. The primary outcome of predefined optimal number of 5-14 oocytes was achieved in 72% of the study and 78% of the controls. This difference was not significant. In the high AMH stratum of the study group given 100 IU rFSH/day 38% had an insufficient (<5) number of oocytes, compared with 6% among the controls given 150 IU rFSH/day (p = 0.03). In the low AMH stratum the study group given corifollitropin had a nonsignificant tendency towards more optimal responses of 73% compared with 53%in the control group. Dose reduction was needed in 2 women (one in each study arm) and cycle cancellation due to insufficient response occurred in 3 (2 in the study and 1 in the control).OHSS was diagnosed in 4 women (2 in each group). Participants answered a daily questionnaire during the luteal phase describing subjective well-being in terms of abdominal distention, abdominal pain, dyspnea and weight. No differences were found between groups. The live birth rates per started cycle were similar (28% and 29%) in the study vs. control group.

Limitations, reasons for caution: This study was powered to determine differences in the distribution of number of oocytes retrieved in the study and control groups, but not to assess possible risk reductions of OHSS as larger data sets would be needed.

Wider implications of the findings: In the high AMH stratum expected to be at risk for excessive responses, 100 IU/day of rFSH seems insufficient when using the antagonist protocol. Further studies should investigate whether 125 IU/day would be better to balance benefits in terms of number of oocytes versus OHSS risk.

Trial registration number: EUDRACT number 2012-004969-40

P-676 Telomere length and telomerase activity in cumulus cells of women with Polycystic Ovarian Syndrome

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Study question: What are the implications of Polycystic Ovarian Syndrome (PCOS) on telomere length and telomerase activity in cumulus cells?

Summary answer: Telomere length in cumulus cells did not differ between PCOS and controls. However, telomerase activity in cumulus cells was higher in the PCOS.

What is known already: Women with PCOS, despite responding well to Assisted Reproduction Treatments (ART), the oocytes usually have low quality and reproductive capacity, which is correlated to oocyte interaction with cumulus cells, in the cumulus-oocyte complex. The low oocyte quality may be related to loss of genomic stability of oocytes, or even cumulus cells, and may lead to a reduction of female fertility. The telomere length and telomerase activity play a fundamental role in maintaining genomic stability, which is considered an important molecular marker of cell viability,

whose alterations are related to apoptosis and/or senescence, maybe also an indicative of oocyte quality.

Study design, size, duration: A prospective case-control study, in which 38 women with PCOS and 61 controls were included from September 2015 to June 2017.

Participants/materials, setting, methods: Age, body mass index (BMI) were measured. Follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, prolactin, insulin, androstenedione, testosterone, free androgen index (FAI), c-reactive protein and homocysteine were evaluated. Telomere length in cumulus cells from immature (ICC) and mature (MCC) oocytes, and leukocytes were evaluated by quantitative real-time polymerase chain reaction (qPCR). Telomerase activity from cumulus cells was evaluated by TRAPeze® XL Kit. Statistical analyses were determined by Wilcoxon test and Spearman correlation.

Main results and the role of chance: Age mean was 33.74 (±4.12) in PCOS and 34.33 (±3.63) in controls (p=.509). The variables BMI (p=.001), LH (p=.015), estradiol (p=.004), insulin (p=.002), testosterone (p<.0001), androstenedione (p=.001), FAI (p<.0001) and c-reactive protein (p=.003) was increased in PCOS group. FSH (p=.0002) was smaller in PCOS group. Prolactin and homocysteine were not different between the groups. The telomeres length in ICC did not differ between PCOS and control groups (1.60 ± 0.56 vs 1.58 ± 0.33; p=.649, respectively). The telomeres length in MCC did not differ between PCOS and control groups (1.61 ± 0.47 vs 1.70 ± 0.43; p=.378, respectively). However, in leukocytes reduced telomeres were observed in the PCOS (p=.025), respectively. The telomerase activity in ICC was higher in the PCOS group than in the control group (1.62 ± 1.49 vs 0.30 ± 0.42; p=.003, respectively). The telomerase activity in MCC was higher in the PCOS group than in the control group (1.39 ± 1.63 vs 0.55 ± 0.84; p=.022, respectively). A positive correlation between telomerase activity and telomere length in the ICC was observed in control group (p=.051). PCOS also presented a positive correlation between telomerase activity and telomere length in MCC (p=.048). Telomere length of leukocytes, ICC and MCC were a positive correlated in both groups.

Limitations, reasons for caution: The limited number of cases of the study group. An increase of sample size is recommended to make the data more robust and confirm the results.

Wider implications of the findings: PCOS seems not to affect telomere content in ICC and MCC, only leukocytes telomeres were reduced in this syndrome. On the other hand, higher telomerase activity ICC and MCC may be required for telomere maintenance in the PCOS.

Trial registration number: N/A.

P-677 HECTD1 influences endocytosis of EGFR through APPL1

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Study question: Whether HECTD1, a newly identified E3-ligase, influences endocytosis of membrane-bound hormone receptors (such as the epidermal growth factor-receptor, EGFR) and how is it regulated?

Summary answer: HECTD1 inhibits degradation of EGFR and thereby influences EGF-signalling pathway. It regulates the recycling pathway through APPL1

What is known already: HECTD1, also known as EULIR, denominates a HECT domain containing E3 ubiquitin ligase. HECTD1 interacts with several membrane receptors, including LH-receptor, FSH-receptor and EGFR. APPL1 is a subpopulation of early endosomes, which regulates the endocytosis pathway.

Study design, size, duration: prospective experimental study, lasting 2 years.

Participants/materials, setting, methods: In HeLa cells HECTD1 expression level is downregulated by HECTD1-specific shRNA. The stable cell lines were selected by puromycin. Immunofluorescence and FACS were used to study the receptor trafficking. EGF receptor degradation and EGF signalling pathway activation were examined by Western blot. Immunofluorescence was used to localize APLL1.

Main results and the role of chance: Our studies demonstrated that HECTD1-knockdown inhibits degradation of EGFR and influences signalling of EGF. Activated MAPK signalling pathway is prolonged. APPL1 localizes in the cytoplasm, under the plasma membrane and in the centrosome. HECTD1 co-localizes with APPL1 6 minutes after EGF-stimulation. After HECTD1-knockdown, APPL1 co-localizes with EGF less in cytoplasm but appears close to the plasma membrane.

Limitations, reasons for caution: Basic experimental research results may not be fully appreciated in complete systems.

Wider implications of the findings: Understanding the regulation of endocytosis of membrane-bound receptors through HECTD1 may help to understand local and temporal differences in sensitivity to endocrine regulation.

Trial registration number: None.

P-678 Cumulative live birth rates (CLBR) after in vitro maturation (IVM) of oocytes in patients with polycystic ovary syndrome (PCOS): does PCOS phenotype have an impact?

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Study question: Which parameters are predictive of CLBR after non-hCG triggered serum-free IVM and is there an impact of the PCOS phenotype?

Summary answer: After IVM, CLBR do not vary significantly between PCOS phenotypes. Calculated free testosterone (FTc) and number of fertilised oocytes are independently correlated with CLBR.

What is known already: In patients with PCOS undergoing conventional assisted reproductive technology (cART), PCOS phenotype is an independent predictor of CLBR. Hyperandrogenic phenotypes of PCOS have significantly poorer cumulative outcomes compared to their normoandrogenic counterparts and compared to patients with polycystic ovarian morphology (PCOM). IVM is a mild-approach, OHSS-free ART alternative and can be offered to patients with polycystic ovaries. Whether clinical outcomes of IVM treatment are influenced by the PCOS phenotype is currently unknown.

Study design, size, duration: Retrospective cohort study of all patients who underwent their first IVM cycle in a university-based tertiary clinic between April 2014 and December 2016. Patients who underwent fertility preservation or preimplantation genetic diagnosis were excluded. In total, 258 cycles were analysed, in 240 of which the cumulative outcome was known.

Participants/materials, setting, methods: Patients with PCOM, PCOS A (hyperandrogenism + ovulatory dysfunction + polycystic ovaries), C (hyperandrogenism + polycystic ovaries) and D (ovulatory dysfunction + polycystic ovaries) between 20-41 years-old underwent non-hCG triggered IVM (30 h) after a short HP-hMG course. Fresh blastocyst transfer was planned if ≥4 good-quality d3-embryos were available. Otherwise, embryos were vitrified at cleavage-stage and transferred in an artificial cycle. Androgens and SHBG levels were measured at intake and cumulative live birth rates were calculated.

Main results and the role of chance: Mean age, body mass index, and antimüllerian hormone concentration were 28.7 ± 3.3 years, 25.0 ± 5.2 kg/m2, and 10.3 ± 6.2 ng/mL, respectively. Mean amount of COCs retrieved, metaphase II oocytes after IVM and 2PN oocytes after ICSI were 20.2 ± 13.0, 9.1 ± 6.4 and 6.0 ± 4.9, respectively. A total of 77 infants were born, all of which were singletons. Overall, CLBR per started cycle was 32.1% (77/240) and CLBR did not vary significantly between PCOS phenotypes, ranging from 23.1% (3/13) in PCOS-C, 31.8% (35/110) in PCOS-D to 37.1% (36/97) in PCOS-A (p = 0.2). In patients with PCOM, CLBR was only 15% (3/20). None of the patients developed OHSS.

Multiple binary logistic regression analysis showed that serum FTc levels and number of 2PN oocytes, with unstandardized coefficients (95% CI) of 0.732 (0.613–0.874) (P = 0.001) and 1.183 (1.076–1.299) (P<0.001), respectively, are strong independent predictors of cumulative live birth.

Limitations, reasons for caution: The relative small sample size of PCOS phenotype C and PCOM may preclude robust conclusions regarding CLBR after IVM in these subpopulations. Other variables, such as needle type, aspiration technique and IVM culture system may also contribute to IVM outcome.

Wider implications of the findings: Cumulative outcomes after HP-hMG primed, non-hCG triggered IVM in selected patients with PCOS are strongly influenced by the endocrine profile of the patient. Whether medical or lifestyle interventions that result in reduced free testosterone levels could improve IVM success rates in hyperandrogenic patients merits further scrutiny.

Trial registration number: not applicable.

P-679 Artificial frozen embryo transfer cycles: how important are the circulating estradiol (E2) levels for live birth rates?

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Study question: Do artificial frozen embryo transfer (FET) cycles require endocrine monitoring for E2 prior to commencing exogenous P administration?

Summary answer: We found no association between late-proliferative phase serum E2 levels and livebirth rates in artificial FET cycles.

What is known already: The use of FET has progressively increased and the need for endocrine monitoring during the proliferative phase in artificial cycles remains unclear. In several studies, late-proliferative phase serum E2 and luteinizing hormone (LH) levels did not seem to predict outcome, however, others have found elevated proliferative serum E2 may negatively impact live birth rates after artificial FET.

Study design, size, duration: All artificial FETs performed in a tertiary centre between 2010-2015 were included in this retrospective study (n = 1341). The same artificial protocol was performed in all cases and blood sampling with a hormone measurement essay was performed 1 or 2 days before exogenously inducing the luteal phase. These cycles were subdivided into 4 groups according to the 25%, 50% and 75% percentiles for late-proliferative serum E2 levels: ≤190 pg/ml (n = 341), 191-257 pg/ml (n = 331), 258-355 pg/ml (n = 338) and ≥356 pg/ml (n = 331).

Participants/materials, setting, methods: We performed mixed-effects multilevel multivariable regression to assess the potential effect of late-proliferative E2 on livebirth rates while adjusting for the following potential confounders: female age, prior livebirths, IVF/ICSI cycle rank, body mass index (BMI), presence of irregular cycles, number of good quality embryos produced, blastocyst versus cleavage stage transfer, total number of days of E2 supplementation, endometrial thickness, need to step-up the administration of E2, and the late-proliferative serum LH and progesterone levels.

Main results and the role of chance: There were no significant differences between these groups in terms of age (31.9 ± 4.7), IVF/ICSI cycle rank (1.8 ± 1.3), number of oocytes retrieved (15.4 ± 9.2), BMI (25.2 ± 5.2), number of prior livebirths (0.4 ± 0.6), blastocyst vs cleavage stage transfer (65.1% vs 34.9%), total dose of E2 administered (76.1 ± 36.1 mg of estradiol valerate) and endometrial thickness (8.5 ± 2.2 mm). There were, however, statistically significant differences among the 4 study groups in terms of number of embryos produced after the fresh cycle (5.2 ± 2.9, 5.3 ± 3.2, 5.6 ± 3.2, 5.7 ± 2.9, p<0.001), the presence of irregular cycles (59.1%, 61.2%, 53.6%, 50.2%, p = 0.016), the need for a exogenous E2 administration step-up (21.7%, 13%, 14.8%, 19.9%, p0.015) and the total number of days of E2 supplementation (14.4 ± 3.7, 13.8 ± 3.2, 13.7 ± 2.8, 14.2 ± 3.3, p = 0.023). Following both univariable and multivariable regression analysis, the level of late-proliferative circulating E2 showed no significant difference in terms of livebirth rates after FET (15.2%, 16.7%, 18.8% and 16%, p = 0.662).

Limitations, reasons for caution: The interpretation of the findings of this study is limited by the retrospective nature of the analysis and the potential for unmeasured confounding.

Wider implications of the findings: According to this large retrospective dataset, the late-proliferative circulating levels of E2 in artificial FET cycles are not associated with the subsequent livebirth rates and, thus, monitoring these levels and using them to guide clinical decision-making (e.g. medication step-up, cycle prolongation or cancelation) is of questionable value.

Trial registration number: NA.

P-680 Changes in the management of poor-responders patients after internal analysis of IVF outcomes according to the Poseidon criteria

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Study question: How did the internal analysis of IVF outcomes in poor-responders patients according to the Poseidon criteria help us to change our management?

Summary answer: Management of poor responders consists to start ovarian stimulation with high-dose of FSH and to cancel the cycle if less than 4 follicles are obtained.

What is known already: The chances of achieving a live birth in the poor responders are low. The definition of poor ovarian responders according to the Bologna criteria is unclear because it mixes different ovarian profiles. That is why the Poseidon group has proposed a new classification defining 4 categories of patients. To our knowledge, no study has reported so far the IVF outcomes according to this new classification.

Study design, size, duration: This is a single-center observational study performed from January 2016 to 2017. 2519 cycles from patients aged between 18 and 42 years old were included. The exclusion criteria were extreme oligoasthenoteratospermia and genetic diseases; classification according to the 4 groups described by the Poseidon classification.

Participants/materials, setting, methods: Ongoing pregnancy (OPR) and miscarriage rates were analyzed according to Poseidon classificationGroup 1 (<35 years, AMH > 2 ng/mL) 498 cycles: 1 A (<4 oocytes) 23 cycles; 1B (4 to 9 oocytes) 165 cycles Group 2 (> 35 years, AMH > 2 ng/mL) 638 cycles: 2 A (<4 oocytes) 64 cycles; 2B (4 to 9 oocytes) 181 cyclesGroup 3 (<35 years, AMH <1.2 ng/mL and AFC<5): 99 cyclesGroup 4 (> 35 years, AMH <1.2 ng/mL and AFC <5): 730 cycles.

Main results and the role of chance: In the good prognosis groups (Group 1 and 2), the pregnancy rate was low in case of few oocytes collected (group 1 A, the OPR is 28.5% (2/7), no miscarriage was reported. In group 2 A, we had no evolutive pregnancy (0/30) and the miscarriage rate was 100% (2/2)). When 4 to 9 oocytes were obtained, the OPR was better in younger group (group 1B, OPR was 28.4% (35/123), miscarriage rate 20.5% (9/44); In group 2B, OPR was 21.4% (28/131), miscarriage rate 39.1% (18/46)). In the worst prognosis groups (Group 3 and 4), regardless of age, OPR was low (group 3, OPR was 7.7% (5/65), miscarriage rate 55% (6/11); group 4, OPR was 16.1% (37/230), miscarriage rate 35.1% (13/37)). in these groups, OPR was not improved by natural cycles compared to high stimulated cycles in the older patients (Group 3: 73 stimulated cycles, OPR was 7.4% (4/54), miscarriage rate 60% (6/10);12 natural cycles, OPR was 25% (1/4), no miscarriage. Group 4: 615 stimulated cycles, 468 pick-up, OPR 16.1% (30/186), miscarriage rate 43.3% (13/30); 115 spontaneous cycles, 96 pick-up, OPR 15.9% (7/44), no miscarriage).

Limitations, reasons for caution: This is an observational retrospective study and the protocols and strategies were specific to each clinician. Some subgroups are too small.

Wider implications of the findings: The Poseidon classification allows to modify medical practice: a maximum number of oocytes is essential because rate of euploid oocytes is constant; the cancellation of a poor cycle allows to propose a stronger appropriate stimulation to recover as many oocytes as possible. Couples can be informed on the expected results.

Trial registration number: not applicable.

P-681 Does the change in needle diameter effect oocyte quality and embryo morphokinetics? A prospective randomized pilot study with sibling oocytes

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Study question: Does the change in needle diameter along the 17-20 G during oocyte collection effect oocyte quality and embryo morphokinetics in compare to 17 G needle?

Summary answer: Beside higher negative value in oocyte score in 17 G needle, no other differences were demonstrated in terms of fertilization, embryo quality or morphokinetics.

What is known already: A smaller diameter needles yielded significant improvement in recovery after ovum pick-up, as well as minimizing bleeding during the procedure and reducing pain afterwards, various needles are available in the field of in vitro fertilization, with the aim of creating a needle that reduces pain without diminishing its stability and ease of operation, a variable diameter 20-17 G needle was introduced to the market (Vitrolife Sweden AB, Goteborg, Sweden). And was found significant decreases in pain and vaginal bleeding compared to the 17 G needle, without affecting the oocyte recovery rate, damaging oocytes or affecting fertilization and clinical pregnancy rates.

Study design, size, duration: Prospective, randomized, blinded pilot study comparing sibling oocytes. 20 patients were prospectively enrolled from August 2016 through January 2017.

Participants/materials, setting, methods: Women undergoing IVF-ICSI, 21 to 39 years old, FSH≤10 IU/l and antral follicular count (AFC) >10 follicles. Comparing oocyte collected with 2 different aspiration needles (17 G/35 mm and 20-17 G/35 mm) in the same patients-Sibling oocytes). Oocyte quality (judged by 5 parameters based on previous publication) and embryo grading including morphology and morpho-kinetics were evaluated. The embryologist was blinded to the needle used for each ovary.

Main results and the role of chance: A total of 256 oocytes were collected, 118 oocytes in the 17 G needle group and 116 in the 20-17 G group. Aspirating with the 17 G needle yielded more oocytes with higher negative score per oocyte compared with the oocytes collected with the 20-17 G needle (2.2 ± 1.39 vs. 1.87 ± 1.69; P = 0.02). Oocytes collected in the cohort with degenerative oocytes (DEG group) immediately after OPU had poorer performance compared to their sibling oocytes from the cohort with no degenerative oocytes(nonDEG group).the DEG group had poorer oocyte scores compared to the nonDEG group(-2.4 ± 1.73 vs. -1.87 ± 1.48; P = 0.02; 95%CI -1.02 to -0.09). Overall fertilization rate was poorer for the DEG group of patients 56.1% versus 70.7% for the nonDEG group (P = 0.05).

However, the amount of good quality embryos were comparable between the DEG vs nonDEG (65.6% vs. 66.4%, respectively).

Furthermore, sub analyzing the cohort with the degenerative oocyte according to the aspiration needle size did not affect fertilization rates or morphokinetic scores (KIDS and alfa ESHRE).

A logistic regression found that the presence of degenerative oocyte in the cohort of sibling oocytes increased the total negative score of the rest of the oocytes in the cohort, ß=-1.3 (95%CI: -1.8 to -0.79); p<0.0001.

Limitations, reasons for caution: Pilot study, small number of participant.

Wider implications of the findings: Our results confirm previous findings that showed no difference in embryo quality and morphokinetics, regardless of the needle used for OPU. This study evaluated embryo quality using morphokinetics parameters after comparing the use of different needle diameters on sibling oocytes.

Trial registration number: NIH number NCT02749773

P-682 Lean PCOS may be a genetically distinct from obese PCOS: lean women with polycystic ovary syndrome and their relatives have no increased risk of T2DM

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Study question: Do lean women with polycystic ovary syndrome (PCOS) and their first, second, and third degree relatives have the same risk of type 2 diabetes (T2DM) as obese women with PCOS?

Summary answer: T2DM risk is increased in obese PCOS women and their first, second, and third degree relatives, but not in lean PCOS women or their relatives.

What is known already: PCOS is a strong risk factor for T2DM, with an approximately 2-fold increase in risk. Heritability of PCOS has been demonstrated in numerous studies, but mode of inheritance has not fit a single model. First degree relatives of women with PCOS appear to share the increased risk of T2DM, suggesting shared susceptibility alleles. However, studies of the T2DM and insulin resistance in relatives of women with PCOS have primarily included overweight and obese probands and relatives.

Study design, size, duration: This cohort study used the Utah Population Database (UPDB), which contains demographic and genealogy data, and medical records from the second largest health caresystem in Utah from 1994 through 2015. PCOS and T2DM cases were identified by ICD-9 codes. Body mass index (BMI) was obtained from drivers' licenses. Lean was defined as BMI <25, and obese as BMI ≥ 30. All first, second, and third degree relatives of PCOS probands in the database were identified.

Participants/materials, setting, methods: PCOS cases were diagnosed at ages 10-54, and had least 3 generations of genealogical data in the UPDB. Controls were matched for sex, birth place, birth residence (urban/rural), and 5-year birth cohort. 463 lean PCOS women and their 1369 first degree, 2179 second degree, and 4612 third degree relatives and 367 obese PCOS women and their 1008 first degree, 1760 second degree and 3422 third degree relatives with linked hospital data were included.

Main results and the role of chance: In the lean PCOS cohort, 13 of 463 probands, 36 of 1369 first degree relatives, 150 of 2179 second degree relatives, and 232 of 4612 third degree relatives had T2DM. The relative risks (95% confidence intervals) for T2DM among lean PCOS women were 1.71 (0.91-2.93) for probands, and 0.77 (0.54-1.06), 1.01 (0.86-1.19), and 1.04 (0.91-1.18) for first, second, and third degree relatives, respectively. In the obese PCOS cohort, 50 of 367 probands, 74 of 1008 first degree relatives, 151 of 1760 second degree relatives, and 219 of 3422 third degree relatives had T2DM. The relative risks (95% confidence intervals) for T2DM among obese PCOS women were 6.85 (5.08-9.03) for probands, and 1.72(1.35-2.16), 1.39 (1.18-1.63), and 1.21 (1.05-1.38) for first, second, and third degree relatives, respectively.

Limitations, reasons for caution: This study includes those who received an ICD-9 diagnosis of PCOS and/or T2DM, and thus those with health insurance may be overrepresented. Details of diagnostic criteria are not available, and so there may phenotypic variability between the lean and obese PCOS cohorts beyond BMI.

Wider implications of the findings: This study suggests that the lean PCOS phenotype is genetically distinct from the obese phenotype, and neither lean PCOS probands nor their relatives share the increased risk of T2DM found in obese PCOS families. BMI should be used for stratification in studies of genes associated with PCOS and T2 DM.

Trial registration number: None.

P-683 Estrogen regulates proliferation of mouse female bone marrow-derived stromal cells

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Study question: To assess the effects of estradiol on the proliferation of the murine bone marrow-derived stromal cells (BMdSCs) according to its concentration.

Summary answer: Estradiol may have a role in the regulation of proliferation of murine BMdSCs by dose- and time- dependent manners.

What is known already: BMdSCs has been known to demonstrate different responses to sex hormones according to gender difference or reproductive aging. Although estradiol has been known to play a major endocrine role on various cells in vivo to female, dose-dependent effect of estradiol on proliferation of BMdSCs has been rarely known.

Study design, size, duration: Femurs of 6-week-old female C57BL/6 mice (n = 20) were collected and washed using fresh media (DMEM).

Participants/materials, setting, methods: The marrow suspension from collected femurs was filtered through sterile Nitex mesh, and retrieved BMdSCs were cultured in in-vitro-conditioned with estradiol at 10^{-5} to 10^{-7} M and estrogen antagonist (ICI-1640), added for 24, 48, and 72 hours. FACS assay for isolated BMdSCs using markers (CD90 and CD105) and RT-PCR for target gene (FSH receptor and LH receptor) were performed after culture.

Main results and the role of chance: Isolated BMdSCs demonstrated enhanced proliferative activity and positively expressed CD90 and CD105. The expression of FSH receptor and LH receptor genes was increased after estradiol treatment and the peak response was observed at 48 hours. This proliferative effect was slightly suppressed by treatment of estrogen antagonist.

Limitations, reasons for caution: To fully understanding the regulatory roles of hormonal effect on proliferation of BMdSCs, further extended study with androgen and corticosteroids should be necessary.

Wider implications of the findings: This experiment implicates hormonal factors could regulate the cell cycles and regenerative capacity of BMdSC population with dose- and time- dependent manners.

Trial registration number: Not available / This study was supported by grants of Ministry of Education, Republic of Korea (2016R1D1A1A02937287 and 2016R1E1A1A01943455).

P-684 Bax-DUB expression regulated by formaldehyde in ovarian cells

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Study question: Could the role of Bax-DUB on formaldehyde-induced apoptosis be implicated in premature ovarian failure (POF)?

Summary answer: Bax-DUB expression is upregulated by formaldehyde in ovarian cells, and Bax-DUB interacts with Bax, participating in deubiquitination of Bax.

What is known already: It has been known that formaldehyde, a ubiquitous environmental pollutant, has a harmful effect on the ovary and may cause POF. Formaldehyde increases oxidative damage and mitochondrial dysfunction that induces mitochondrial apoptosis. DUBs can reverse protein degradation by cleaving the peptide or isopeptide bond between ubiquitin and its substrate proteins.

Study design, size, duration: The expression levels of Bax-DUB were determined by the treatment of ovarian cells with different concentrations of formaldehyde, and the protein-protein interaction along with biological roles of Bax-DUB in ovarian cells was investigated.

Participants/materials, setting, methods: Ovarian cells were treated with formaldehyde in a dose-dependent manner, and it was found that Bax-DUB was upregulated by formaldehyde. We studied the protein-protein interaction through yeast two-hybrid, GST pull-down, immunoprecipitation, and immunocytochemical assays. Ubiquitination and deubiquitination assays were performed to investigate cellular functions of Bax-DUB in ovarian cells.

Main results and the role of chance: The expression level of Bax-DUB was increased by formaldehyde in ovarian cells. Bax is known as a pro-apoptotic factor to initiate mitochondrial apoptosis and is increased by formaldehyde. Therefore, we investigated relationship between Bax-DUB and Bax, and identified that Bax-DUB binds to Bax directly. Moreover, the ubiquitination level of Bax is decreased by Bax-DUB.

Limitations, reasons for caution: The results of this study were limited by the conduct of a cell line.

Wider implications of the findings: This is the first attempt to explain the pathogenesis of POF in the ubiquitin-proteasome system. The results of this study suggest that Bax-DUB may enhance the effect of mitochondrial mediated apoptosis by regulating the function of Bax, and deplete ovarian cells as early as possible to cause POF.

Trial registration number: not applicable.

P-685 Negative progesterone elevation threhold level on trigger day is different in subclasses of patients

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Study question: Influence of progesterone elevation (PE) and different progesterone threshold values at trigger day in different groups of fresh cleavage or blastocyst stage embryo transfer.

Summary answer: In good prognosis patients the progesterone impact starts when the serum level is > 1 ng/ml. In other women when it is > 1,75 ng/ml.

What is known already: Endometrial receptivity play a substantial role in implantation and a serum PE measured on the day of human chorionic gonadotrophin (hCG) administration has been associated with a lower pregnancy rate in fresh cycles, although threshold levels and percentage of success reduction are still objects of debate. There is not an optimal PE threshold to predict the IVF cycle outcome, although most studies indicate a value ≥ to 1.5 ng/ml to reduce the pregnancy rate. Several studies indicate that embryo transfer at the blastocyst stage can at least partially reverse the negative impact of high progesterone levels.

Study design, size, duration: This study is a single-center retrospective study, performed in a tertiary care academic Fertility Center. We analyzed all fresh embryo transfer, at cleavage (CS) and blastocyst (BS) stage, performed from January 2012 to December 2015. We, therefore, separately analyzed all the first thawing transfer performed after cancelled transfer cycles for any reason and elevated progesterone values (≥1.5 ng/ml).

Participants/materials, setting, methods: We report of 6,484 fresh embryo transfers performed during the study period: 6,098 cleavage, 386 blastocyst stage transfers and We included 108 thawing cycle. For each cycle, we analyzed serum progesterone level at trigger day. Progesterone assay was performed by a Chemiluminescent Micro particle Immunoassay (CMIA) with an analytical sensitivity ≤ 0.1 ng/mL. The impact of progesterone level on clinical pregnancy rate (CPR) and live birth rate (LBR) were explored using multiple logistic regression.

Main results and the role of chance: After the adjustment for maternal age, number of retrieved oocytes and transferred embryos, in CS transfers CPR shows the first significant decrease at progesterone ≥1 ng/ml (OR 0.83 95%CI 0.69-0.99 P = 0,039). An additional CPR decrease is observed with levels ≥1.75 ng/ml (OR 0.50 95% CI 0.36–0.68 P<0.001). This difference was confirmed by a statistically lower LBR in PE cycles. In good prognosis patients (<38 years and retrieved oocytes >8) the adverse effects of elevate progesterone is already evident when progesterone reaches 1 ng/ml (38.2% vs 30.7%, OR 0.72 95%CI 0.58–0.88 P = 0.001). In patients >38 years or <8 retrieved oocytes the influence of progesterone level become evident only from 1.75 ng/ml (23.1% vs 13.1% OR 0.50 95%CI 0.32–0.78 P = 0.002). In the BS group, only a progesterone level over 1.75 ng/ml halved the CPR (OR 0.43 95%CI 0.19-0.98 P = 0.045) and LBR (OR 0.41 95%CI 0.16-1.05, P = 0.062). In the first frozen embryo transfer after PE freeze only cycles, the CPR (OR 1.19 95%CI 0.78-1.82 p = 0.407) and LBR (0.80 95%CI 0.48-1.34, p = 0.401) appeared not significantly different than in low progesterone level fresh transfers.

Limitations, reasons for caution: This is a retrospective analysis, with all the inherent limitations and bias of such a study. The results of vitrified-warmed freeze only cycles need to be confirmed in a larger sample.

Wider implications of the findings: Our data show that PE at trigger day is significantly inversely related to the fresh cycle outcome in CS and BS transfers, although the PE threshold appear to be higher in BS. Our data confirm that elective freezing strategy rescue the negative effect of PE on cycle outcome.

Trial registration number: N/A.

P-686 Cross-talk between Bcl2l10 and Aurka in regulation of meiotic spindle assembly

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Study question: How does Bcl2l10 regulate expression and activity of Aurka (Aurora kinase A)?

Summary answer: Bcl2l10 and Aurka regulate each other for their expression and activity via transcription factor of Aurka expression and PP1 (protein phosphatase 1) in mouse oocytes.

What is known already: Previously, we firstly identified the non-apoptotic function of Bcl2l10, a member of Bcl-2 gene family, that Bcl2l10 was expressed in meiotic spindle and RNAi (RNA interference)-based Bcl2l10 silencing caused meiotic arrest at Metaphase-I with abnormal spindle assembly. Aurka is a pivotal kinase that required for centrosome maturation and spindle assembly in mitosis as well as meiosis. Expression of Aurka decreased at transcription and translation levels, whereas phospho-Aurka increased by depletion of Bcl2l10 in mouse oocytes. In addition, activity of Aurka was also reduced by Bcl2l10 RNAi. Consequently, we firstly found that Bcl2l10 is an upstream regulator of Aurka in oocytes.

Study design, size, duration: Phospho-Aurka can be dephosphorylated by PP1. Because Bcl2l10 RNAi increased auto-phosphorylation of Aurka on Threonine 288, we confirmed whether changes in PP1 activity mediated that increase in Aurka phosphorylation after Bcl2l10 RNAi. Oocytes were collected from C57BL/6 mice. Changes in PP1 activity were confirmed by western blot analysis using phospho-PP1 specific antibody. For inhibition or activation of Aurka activity, we treated oocytes with MLN8054 or okadaic acid, respectively.

Participants/materials, setting, methods: Bcl2l10 RNAi-treated MI oocytes were obtained by microinjection of Bcl2l10 double-stranded RNA and non-treated MI-oocytes were used as control. MLN8054 or okadaic acid were added to culture medium and oocytes were incubated for 16 hours. RT-PCR was carried out by using cDNAs equivalent to that of single-oocyte. For western blot analysis, 200 oocytes of each group were used.

Main results and the role of chance: As a result of RT-PCR, transcripts of E4TF1, TRAP220/MED1 and HIF-1α decreased by Bcl2l10 RNAi. In addition, protein expressions of E4TF1 and HIF-1α were decreased whereas TRAP220/MED1 was not detected. Phosphorylation of PP1 at Threonine 320, an inactive form of PP1, increased after Bcl2l10 RNAi treatment indicating that PP1 activity was inhibited by Bcl2l10 RNAi. It suggests that Bcl2l10 regulates the phosphorylation of Aurka through regulation of PP1 activity. By treatment MLN8054 (Aurka inhibitor) or okadaic acid (Aurka activator) during in vitro oocyte maturation, phosphorylation of Aurka at Threonine 228 decreased or increased, respectively. Interestingly, Bcl2l10 expression was enhanced by MLN8054 and was reduced by okadaic acid. Therefore, our data suggests that a novel form of reciprocal regulation exists between Aurka activity and Bcl2l10, forming a feedback loop in mouse oocytes.

Limitations, reasons for caution: This study was performed with mouse oocytes and results need to be validated for human oocytes.

Wider implications of the findings: In the present study, we firstly identified the association between Bcl2l10 and Aurka in meiotic spindle assembly. It is known that defects in spindle assembly are the major contributor to aneuploidy in oocytes. Therefore, this study may provide new theoretical basis about high rates of aneuploidy in human eggs.

Trial registration number: No required.

P-687 Does endometriosis influence the embryo quality and/or development? Insights from a retrospective study

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Study question: To evaluate the impact of endometriosis on embryo development at cleavage stage.

Summary answer: We did not find differences in the number/quality of day 3 embryos but we detected a lower ongoing pregnancy rate in women with endometriosis.

What is known already: The impact of endometriosis on fecundity and IVF outcomes is still controversial. Some studies suggested that endometriosis could influence IVF outcomes since oocytes may be more difficult to be retrieved and fewer embryos could be obtained compared to women without endometriosis. Poor oocyte and embryo quality has been as well postulated. Moreover, endometriosis has been not consistently associated with lower implantation rates and lower pregnancy rates. Nevertheless, the reasons to explain the suboptimal performance of IVF in endometriosis patients are poorly understood and can only be hypothesized.

Study design, size, duration: This is a retrospective study including patients undergoing IVF at the San Raffaele Hospital from 2013 to 2017. Endometriosis were laparoscopically diagnosed in all patients before IVF treatment.

A total of 430 cycles from women with endometriosis were studied and matched with cycles of women without the disease (n = 854) according to age, number of oocytes retrieved and time period of the treatment. A total of 3818 embryos at cleavage stage (day 3) have been analyzed.

Participants/materials, setting, methods: Embryo quality at day 3 in terms of number of cells, cell fragmentation and symmetry was evaluated. Principal components summarizing baselines variables (age, BMI, seminal parameters, number of oocytes retrieved, number of oocytes available) were used as covariates. Student's test, Mann-Whitney U test and logistic regression model were used as appropriate. All the results are presented as mean ± SD, OR (95% CI) or median (interquartile range)

Main results and the role of chance: The number of oocytes retrieved in both groups were similar (5.8 ± 4.4 controls vs 5.8 ± 4.6 endometriosis, p = 0.84). We did not find as well any statistically differences in the percentage of MII oocytes [80% (56-100%) controls vs 75% (50-100%) endometriosis patients, p = 0.25] and in fertilization rate [80% (57-100%) controls vs 80% (61-100%) endometriosis patients, p = 0.29]. Overall, in cycles from endometriosis women we did not find any difference regarding the number of embryos obtained at cleavage stage (3.5 ± 2.6 controls vs 3.7 ± 2.6 endometriosis patients, p = 0.47). In addition, the percentage of top/good quality embryos was as well similar [56% (25-100%) controls vs 50% (17-88%) endometriosis patients, p = 0.20]. Besides, we did not find any difference in the blastulation rate, excluding cycles in which transfers were performed or embryos were frozen in day 3. Despite similar fertilization rate and number/quality of embryos obtained, we found a reduction in ongoing pregnancy rate in women with endometriosis compared with the control group (24.2% in controls vs 17.7% in endometriosis group, corrected OR = 0.60; 95% CI 0.40-0.90, p = 0.014) after adjusting for the number and quality of transferred embryos, the day of transfer (cleavage or blastocyst stage) and principal components.

Limitations, reasons for caution: The main limitation of our study is its retrospective design; however, no clinical decision/intervention has been done between the time of fertilization and day 3 so that this study may be comparable to a prospective one. In both groups, the effect of other causes of infertility cannot be excluded.

Wider implications of the findings: These results indicate that women with endometriosis undergoing IVF treatments may expect a similar embryo quality than women without the disease. The results of this study could help in understanding whether embryo or endometrial factor represents the main cause for the poor success in ART in patients with moderate/severe endometriosis.

Trial registration number: not applicable.

P-688 GnRH antagonist administration in the luteal phase for the prevention and management of severe OHSS: a systematic review and meta-analysis

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Study question: Is luteal GnRH antagonist administration effective for the prevention and management of severe OHSS?

Summary answer: Luteal GnRH-antagonist administration is not effective for the prevention of severe OHSS, while appropriate trials evaluating its use for outpatient management of OHSS are lacking.

What is known already: Despite the increasing use of GnRH agonist trigger in high risk for OHSS patients, hCG administration remains the most popular triggering option, and as a result a significant proportion of high-risk women (9-38%) will still develop severe OHSS. Luteal GnRH-antagonist administration has been used as a method of OHSS prevention, as well as tertiary management of established severe OHSS at an outpatient level via a luteolytic mechanism.

Study design, size, duration: Medline, Scopus, Google Scholar, Cochrane and Clinicaltrials.gov databases were systematically searched for articles published up to December 2017, along with the references of all articles that were retrieved in full text. Trials referring to prevention or treatment of OHSS by administering GnRH antagonists in the luteal phase of women undergoing ART who were either at risk for developing OHSS or were diagnosed with severe OHSS were considered eligible for inclusion.

Participants/materials, setting, methods: Fourteen studies with a total 1029 patients were considered eligible for inclusion. Pooled odds ratios (OR) and 95% confidence intervals (CI) were calculated using random effects model due to significant heterogeneity in the methodological characteristics of the included studies. Higgin's I^2 was utilized for statistical heterogeneity assessment. Statistical meta-analysis was performed using RevMan 5.3.

Main results and the role of chance: Prevention of early OHSS: Four eligible studies (1 RCT, 1 case-control, 2 retrospective) with 409 high-risk patients (GnRH-antagonist n = 197 versus control n = 212) were included in the meta-analysis. GnRH-antagonist was administered from Day 0 or Day 1 for 3-5 days. The incidence of OHSS was not affected by GnRH-antagonist administration (Mild OHSS: OR = 1.58%, 95% CI:0.49-5.15, p = 0.44, I^2 = 79%; Moderate OHSS: OR = 0.92%, 95% CI:0.41-2.05, p = 0.84, I^2 = 56%; Severe OHSS: OR = 0.39%, 95% CI:0.05-3.16, p = 0.37, I^2 = 54%). Clinical pregnancy rates were similar (229 patients; OR 1.33 95% CI 0.71 to 2.51, p = 0.37; I^2=%, p = 0.56) in GnRH-antagonist and control group.

Management of established early severe OHSS (tertiary management): Ten studies: 3 prospective observational, 2 retrospective and 5 case-series; 620 patients) were considered eligible. GnRH-antagonist was administered from the day of severe OHSS diagnosis for 2-4 days. The studies used different methodology and inclusion criteria, rendering a meta-analysis not feasible. In the systematic review conducted, the majority of studies supported that luteal GnRH-antagonist administration prevented patient hospitalization and resulted in rapid regression of established severe OHSS. OHSS resolution was demonstrated by a significant decline of ovarian volume, ascites, haematocrit, white blood cell count, serum oestradiol and progesterone, making patient management on an outpatient basis feasible.

Limitations, reasons for caution: The retrospective nature of most of the included studies, their high heterogeneity and low quality, as well as the limited number of patients treated constitute significant limitations. Furthermore, the definition of OHSS and the therapeutic protocol of GnRH- antagonist administration varied both for prevention and for treatment of OHSS studies.

Wider implications of the findings: Although available evidence is insufficient, luteal GnRH-antagonist administration does not appear to be effective for the the prevention of severe OHSS. On the other hand, GnRH-antagonist appears to result in rapid regression of established severe OHSS, but appropriate trials evaluating its use for outpatient management of OHSS are lacking.

Trial registration number: Not applicable.

P-689 Follicular flushing is associated with a significantly higher oocyte recovery rate compared to no flushing: a randomized controlled trial

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Study question: Is follicular flushing associated with a higher oocyte recovery rate compared to no flushing?

Summary answer: Follicular flushing is associated with significantly higher oocyte recovery rates compared to no flushing.

What is known already: There is an ongoing debate regarding the effectiveness of follicular flushing during oocyte retrieval. Current evidence suggests that follicular flushing does not seem to improve live birth rate, clinical pregnancy rate or the number of oocytes retrieved, but to significantly increase the duration of oocyte retrieval compared to no flushing. Nevertheless, follicular flushing continues to be a routine procedure in many IVF centres.

Study design, size, duration: In this randomized controlled trial, conducted from November 2017 until January 2018, the right and left ovary from each patient were randomized (with a computer-generated randomization list) to be aspirated using either follicular flushing or no flushing. The primary outcome was oocyte recovery rate defined as the number of oocytes retrieved/number of follicles aspirated (>11 mm). Assuming a 10% difference in recovery rate, a sample size of eighteen patients was required to yield 80% power.

Participants/materials, setting, methods: Patients aged <42 years, with intact ovaries, each containing at least four follicles > 11 mm on the day of hCG administration were included in the study. Patients with endometriotic cysts were excluded. Oocyte retrieval was performed 35–36 h after hCG by transvaginal ultrasound-guided aspiration using the same double lumen needle (16 G, Casmed International Ltd, UK) and the same digitally adjusted aspiration vacuum for both ovaries. Outcomes were analyzed using paired t-test.

Main results and the role of chance: A total 20 patients were included in the study. Baseline characteristics, expressed as mean (SD), were: age 34.6 years (4.7), BMI 24.1 kg/m^2 (5.2), duration of infertility 4.1 years (3.0), number of previous attempts 1.0 (1.1), AFC 8.8 (5.8), AMH 3.1 (2.7), basal FSH 7.2 (1.9), basal LH 5.1 (1.9), basal oestradiol 43.8 (13.8) and basal progesterone 0.7 (0.6).

Oocyte recovery rates [mean (95% CI)] [74.2% (65.0-83.4) versus [42.7% (30.0-55.5), p<0.0001], number of COC retrieved [7.7 (5.1-10.2) versus 4.1 (1.7-6.5), p<0.0001], number of metaphase-II oocytes [6.8 (4.6-8.9) versus 4.0 (1.7-6.3), p<0.0001] and number of fertilised oocytes [4.1 (2.9-5.4) versus 2.2 (1.2-3.1), p = 0.001] were significantly higher using follicular flushing compared to no flushing. Maturation rates [90.2% (82.5-97.9) versus 87.8% (75.6-99.9)], fertilization rates [64.5% (53.4-75.6) versus 65.3% (45.5-85.1)], proportion of embryos transferred [34.1% (6.4-61.9) versus 25.8% (-3.4 – 55.1)] were similar in the flushing and no flushing groups. There were no cases of bleeding in either group of the present study.

Limitations, reasons for caution: A double-lumen needle was used in both "flushing" and "no-flushing" groups. The use of a single-lumen aspiration needle in the non-flushing group might have altered the results obtained, although this is not supported by the published literature. The current study was powered to compare retrieval rates but not pregnancy achievement.

Wider implications of the findings: Follicular flushing was associated with a significantly higher oocyte recovery rate, number of COCs retrieved, number of metaphase-II oocytes, number of 2PNs but with similar oocyte maturation, fertilization rates and proportion of embryos transferred compared to no flushing in normal/high responders.

Trial registration number: NCT03354858

P-690 The acceleration of reproductive aging in MMTV-TGF-alpha mice

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Study question: Does MMTV-TGF-alpha mice present diminished ovarian reserve during reproductive lifespan and does TGF-alpha gene interfere with mTOR signal pathway during ovarian aging?

Summary answer: Protein and mRNA expression levels of mTOR and P-mTOR were gradually decreased from 10 to 18 week, but increased at 50 week in MMTV-TGF-alpha ovary.

What is known already: TGF-alpha is an important growth factor regulating follicle development and tumorigenesis. TGF-alpha detected in granulosa cells of developing follicles, atretic follicles and theca-lutein cells. mTOR is a key regulator for proliferation, growth, differentiation, and apoptosis. Previously, we have shown that mTOR has regulatory role during follicular development and inhibition of mTOR induced a reduction in granulosa cell proliferation and follicle growth. mTOR signaling also plays a major regulatory role in aging since suppression of this pathway extends lifespan in model organisms.

Study design, size, duration: Total of 24 MMTV-TGF-alpha female mice which overexpreses TGF-alpha were used. Animals were ad-libitum fed and sacrificed and then, ovaries were removed at 10, 18, 50 and 82 weeks of mouse age.

Participants/materials, setting, methods: Ovaries of 10, 18, 50 and 82 week old MMTV-TGF-alpha mice were dissected and fixed in 4% paraformaldehyde and embedded in paraffin. 5 µm slides were taken from paraffin blocks and stained with hematoxylin and eosin staining in order to examine the morphological changes in ovaries. mTOR and P-mTOR protein and mRNA expression levels were detected using western blot and q-RT-PCR,respectively, for each experimental group.

Main results and the role of chance: 10 week old MMTV-TGF-alpha mouse ovary presented numerous developing follicles from different follicular developmental stage. However, compared to the usual healthy mouse ovary, MMTV-TGF-alpha ovary had smaller antrum spaces in secondary follicles and did not exhibit a healthy follicular structure. The number of infiltration of luteinized cells were higher, while the number of developing follicles were lower at 18 week old ovaries. Compared to the other groups (10, 18, and 82 week), 50 week old ovary had decreased number of developing follicle and increased number of atretic follicles. In addition, diminished number of developing follicules and increased number of atretic follicles were detected in 82 week old mouse ovary. Protein and mRNA expression levels of m-TOR and P-mTOR were gradually decreased from week 10 to 18 week, but they were increased at 50 week. Then, expression levels were decreased again towards to week 82 of mouse age. Therefore we suggest that TGF-alpha may have relation with mTOR signal pathway during ovarian aging and over-expression of TGF-alpha may cause acceleration of diminished ovarian reserve and premature ovarian failure.

Limitations, reasons for caution: The best way to explain interaction between TGF-alpha and mTOR signal pathway might be use TGF-alpha homozygote/heterozygote knock-out mouse ovary.

Wider implications of the findings: TGF-alpha and/or mTOR might be targets for developments of drug which could be used in infertility.

Trial registration number: Embryology/(female (in) fertility)

P-691 Altered endometrial receptivity in infertile females with polycystic ovarian syndrome (PCOS) as compared to fertile counterparts: analogy of protein expression profiles

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Study question: To unravel the proteins involved in compromising the receptivity and hampering the decidualization process in the endometrium of females with polycystic ovarian syndrome.

Summary answer: Proteins involved in cell proliferation, differentiation, development, signaling, mitochondrial metabolism, DNA repair, apoptosis and post-transcriptional modification may play key roles in endometrial programming for receptivity.

What is known already: Various expression studies have revealed the profiles of genes underlying unique cellular and molecular mechanisms involved in endometrial shedding, repair, regeneration and remodelling. Endometrium in PCOS females is known to be compromised for receptivity due to progesterone resistance with elevated levels of androgens and/or insulin. However dysregulation of specific downstream pathways and involved proteins need to be elucidated in detail for the development of biomarkers related to infertility and pregnancy disorders in PCOS women.

Study design, size, duration: 12 infertile PCOS and 12 healthy fertile subjects within reproductive age group (24–36 years) were recruited from Gynecology and Obstetrics OPD of HAH Centenary Hospital, Jamia Hamdard, during the period (june 2015 to august 2016) after approval of JHIEC for clinical studies. Endometrial biopsies were collected during secretory phase in oligo-ovulatory PCOS, anovulatory PCOS (progesterone withdrawal bleeding) and normal subjects after written informed consent was taken from all subjects enrolled in the study.

Participants/materials, setting, methods: Tissue was lysed and protein profiles were generated though two-dimensional gel electrophoresis (GE Lifesciences) and analyzed with PD-QUEST analysis software (Bio-Rad). Differential protein spots were identified through MALDI-TOF MS protein mass fingerprinting (Ultraflex III instrument). This was followed by RNA isolation (MiniSure Spin total RNA isolation kit, Nucleopore) and cDNA synthesis using RevertAid^{TM} H Minus first strand cDNA synthesis kit (Thermo Fisher Scientific). Relative expression analysis was performed using real time-PCR (Light cycler 480, Roche)

Main results and the role of chance: Relative expression of 13 genes was calculated with reference to GAPDH (reference gene). Altered expression in genes UCHL1, MYO3A, TGM1, CCNB1, AR6P1, PARP6, CRBB2, PIGX, DUS2L, THIL, ZNHI1, RAM and P2YP12 was found to be associated with infertile endometrial grounds in PCOS females. Besides basic cellular processes like proliferation, differentiation and development; cell cycle regulatory, DNA repair and apoptotic processes seemed to be involved in decreased endometrial receptivity in case of PCOS females. Mitochondrial metabolism, cell signaling and cellular transport were some other key affected processes. Regulation at post transcriptional level also seem to control many important downstream processes. The observed differences in gene expression profiles provide a roadmap into the intricacies involves in endometrial remodelling and receptivity.

Limitations, reasons for caution: Small sample size and unicentric nature of the study are the main limitations.

Wider implications of the findings: These findings have important diagnostic and prognostic implications in case of infertile PCOS females. Moreover the study also provide insights into endometrial cancer risk in infertile PCOS subjects. Further studies are required to establish the role of these proteins in the regulation of pathways associated with endometrial programming and remodeling.

Trial registration number: None.

P-692 Increased apoptosis and inflammation are key factors in endometrial dysfunction of PCOS patients

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Study question: Which phenomena could result in impaired endometrial function in women with PCOS?

Summary answer: Apoptosis and inflammation pathways play important roles in endometrial dysfunction of PCOS patients.

What is known already: Increased cytokine expression and inflammatory responses due to intense leukocyte immigration could impede endometrial

preparation and manifest implantation failure and recurrent miscarriage. Besides, overexpression of p53-dependant apoptosis genes demonstrated excess cell death in endometrial cell in PCOS patients which could be caused by higher inflammation and following ROS production. Generally, it seems that reduced implantation rate in PCOS might be due to insufficient endometrial preparation caused by more intense inflammation and apoptosis than normal proliferative phase endometrium.

Study design, size, duration: This study was genomic analysis that 10 human endometrial biopsies taken from pcos patients and 10 from healthy fertile women in the proliferative phase. All participants are in reproductive age with normal menstrual cycles.

Participants/materials, setting, methods: Total mRNA were extracted from endometrial tissues from pcos patients (n = 10) and healthy fertile individuals (n = 10) during proliferative phase. The expression profile of female infertility was investigated using qRT-PCR array.Informed consent was obtained from patients. All measurements were performed in triplicates on independent biological replicates.

Main results and the role of chance: Data of PCR array demonstrated high expression of apoptosis inducing factors such as IL1B, TNF and TNFRSF and apoptosis promoting factors like TP53, BAX and Bcl2 in PCOS group, while the expression of CASP3 was not different significantly in both groups. Beside apoptosis genes, cytokine genes expression was higher in PCOS group. overexpression of inflammatory factors like CCL5, CXCL12, CLDN4 and ICAM1 confirmed leukocyte immigration in endometrial tissue and inflammation. In inflammation, TNF, IL6 and IL1 activate NF-kB which could enhance DNA hypermethylation and chromosomal instability. Following DNA hypermethylation, epigenetic inactivation of tumour suppressors and overexpression of Bcl2 which suppresses DNA repair might reduce endometrial cell quality and impair its function in implantation.

Limitations, reasons for caution: The main limitation of this study is a low number of human endometrial samples for array analysis.

Wider implications of the findings: This study provide novel data about impeded endometrium preparation in proliferative phase of PCOS patients as compared to normal women which might affect endometrial receptivity in women with PCOS.

Trial registration number: N/A.

P-693 Effect of maternal separation stress and use of antidepressants on rat ovarian reserve

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Study question: does maternal separation and consequent antidepressant use effect ovarian reserve in rat ovaries?

Summary answer: Maternal separation may cause diminish of ovarian reserve. Afterwards antidepressants use have different effects on ovary depending on Agmatine treatment or Imipramine treatment.

What is known already: Premature ovarian insufficiency (POI) occurs when primordial follicle pool of a female is depleted early in her reproductive life leading to the cease of menstrual cycle and fertility. The premature activation of primordial follicles can occur due to many reasons such as genetic background, diseases, drug use or exposure to harmful materials. There might be a relationship between the use of antidepressants and the premature depletion of ovarian reserve in female rats using a depression model. Therefore, we aimed to determine the follicle reserve of the ovaries in the depression model created by maternal separation in rats with antidepressants used.

Study design, size, duration: Totaly 20 pups were used in this study. Pups separated from their mother on their second postnatal day for 4 hours every day for 15 days and when they were grown to 60 days old they were given antidepressants Agmatine and Imipramine for 15 days until dissection. Dissected ovaries were processed, embedded in paraffin and sectioned using microtome. The sections were then stained with hematoxylin and eosin for morphological analyzes and imaged using light microscope.

Participants/materials, setting, methods: Dissected ovary tissues were processed and sectioned at 5 µm. Sections were stained using hematoxylin and eosin for morphological analyzes and follicle counting. Afterwards mTOR and P-mTOR expression were detected in serial sections using immunofluorescence staining.

Main results and the role of chance: Morphological results presented that maternal separation may cause diminish of ovarian reserve. Rats were separated from their mother as pups but not treated with antidepressants later in life would have smaller ovaries and more corpus luteum then control ovaries that were kept together with mother. Afterwards antidepressants use have different effects on ovary depending on Agmatine treatment or Imipramine treatment. Agmatine treatment helps decrease corpus luteum numbers so may maintain follicles from reserve in each ovarian cycle in rats. In addition to that decreased number of Graafian follicle numbers in Agmatine treated group could indicate that, activation of oocytes decrease along with their full maturation therefore number of Graafian follicles decrease and ovarian reserve is maintained. mTOR expression was detected in oocyte and granulosa cells in rat ovary from each experimental group. P-mTOR expression observed specifically in oocyte nucleus and in mitotic dividing granulosa cells in rat ovaries. Even though no significant change is observed in mTOR and P-mTOR levels in immunofluorescence staining, Imipramine has detrimental effects on ovarian reserve as it increases corpus luteum numbers. Therefore, further functional studies will provide an insight into whether mTOR signal pathway have relation with Agmatine and Imipramine treatment in model of depression.

Limitations, reasons for caution: Anti-mullerian hormone level might show as indicator of diminished ovarian reserve. We will confirm mTOR and P-mTOR expression results with western blot and qRT-PCR.

Wider implications of the findings: Tricyclic antidepressant Imipramine has more severe effects that Agmatine on ovarian reserve.

Trial registration number: not applicable.

P-694 Does early respons on ovarian stimulation using corifollitropin alfa versus hp-hMG influence live-birth rates in a GnRH antagonist protocol?

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Study question: Does early respons on ovarian stimulation using either corifollitropin alfa (CFA) or highly purified human postmenopausal gonadotrophin (hp-hMG) in an antagonist protocol influence live-birth rates?

Summary answer: No statistically significant difference in terms of clinical outcomes between early versus normal responders after ovarian stimulation with CFA or hp-hMG was found.

What is known already: A single injection of CFA for the first 7 days of ovarian stimulation provides similar clinical outcomes when compared to daily injections of rFSH, namely with respect to the number of oocytes and live-birth rates. A retrospective analysis has also shown that early responders (women who fulfill the final maturation hCG trigger criteria prior to or on stimulation day 8) have comparable number and size of pre-ovulatory follicles, oocytes, good-quality embryos and ongoing pregnancy rates, when compared to those who perform stimulation for >8 days. However, comparative information between early and late responders following CFA versus hp-hMG is lacking.

Study design, size, duration: This is a retrospective analysis of a cohort of fresh IVF/ICSI cycles in GnRH antagonist protocol performed in a single tertiary IVF center, during the period comprised between January 2011 and January 2017.

Participants/materials, setting, methods: Patients (aged ≥35 to ≤42 years) received either a single dose of 150 µg CFA or daily 300IU of hp-HMG for the first 7 days of stimulation. From day 8 on, treatment continued with a daily dose of hp-hMG (300IU/d) until the day of trigger. We categorized patients as "early responders" if they reached at least three follicles ≥17 mm

before day 8 of stimulation and as "normal responders" those that reached the criteria after 8 days.

Main results and the role of chance: A total of 916 cycles were divided in 4 groups: group A (CFA early responders, n = 116), group B (CFA normal responders, n = 344), group C (hp-hMG early responders, n = 127) and group D (hp-hMG normal responders, n = 309). There were no differences in demographic characteristics among the groups regarding age, AFC and BMI. Patients who needed 8 or more stimulation days needed on average 3 extra days until triggering in both groups (CFA-group 10.1 total days vs hp-hMG-group 9.5 total days; p = 0.2). There were no differences among the four groups in terms of total number of retrieved oocytes (6.0 ± 5.3, 5.7 ± 3.3, 5.6 ± 4.9, 5.7 ± 3.7, p = 0.9), number of mature oocytes (4.4 ± 4.3, 4.7 ± 2.7, 4.0 ± 3.0, 4.4 ± 3.2; p = 0.22), and number of embryos transferred (1.5 ± 0.8, 1.6 ± 0.6, 1.5 ± 1.0, 1.5 ± 0.9; p = 0.3). Live-birth rates were 22.4%, 20.4%, 17.3% and 15.6% (p = 0.28) respectively, for the four study groups.

Limitations, reasons for caution: We acknowledge that this is a retrospective data analysis. Although a large dataset was analyzed, prospective studies are needed to confirm these results.

Wider implications of the findings: The duration of ovarian stimulation to reach the criteria for triggering final oocyte maturation did not seem relevant for the clinical outcome.

Trial registration number: not applicable.

P-695 NLRP3 expression is upregulated in ovarian granulosa cells in a mouse model of polycystic ovary syndrome

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Study question: TLR3 and NLRP3 inflammasome contribute to the pathogenesis of Type 2 diabetes in functioning as sensors for metabolic stress. Whether TLR3 and/or NLRP3 are involved in the pathogenesis of PCOS.

Summary answer: By immunohistochemical analysis, expressions of TLR3 and NLRP3 are increased in granulosa cells in the PCOS mice.

What is known already: Elevated TLR3 is involved in dysfunction of pancreatic β-cells. NLRP3 inflammasome activation is upregulated in type 2 diabetes. TLR3 activation is one of the most important signals that can activate NLRP3 inflammasome. Most PCOS women are suffering from insulin resistance. High fat diet (HFD) and letrozole (LET) treatment have successfully induced reproductive and metabolic phenotype of PCOS in mice.

Study design, size, duration: Four week-old, C57BL/6 N female mice were implanted subcutaneously with a placebo or 3 mg LET pellet with or without HFD (60% calories from fat) feeding (n = 8 for control and HFD group, n = 6 for LET and LET plus HFD group). Seven weeks after treatment, the mice were anesthetized with chloral hydrate, blood was collected via retro-orbital bleeding, the parovarian fat pad, the ovaries were dissected, fixed in 4% paraformaldehyde.

Participants/materials, setting, methods: The mice were weighed weekly. A glucose tolerance test was performed on the mice 3 weeks after the treatment. Blood triglyceride, total cholesterol were measured. Paraffin-embedded fats and ovaries were sectioned and stained with hematoxylin and eosin. Expression of TLR3 and NLRP3 in the ovaries were evaluated by immunohistochemical staining. Numbers of follicle and corpus luteal in each ovary were recorded, and diameters of fat cells were measured by using the CellSens software.

Main results and the role of chance: From 3 weeks after treatment, the LET + HFD and LET mice exhibited significantly more weight gain than control and HFD mice (P < 0.05). LET + HFD and LET mice contained more large antral follicles than HFD and control mice. The number of corpora lutea was significantly lower in LET and LET + HFD mice than in control and HFD mice (P < 0.01). Fasting glucose levels in LET and LET + HFD mice were significantly higher than control mice (P < 0.05). LET + HFD mice resulted in markedly increased serum glucose levels at 60, 120 and 180 min after administration of glucose compared with control and LET mice (P < 0.01). LET + HFD mice exhibited significantly higher serum triglyceride and total cholesterol levels than control, HFD, and LET mice (P < 0.05). The average diameter of adipocytes was significantly larger in LET + HFD mice than control and LET mice (P < 0.01). Integral optical density (IOD) of TLR3 in ovary section of LET and LET + HFD mice was higher than HFD and control (P > 0.05). IOD of NLRP3 in LET and LET + HFD mice was significantly higher than control (P < 0.05).

Limitations, reasons for caution: In this study, expression of TLR3 and NLRP3 in the ovary was shown only in mouse species evaluated by immunohistochemistry. Further study needs to be carried out in human beings. Whether increased NLRP3 correlates with activation of caspase-1 then results in pyroptosis of granulosa cell still need to be clarified.

Wider implications of the findings: The significant body weight gain, dyslipidemia and ovarian morphology change in mice is caused mainly by LET. TLR3 and NLRP3 mediated inflammation might involved in the pathogenesis and etiology of PCOS. NLRP3 might be used as potential therapeutic target in this disease.

Trial registration number: basic research, no trial registration number.

P-696 Dual trigger with gonadotropin-releasing hormone agonist (GnRHa) and standard dose of human chorionic gonadotropin (hCG) to improve oocyte maturation rate (MR)

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Study question: Does dual trigger with GnRHa and standard dose of hCG in IVF/ICSI cycles increase MR in patients with a history of >25% of immature oocytes?

Summary answer: The proportion of mature oocytes retrieved was significantly higher after a dual trigger in comparison with the patient's previous cycle.

What is known already: Oocyte maturation for IVF/ICSI cycles is commonly induced by hCG to mimic natural luteinizing hormone (LH) surge. After controlled ovarian hyperstimulation (COH), few oocytes are arrested at immature stages. If >25% of retrieved oocytes are immature, affected patients have poorer IVF outcomes than those with <25%. GnRHa has been explored in many clinical scenarios, primarily to prevent ovarian hyperstimulation syndrome. One advantage of using GnRHa to trigger oocyte maturation is the induction of a more physiological release of LH/FSH (Follicle-Stimulating-Hormone). Recently, a dual trigger has been proposed as a potential treatment in patients with a history of immature oocytes retrieved.

Study design, size, duration: This is a retrospective, case-control study performed between December 2008 and December 2017. A total of 70 patients, who experienced high oocyte immaturity rate (>25%) during their first IVF/ICSI cycle (analyzed as control group) and received a dual trigger for their subsequent cycle (dual trigger group), were involved in the study. Only patients with at least one oocyte retrieved were included.

Participants/materials, setting, methods: Initial COH cycle was performed using agonist/antagonist protocols, and ovulation was triggered with hCG. During the subsequent cycle, patients received antagonist protocol and dual trigger using triptorelin 0.2 mg and hCG. Primary endpoint was MR (number of metaphase-2 (M2) oocytes/ retrieved oocytes). Secondary endpoints were (i) fertilization (ii) D2-top-quality-embryo (D2-TQE: 4 blastomeres, <20% cytoplasmic fragmentation) and (iii) cumulative clinical pregnancy rates (including fresh and frozen-thawed transfers during the study period). Outcomes were compared using paired-sample analysis.

Main results and the role of chance: Briefly, patients' mean age was 32.9 (control group) versus (vs.) 34.3 years (dual trigger group) (p<0.0001). A mean of 11.3 and 12.3 oocytes were retrieved in control and case groups, respectively (p = 0.26). A significant increase in number of M2 oocytes and consequently MR was achieved in case of dual trigger (8.9; 73.0%) when compared to control group (6.0; 50.3%: p<0.0001). Hence, numbers of fertilized oocytes (6.2 vs. 3.8; p<0.0001) and D2-TQE (2.2 vs. 1.6; p = 0.015) were significantly higher after dual trigger. However, fertilization (61.3 vs. 68.0%; p = 0.19) and

D2-TQE rates (33.5 vs. 36.7%, p = 0.4) per mature oocyte were statistically similar when compared between control and dual trigger groups, respectively. During the study period, cumulative clinical pregnancy rate yielded 50% (35/70) in control group vs. 64.3% (45/70) in dual trigger group (p = 0.08).

Limitations, reasons for caution: Cases of high oocyte immaturity rate are rare, explaining the long inclusion period in this retrospective study. Besides, baseline characteristics of patients were heterogeneous; especially antagonist and agonist protocols were included in control group. Only randomized studies, analyzing both biological and clinical outcomes, would confirm the efficiency of this strategy.

Wider implications of the findings: Dual trigger seems efficient for managing patients with high oocyte immaturity rate. Moreover, oocyte ability to be fertilized and develop into TQE might not be impaired by this treatment. If these results were confirmed, dual trigger, permitting to obtain more M2-oocytes, might optimize the chances of pregnancy in such patients.

Trial registration number: Not applicacable.

P-697 Oocyte quality in patients with polycystic ovary syndrome (PCOS): impact on the extended culture and live birth rates

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Study question: Does the oocyte quality in patients with PCOS affect the extended culture and live birth rates?

Summary answer: PCOS does not seem to affect the rate of useful blastocysts when compared to normo-ovulatory patients.

What is known already: PCOS and its subsequent fertility disorders cause difficulties in the management of the assisted reproductive technologies. Some authors have hypothesized that PCOS patients have a lower oocyte and embryo quality due to modifications of intra- and extra-ovarian factors. Nonetheless, few studies have investigated the biological parameters in PCOS patients after IVF attempts and specifically the extended culture.

Study design, size, duration: This is a single-center cohort study performed from October 2015 to December 2017. Patients with PCOS (Rotterdam criteria) and control normo-ovulatory patients (defined as patients presenting tubal, male or idiopathic infertility, with regular cycles and AMH basal level between 2 and 4 ng/ml), who underwent IVF with extended culture to day 6 were included. Were exclude: woman's age > 37 years, severe endometriosis, cycle rank > 3 and severe male factor (<1 million/mL).

Participants/materials, setting, methods: 59 PCOS and 114 control patients were included. The main endpoint was the rate of useful blastocysts obtained (transferred and/or frozen) and secondaries were the oocyte maturity rate and the cumulative live birth rate per ovum pick up.

Results were compared using chi-square test and Student t-test. Confounding factors were evaluated by a logistic regression model. The P value was considered significant when <0.05. The results were expressed by mean ± standard deviation or percentage.

Main results and the role of chance: The mean age of the patients was 31.5 ± 3.1 and 32.4 ± 3.0 years old, respectively, for the PCOS and the control groups (p = 0.06). While the total dose of FSH administered and the number of days of stimulation were similar, the average number of oocytes retrieved was significantly higher in the PCOS group compared with controls (15.7 ± 7.3 versus 12.9 ± 7.1, p = 0.02). The oocyte maturity rate was statistically lower in the PCOS group compared to controls (74.3% versus 83.1%, p = 0.005). The rate of useful blastocysts was similar between the PCOS and the control groups (50.1% versus 53.6%, p = 0.62). The cumulative live birth rates per ovum pick up were not statistically different (31.7% in the PCOS group versus 37.2% in the control group, p = 0.50; Odds Ratio = 0.78; 95% Confidence Interval [0.38; 1.59]).

Limitations, reasons for caution: This preliminary results need to be confirmed by larger studies and prospective randomized controlled trials.

Wider implications of the findings: Our study confirms that PCOS patients have significantly more retrieved oocytes but a lower maturity rate thus leading to a similar number of mature oocytes compared to normo-ovulatory patients and the chances of obtaining useful blastocysts were similar. Pregnancy and birth rates were comparable to those of the normo-ovulatory patients.

Trial registration number: I certify that there is with any financial organization regarding the material discussed in the study.

P-698 To compare clinical and metabolic effects of metformin versus combined therapy with metformin and myoinositol and D- chiro-inositol in PCOS women: A randomized control trial

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Study question: Does addition of Inositols have a role in improving clinical and metabolic parameters in women with PCOS?

Summary answer: Inositols have shown to reduce insulin resistance, it improves insulin sensitivity of target tissues and positively affects clinical and metabolic parameters in women with PCOS.

What is known already: PCOS is one of the most common endocrine disorders (6-10%) affecting women of reproductive age. Insulin resistance plays a pivotal role in etiopathogenesis of PCOS. To prevent long term health consequences of PCOS, besides life style modifications, use of insulin sensitizers has been proposed. Metformin has been commonly used. Recently, inositols-Myoinositol (MI) and D-chiro-inositol (DCI) have shown to be an efficient and safe alternative in PCOS management.

Study design, size, duration: This randomized controlled trial was conducted in Gynae OPD at All India Institute of Medical Sciences, Rishikesh from January 2017 till December 2017. Patients with PCOS were randomized into two groups, 30 in group I (Metformin) and 32 in group II (Metformin and MI and DCI).

Participants/materials, setting, methods: Group I received Metformin 500 mg twice a day for 3 months & in Group 2 received metformin with Myoinositol 550 mg + D-chiro-inositol 150 mg twice daily for 3 months. Clinical parameters like menstrual cycle regularity, acne, hirsutism, BMI, waist & hip circumference were compared at baseline and after 3 months of therapy. Similarly fasting blood sugar, fasting insulin levels and HOMA-IR index was compared at baseline and after 3 months of therapy.

Main results and the role of chance: Baseline characteristics were similar in two groups. Mean age was 28.35 +/- 2.74 & 27.12 +/- 3.34 years in Group I and II respectively. Mean BMI was 27.71 +/- 3.60 & 27.38 +/- 3.92 kg/m2 in Group I and II respectively. There was no improvement in acne score. However, after receiving treatment for 3 months statistically significant improvement was seen in Group 2 (metformin + MI +DCI) in their clinical parameters like weight (p < 0.02), waist circumference (p < 0.0155) & hip ratio (p < 0.00045). A significant improvement was seen in menstrual cycle length and bleeding days in Group II (p < 0.01). HOMA-IR index also showed a statistically significant improvement after 3 months of therapy in Group II (2.83 +/- 1.29 vs 1.62 +/- 0.59, p < 0.03).

Limitations, reasons for caution: Main limitation of this study is small sample size. Large randomized control trials are required to explore above hypothesis.

Wider implications of the findings: Using a comprehensive, detailed assessment of clinical parameters our study has shown a beneficial effect of Metformin in combination with MI + DCI in women with PCOS & insulin resistance. Combined therapy may have a role in PCOS patients.

Trial registration number: Applied for.

P-699 Impaired follicular development and ovulation in PCOS mouse model can rescued by rapamycin treatment

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Study question: Does Rapamycin (Inhibitor of mTOR) treatment suppress proliferation of granulosa cells and decrease LH level in treatment of PCOS?

Summary answer: Rapamycin may have important role in treatment of PCOS.

What is known already: Polycystic ovary syndrome (PCOS) is a complex reproductive and endocrine disorder. The target of rapamycin (TOR) gene product has been implicated in the control of cell growth and proliferation, protein synthesis, and autophagy. mTOR consist of 2 different protein complex as TOR complex 1 (mTORC1) and TOR complex 2 (mTORC2). Rapamycin is an inhibitor of mechanistic target of rapamycin pathway. Phosphorylation of serine residue 2448 in mTOR has been shown to correlate with the activation status of mTOR and proliferation of granulosa cells, and mTOR has functional role in granulosa cells during successful folliculogenesis and ovary with PCOS.

Study design, size, duration: 5 groups were mainly designed: Control (no treatment), PCOS (injection of DHEA (6 mg/100 g BW in 0.1 ml of sesame oil) (s.c) for 20 consecutive days), Vehicle (daily (s.c) sesame oil alone injection), Rapamycin (5 mg/kg in DMSO), Rapamycin Vehicle (daily (s.c) DMSO alone injection).

Participants/materials, setting, methods: We used hematoxylin-eosin staining for morphological eveluation to show the effect of Rapamycin treatment on PCOS mouse ovary. 17-Beta estradiol, progesterone and Luteinizing hormone levels detected by using ELISA. Additionally TUNEL and follicle counting methods were used for determine of follicle reserve in each experimental groups. mTOR signal pathway proteins and genes were presented with western blot and quantitative Real Time PCR, respectively.

Main results and the role of chance: it is the first time mTOR pathway (TORC1 ve TORC2) and activation products are presented at protein and mRNA levels in Rapamycin treated-PCOS mouse model. We detected that Rapamycin treatment can improve follicular development, decreased LH level and provide ovulation, and corpus luteum structure can occur again in PCOS mouse ovary ($p<0.05$). Additionally, we detected decrease number of atretic follicle and save primordial follicle reserve ($p<0.05$) after Rapamycin treatment. Therefore Rapamycin may be use as a potential therapeutic strategy for the treatment of PCOS.

Limitations, reasons for caution: Different treatment dosages might use for treatment model of POCS.

Wider implications of the findings: We suggested that Rapamycin may have a new approach to PCOS treatment and understanding the mechanism of dominant follicle selection and anovulatory female infertility.

Trial registration number: not applicable.

P-700 Cumulative live birth rates in women with low ovarian reserve are higher with conventional IVF stimulation than after repeated natural cycle IVF attempts

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Study question: To determine the comparative efficacy of stimulated IVF (stimIVF) versus natural cycle IVF (ncIVF) in women with predicted poor ovarian reserve (POR).

Summary answer: The stimIVF group had fewer visits, fewer cycles cancelled and higher egg yield (all $P<0.001$) and higher cumulative live birth rate (18.2% vs. 10.3%, $P<0.03$).

What is known already: The conveyor belt of follicle recruitment in POR, and how many follicles might develop in any one treatment cycle, is unpredictable and variable. Such women have a poor prognosis and clinics are often reluctant to treat them because of the perceived effect on the clinic's success rates. In the UK, ncIVF is not counted in a clinic's pregnancy results so it offers an alternative, also avoiding expensive FSH injections and the possibility of back-to-back treatment cycles if a cycle is unsuccessful. However, a maximum of 2 oocytes is achievable.

Study design, size, duration: Simultaneous, parallel 2-centre cohort study in cycles from January 2015 – December 2016.

501 women underwent 699 stimIVF cycles (flare protocol with Gonal-F) at GCRM (Glasgow), and 55 women underwent 116 ncIVF cycles at Nurture (Nottingham).

The number of visits required, the cycle cancellation rate, the egg yields, live birth rates (LBR) per fresh cycle and cumulative LBR were determined.

Participants/materials, setting, methods: Both centres are private IVF units but take a different approach to treatment of poor ovarian responders. GCRM (Glasgow) favours conventional stimulation flare protocol with Gonal-F (225 IU/day if weight < 80Kg, 300 IU/day if weight > 80Kg). Nurture (Nottingham) favours ncIVF with no FSH but starting Cetrotide and daily ultrasound scans when the lead follicle is greater than 13 mm diameter. Both trigger using Ovitrelle and oocyte retrieval is performed 35-37 hours post-trigger.

Main results and the role of chance: There was no difference in BMI between the groups but the stimIVF patients were one year younger ($P<0.01$). They had a higher AMH level ($P < 0.001$) but were more likely to smoke ($P<0.001$).

The stimIVF group had fewer treatment cycles per patient (1.4 vs. 2.1, $P < 0.01$). Furthermore, they had fewer scans (and hence fewer clinic visits), fewer cycles cancelled, higher egg yields per egg retrieval (3.6 ± 2.7 vs. 0.9 ± 0.5) with more embryos available for transfer (all $P<0.001$). Only 4% of the stimIVF patients retrieved no eggs compared to 18% in the ncIVF group although this was not statistically significant ($P = 0.11$).

The fresh live birth rate (LBR) per cycle start was not different (16.6% v. 10.3%, $P = 0.09$). However, the stimIVF group had a greater chance of having frozen embryos and when subsequent frozen embryo transfers were taken into account, the cumulative LBR was higher (18.2% vs. 10.3%, $P<0.03$).

Limitations, reasons for caution: This was neither a matched nor randomised study. The stimIVF group were more likely to smoke but had a higher AMH. However, the difference in AMH does not account for the difference in the egg yields between the groups.

Wider implications of the findings: This is a poor prognosis group and both approaches have their merits. The avoidance of FSH injections in ncIVF must be balanced against the need for more scans (the increased burden of more clinic visits), the psychological impact of greater likelihood of cycle cancellation and the lower cumulative LBR.

Trial registration number: N/A.

P-701 Optimal number of oocytes need to complete family per retrieval

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Study question: To determine the optimal number of oocytes per retrieval needed to obtain at least two live births while practicing safe and effective stimulation.

Summary answer: Retrieving12-16 oocytes per cycle is optimal for completing family without wastage of surplus embryos as compared to retrieving more than16 or less than 8 oocytes.

What is known already: The practice is globally shifting to mild stimulation and elective single embryo transfer claiming to attain pregnancy rates similar to conventional stimulation and embryo transfer, but the desire to complete family and need for multiple retrieval cannot be overlooked. Aggressive stimulation to obtain too many oocytes, is associated with risk of OHSS, diminished receptivity because of high estradiol levels. On the other hand mild stimulation with multiple stimulation cycles, associated surgical and anaesthetic risks, financial and emotional burden.Too many cryopreserved embryos burden the lab and

couples financially. Generally they are written off by couples after achieving two live births.

Study design, size, duration: A prospective cohort study conducted in an IVF centre of tertiary hospital. All women less that 36 years with primary infertility undergoing IVF between 1st January 2016 and 31st December 2016.

Participants/materials, setting, methods: Women were stimulated with conventional antagonist protocol. All the participants meeting the inclusion criteria were divided in 3 groups Group A: 8-12, Group B: 12–16 and Group C with ≥ 16 oocytes. Women with < 8 retrieved oocytes or with single embryo transfer were excluded. The primary outcomes cumulative pregnancy rate (CPR) and family completion rate (≥ 2 live births) were calculated for each group. Statistics done using chi square test.

Main results and the role of chance: A total of 782 women meeting the inclusion criteria were divided in Group A (N = 186) Group B (N = 308) Group C (N = 288) based on number of oocytes as described. The number of fresh cycles were least in Group C (39%) due to elective freezing to prevent OHSS. The family completion rate was comparable in group B and Group C which was significantly more than that in Group A (23%, 26%, 13% respectively p = 0.0005,0.0007, 0.34). There would be significantly more number of patients with unused embryos in group C compared to Group B (15% vs 9% p = 0.03).

N = 782	Group A 8-12 N = 186 (%)	Group B 12-16 N = 308(%)	Group C > = 16 N = 288 (%)
Fresh ET cases	162 (87%)	211 (89%)	112 (39%)
Retrievals leading to a 1 Live Birth	87(47%)	179 (58%)	184 (64%)
Retrievals leading to 2 Live Birth	20(11%)	52 (17%)	55(19%)
Patients with 1 live birth with frozen embryos remaining	14 (8%)	74 (24%)	83 (29%)
Patients with 2 live birth with frozen embryos remaining	3 (2%)	29 (9%)	43(15%)
Expected no. patients to achieve a 2nd child from their remaining frozen embryos	4(2%)	18 (6%)	20 (7%)
Family Completion Achieved	24(13%)	70(23%)	75(26%)

Limitations, reasons for caution: A better design would be comparison of multiple stimulation cycles in women with low oocyte yield. Small sample size and short follow up too are limitations.

Wider implications of the findings: Stimulate mildly to retrieve 12-16 oocytes as LBR are better in fresh cycles with just sufficient number of frozen embryos needed for family completion compared to too many oocytes posing risk of OHSS and high cost of unused cryopreservation embryos without any increase in number of women with completed family.

Trial registration number: Trial registration number: MCDH/2015/58

P-702 The impact of polycystic ovary syndrome (PCOS) on early- and late-morphokinetic parameters: a retrospective analysis of 3938 time-lapse (TL) monitored embryos

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Study question: Is there an effect of PCOS on either early- and/or late-morphokinetic parameters obtained during embryonic culture to blastocyst stage in a TL-monitored incubator?

Summary answer: When comparing embryos derived from PCOS patients with those from other diagnoses no significant effect of PCOS on either early- and/or late-morphokinetic parameters was noted.

What is known already: PCOS contributes to both anovulation and poor embryonic quality, as well as an increased risk for early pregnancy loss. Emerging TL studies have raised suspicions that PCOS may influence certain TL-morphokinetic parameters, such as the duration of the 4th cleavage division and the time to reach the morula stage. Whether PCOS affects the timing of additional early- and/or late-morphokinetic parameters thought to predict blastocyst formation and implantation remains unclear.

Study design, size, duration: Data from 3938 embryos, derived from 579 IVF cycles taking place at a major academic center between 9/2013 and 9/2016, were retrospectively reviewed. All embryos were cultured to the blastocyst in a TL-monitored incubator. Embryos derived from PCOS were compared to i) those from patients with all other diagnoses (225 and 3713 embryos, respectively), and ii) in a sub-analysis to those with either idiopathic or tubal factor infertility (TFI), in regards to certain morphokinetic parameters.

Participants/materials, setting, methods: Groups were compared in regards to the following TL-morphokinetic parameters: time-period from i) pronuclei fading (tPN*f*) to 1st-cytokinesis (P_1), ii) 2- to 3-cells (P2), iii) 3- to 4-cells (P3), iv) 4- to 5-cells (P4), v) tPN*f* to early-blastulation (P_{SB}).

Mann-Whitney *U*-, χ^2-tests, and multilevel mixed-effects regression models [adjusted for potential confounders: age, body mass index (BMI), basal-FSH, fertilization method] were used to assess mean differences [AMD (95% CI)] between groups. P<0.05 was considered significant.

Main results and the role of chance: Median (interquartile range) age, BMI, basal-FSH were 33.1(31.6-36.6) vs. 35.6(32.9-38.8) years, p = 0.01; 24.5(22.3-28.1) vs. 23.1(21.2-26.5) kg/m^2, p = 0.06; 5.4(5.0-6.5) vs. 7.0(5.9-8.3) IU/L, p<0.01 for PCOS vs. non-PCOS diagnosis, respectively. When all embryos were included in the analysis, no difference was noted in any of the morphokinetic parameters between the groups [AMD (95% CI) in hours: 0.1 (-0.2-0.3), p = 0.54; -0.3(-1.3-0.7), p = 0.53; -0.4(-1.4-0.6), p = 0.46; -0.3 (-1.8-1.1), p = 0.66; 1.3(-1.6-4.3); p = 0.38; for P_1, P_2, P_3, P_4 and P_{SB}, respectively]. Furthermore, when usable (selected for either transfer or cryopreservation) were analyzed separately from discarded embryos, the results did not change.

Similarly, in a sub-analysis comparing PCOS-embryos to those derived from couples with idiopathic infertility, no significant differences were noted in any of the studied morphokinetic parameters [AMD (95% CI) in hours: 0.10(-0.18-0.38), p = 0.48; -0.3(-1.3-0.7), p = 0.53; -0.4(-1.4-0.6), p = 0.46; -0.3(-1.8-1.1), p = 0.66; 1.3(-1.6-4.3), p = 0.38; for P_1, P_2, P_3, P_4 and P_{SB}, respectively]. However, when comparing PCOS to TFI-embryos (no oocyte factor infertility), P_1 duration was significantly longer and P_2 significantly shorter in the former subgroup [AMD (95%CI): 0.36(0.07-0.66) hours, p = 0.02; 1.25(0.44-2.06) hours, p<0.01, respectively]. Differences persisted for the most part, even when analyzing usable embryos separately from discarded ones. All other morphokinetic parameters did not differ between PCOS and TFI groups.

Limitations, reasons for caution: Limitations include mainly the retrospective design of the study and the inclusion of only those morphokinetic parameters thought to predict blastocyst development. Furthermore, insulin resistance and/or metformin treatment were not taken into consideration. Time periods were normalized to tPN*f* to account further for fertilization method, as needed.

Wider implications of the findings: No obvious effect of PCOS was noted on either early- or late-morphokinetic parameters assessed by TL-microscopy, except when PCOS embryos were compared to a subgroup with no obvious contributing oocyte factor, a finding suggestive of a subtle impact of PCOS on oocyte quality.

Trial registration number: Not applicable.

P-703 Impact of serum progesterone on embryo quality and cumulative live birth rate in oocyte donation cycles

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Study question: Does late follicular elevated serum-progesterone (LFEP) during ovarian stimulation for oocyte donation have an impact on embryo quality (EQ) and cumulative live birth rates (CLBRs)?

Summary answer: LFEP does not have an influence on embryo quality and CLBR in oocyte donation cycles.

What is known already: Ovarian stimulation promotes the production of progesterone (P) which has been demonstrated to have deleterious effect on IVF outcomes when elevated during the follicular phase. While there is robust evidence that this elevation results in (epi)genetic changes impairing endometrial receptivity, the impact on EQ remains a matter of debate. The oocyte-donation model is the best tool to assess the effects of LFEP on EQ from those on endometrium receptivity separately. Previous studies in oocyte donation cycles investigating the influence of elevated P on pregnancy outcomes in oocyte recipients gave conflicting results.

Study design, size, duration: This was a retrospective analysis including all GnRH antagonist down-regulated cycles for fresh oocyte donation that took place in a tertiary referral university hospital between 2010-2017. A total of 397 donor-acceptor cycles were included. Each donor was included only once in the analysis and could be associated to a single acceptor.

Participants/materials, setting, methods: The sample was stratified according to the following serum P levels on the day of ovulation triggering: ≤1.5 ng/mL and >1.5 ng/mL. The primary outcome parameter was the top-quality embryo rate on day 3 and the secondary outcome was CLBR defined as a live-born delivery after 24 weeks.

Main results and the role of chance: 397 fresh oocyte donation cycles were included in the analysis, of which 314 (79%) had a serum P ≤ 1.5 ng/ml and 83 (20.9%) had a serum P>1.5 ng/ml. The average age of the two oocyte donor groups was 31.4 ± 4.7 and 30 ± 4.6 years, respectively. The mean number of oocytes retrieved was significantly higher in the elevated P group with 16.6 ± 10.6 vs 11.5 ± 6.7 in the P ≤ 1.5 group (p<0.001). Maturation rate, fertilization rate and mean age of the acceptors did not vary significantly between the two study groups (p = 0.622; p = 0.767; p = 0.889, respectively). The total number of good quality embryos on day 3 was significantly higher in the elevated P group with 6.1 ± 4.4 vs 8.7 ± 6.3, p<0.001); however, the good quality embryo rate and the utilization rate (defined as the number of transferred or cryopreserved embryos to the number of fertilized oocytes) were similar between the two arms (p = 0.386 and p = 0.098, respectively). Furthermore, multivariable regression analysis accounting for donor age and number of oocytes retrieved as potential confounders showed that CLBR was not negatively influenced by LFEP (60.7% for P ≤ 1.5 ng/ml and 71.2% for P>1.5 ng/ml, p = 0.115).

Limitations, reasons for caution: This is an observational study based on a retrospective data analysis. Better extrapolation of the results could be validated by performing a prospective analysis.

Wider implications of the findings: This is the first study providing evidence that LFEP in oocyte donation programs does not influence embryo quality neither cumulative live birth rates in the oocyte recipient.

Trial registration number: not applicable.

P-704 Effects of progesterone on the regulation of luteinising hormone surge in adult female rat

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Study question: Our clinical studies have shown that progesterone acts as an effective alternative for blocking premature LH surges in COH. What is the underlying mechanisms of progesterone blocking LH surge?

Summary answer: Our results indicate progesterone prevents premature LH surges during superovulation in women and rats. The hypothalamic AVPV and ARC nuclei may be targeted for this.

What is known already: Premature LH surges are a major cause for cycle cancellation during controlled ovarian stimulation (COH) in women undergoing IVF. Efforts to minimize the occurrence of premature LH surges have mainly relied on the use of GnRH agonist/antagonist, which exert greater risk of side effects. Our clinical studies have shown that progesterone acts as an effective alternative for blocking premature LH surges in COH. Early studies indicate the hypothalamus mediates the feedback effect of progesterone on regulation of LH level. However, the underlying mechanisms remain unknown, which exert difficult for clinical doctors to use this protocol.

Study design, size, duration: This study included a total of 64 adult female Sprague-Dawley rats, who were given a single intraperitoneal injection of pregnant mare serum gonadotropin to induce ovarian hyperstimulation followed by either progesterone or oil injection for 2 days.

Participants/materials, setting, methods: The collected data considered the LH surge and pulse, progesterone and estradiol dynamic during hyerovulation. Also, the follicle development and embryological result were examined. We tested the hypothesis:1, progesterone can prevent premature LH surge during superovulation without affecting oocyte quality in rat model; 2, Progesterone may prevent premature LH surges via progesterone receptors in the hypothalamic arcuate (ARC) and/or anteroventral periventricular (AVPV) nuclei; loci known to be involved in LH pulse and LH surge generation.

Main results and the role of chance: Our results indicated that progesterone prevents premature LH surges during superovulation in rats,without affect the number of oocyte retrieved and further embryonic development, These results coincide with our clinical observation. Meanwhile, progesterone administration prolonged the estrous cycle (P<0.05) in SD rats. Onset of the LH surge was significantly delayed when progesterone was administrated with PMSG(P<0.05). Furthermore, bilateral microinjections of anti-progestin RU486 (5 ng/μl, 0.8 μl) into the AVPV and ARC influence LH levels. Therefore, The hypothalamic AVPV and ARC nuclei may be targeted for progesterone regulation of LH surges in the rat.

Limitations, reasons for caution: Transferability of our findings is challenged by the possible variation of hypothalamus nuclei corodinates, but positively affected by our intra-nuclei injection technique and observation on microscopic histological inspection of stained brain sections to eliminate animals with inappropriate bilateral cannulae placement.

Wider implications of the findings: Our present study enhances the current understanding of the roles of progesterone in the regulation of the GnRH/LH surge:1) progesterone can effectively blocking LH surges during superovulation while maintaining normal follicle development ;2.The hypothalamic AVPV and ARC nuclei may be targeted for progesterone blockade of LH surge in rats.

Trial registration number: Not applicable.

P-705 Retrospective analysis of DuoStim cycles shows similar overall performance and oocyte quality between follicular and luteal phase stimulation

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Study question: Is there any difference in the overall performance of follicular and luteal phase stimulation among patients undergoing double ovarian stimulation (DuoStim)?

Summary answer: DuoStim shows similar results with respect to the number of retrieved oocytes, fertilization and blastocyst formation rates in both the follicular and luteal phase stimulation.

What is known already: Previous studies have demonstrated that several waves of cyclic development of antral follicles may coexist within the same menstrual cycle. Moreover, it has been shown that follicles formed during the luteal phase have a similar ovulation potential. Often, physicians are compelled to perform several stimulation cycles to obtain more eggs. Thus, the DuoStim

protocol could be an attractive alternative to obtain a higher number of oocytes in a shorter period, declining the time-to-pregnancy. It also emerges as an interesting option for emergency fertility preservation in oncologic patients. However, more studies are needed to validate its feasibility.

Study design, size, duration: Retrospective analysis of DuoStim cycles performed over the past year in IVI-RMA Global clinic, Madrid, Spain. We aimed to evaluate the potential usefulness of the DuoStim protocol by comparing oocyte quality and overall performance between follicular and luteal phase stimulation within the same menstrual cycle.

Participants/materials, setting, methods: We included 25 women who underwent a total of 30 cycles using the DuoStim protocol in 2017 at IVI-RMA Global clinic, Madrid, Spain. Data containing baseline and demographic characteristics along with the variables of interest from both the follicular and the luteal stimulation were exported from our institutional repository software platform. Statistical analysis included ANOVA test for variables with normal distribution and Mann-Whitney U test whenever the distribution was not normal.

Main results and the role of chance: Baseline characteristics revealed that the median values of age, AMH, AFC and IMC were 39.2 years, 1.34, 5.88 and 20.8 kg/m^2, respectively. Thus, our study population was mainly composed of women with advanced age and low ovarian reserve. In fact, all patients presented with very poor reproductive prognosis and multiple IVF failures. Variables of interest presented a normal distribution, except for the blastocyst formation rate. ANOVA test demonstrated no significant differences between the follicular and luteal stimulation, respectively, regarding the median of: days of stimulation (10.53 vs. 11.83; $p = 0.077$), amount of gonadotropin administered (2059.26 UI vs. 2603.45 UI; $p = 0.061$), retrieved oocytes (4.83 vs. 5.17; $p = 0.737$), MII oocytes (3.93 vs. 4.20; $p = 0.741$) and fertilization rate (60.75% vs. 62.76%; $p = 0.830$). Likewise, Mann-Whitney U test did not show any difference with respect to the blastocyst formation rate (20.78% vs. 26.62%; $p = 0.260$). There was a trend to use greater amounts of gonadotropin during the luteal phase, although not statistically significant. In conclusion, the use of the DuoStim protocol exhibited encouraging results irrespective of age and ovarian reserve. Similar findings were reported by other few studies comprising patients with a distinct clinical profile. Randomized clinical trials are needed to legitimize its use in daily practice.

Limitations, reasons for caution: Unequivocal limitations are essentially related to the retrospective design. Therefore, the study is prone to sources of error due to confounding factors. Nevertheless, considering the scarcity of prospective data in literature, our results provide important insights on the effectiveness of the DuoStim.

Wider implications of the findings: This work contributes to confirm the efficiency of the DuoStim protocol. It may be especially convenient for oncologic patients or those with a poor reproductive prognosis pursuing infertility treatments. Not only it reduces the time to obtain a higher number of eggs, but it also attenuates the couple´s anxiety.

Trial registration number: Not clinical trial.

P-706 Primary subfertility is significantly associated with higher levels of Thyroid Stimulating Hormone within the normal range preceding In Vitro Fertilization

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Study question: Is the distribution of Thyroid Stimulating Hormone (TSH) levels within the normal range different in primary or secondary subfertile women without thyroid hormone substitution?

Summary answer: In women with primary subfertility preceding In Vitro Fertilization (IVF) the prevalence of higher TSH values is significantly increased compared to secondary subfertile women.

What is known already: Among healthy fecund women, TSH levels ≥ 2.5 mIU/L were not associated with fecundity, pregnancy loss, or live birth (Plowden, et al., 2016). While in subfertile women the prevalence of TSH levels 2.5 mIU/L -4.5 mIU/L is much higher (20%) than in the general population (5 %) we don't know the influence of primary or secondary subfertility on this distribution. In the context of the discussion whether the cut-off level for TSH for subfertile women should be 2.5 mIU/L or 4.5 mIU/L we should fill this gap of knowledge and precise predicting factors for and define individual thyroid (dys) function.

Study design, size, duration: In a cohort study in women starting In Vitro Fertilization (n = 858) or Intra Cytoplasmatic Sperm Injection (n = 843) between the first of January 2008 and the first of March 2012 the distribution of TSH levels (0.3-2.49 and 2.50-4.5 mIU/L) was compared in primary and secondary subfertility women.

Participants/materials, setting, methods: Women aged 22-45 years with TSH 0.3–4.5 mIU/L without thyroid hormone substitution were included at VU University Medical Center, Amsterdam, the Netherlands, an Iodine-sufficient area. Chi-square tests were used to compare groups of patients based on TSH values using 2.5 mIU/l as a cut-off, and primary or secondary subfertility.

Main results and the role of chance: In primary subfertile women TSH is significantly increased preceding IVF compared to secondary subfertile women ($p = 0.006$): of the primary subfertile women 23.4 % has TSH levels in the higher reference range, while of the secondary subfertile women 16 % has TSH levels in the higher reference range.

Limitations, reasons for caution: Unknown values of free thyroxine and thyroid peroxidase antibodies limit the clinical interpretability.

Wider implications of the findings: Increased prevalence of higher TSH levels in primary subfertile women preceding IVF corresponds with Plowdens data that increased TSH values are of less importance in previous pregnant women. In primary subfertile women preceding IVF on the other hand these increased TSH values might disclose thyroid dysfunction.

Trial registration number: not applicable.

P-707 Caenorhabditis elegans, a model for polycystic ovary syndrome

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Study question: Can C. elegans be used as a model for POCS suitable for drug screening and gene target identification?

Summary answer: We have found drugs, natural extracts and genes that when inactivated reduce the infertility of a C. elegans POCS model.

What is known already: PCOS is the most common endocrine and metabolic disorder affecting women in reproductive age. Although the ethology is complex, in most cases this syndrome is related to the insulin signalling pathway. In fact, two-thirds of adult women with PCOS suffer from insulin resistance and hyperinsulinemia. Reduction of the insulin signalling pathway reduce fertility in all biomedical model organism, including invertebrate like Drosophila and the nematode Caenorhabditis elegans. C. elegans is a simple model organism that allow easily to search in high through put scale for genes, drugs and natural extracts that suppresses the reduction of fertility.

Study design, size, duration: We have selected C. elegans strains affected in the insulin pathway which generate a strong reduction of the fertility. Specifically, a mutant in the homologue gene to the insulin receptor and a mutant in the phosphatidylinositol-3-kinases.

By using high through put RNAi screening we have tested reduction of activity in 16.000 genes in the PCOS models and observed if the infertility is reduced. Furthermore, we have treated with 250 edible plant extracts and 50 purified compounds.

Participants/materials, setting, methods: C. elegans allows high through put screening of plant extracts and purified compounds. Plant extracts were collected by our group and hydrophilic and hydrophobic extract were generated of each extract.

RNAi screening were performed, feeding C. elegans with bacteria that produce double strand RNAi of target genes.

In each case more than 10 individuals were treated and in three days all the brood size was counted.

Main results and the role of chance: From 16.000 RNAi assays we have found that inactivation of 42 genes suppress the infertility of the PCOS model. We are currently analyzing those genes that are involved in different biological processes among them oxidative stress and cell cycle control.

Some of those genes are targeted of already identified drugs, which we are now testing for suppression of the infertility.

Two extracts form edible plants have also demonstrated a suppression of the infertility of the PCOS models. We are now purifying the active principle.

Limitations, reasons for caution: C. elegans model is a very suitable to perform high through put screening of drugs, compounds or RNAi; However; results needs to be validates in a mammal model.

Wider implications of the findings: Gen identification of suppressor of the infertility of the C. elegans model for PCOS will allow to better understand the nature of the reduction of fertility.

Identification of compounds or extracts can be of interest for future treatment of the disease.

Trial registration number: Not application.

P-708 Could polymorphisms of some hormonal receptor genes, involved in folliculogenesis help in predicting patient response to controlled ovarian stimulation?

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Study question: Are single-nucleotide polymorphisms (SNPs) in genes involved in folliculogenesis associated with serum hormonal levels and recombinant FSH (rFSH) dose used in controlled ovarian stimulation (COS)?

Summary answer: FSHR rs1394205 was only SNP that was positively associated with serum AMH level and used rFSH dose per retrieved oocyte.

What is known already: Despite of development in diagnostics, unexpected ovarian response in patients undergoing COS could still occur. For COS in an in vitro fertilization (IVF) programme, exogenous gonadotrophins are administered to patients. Numerous studies were focused on genetics of ovarian hormonal receptors but the results are contradictory. It is suggested that specific SNPs of hormonal receptor genes from granulosa cells influence foliculogenesis, oocyte development and maturation. Ovarian response to COS is usually determined by the number of growing antral follicles and consequently by the number of retrieved oocytes and is characterized as low, normal, or high.

Study design, size, duration: In a cross-sectional study (March–July 2015) 60 IVF patients were treated by GnRH antagonist and rFSH. Patients of advance age (>39 years), PCOS, or endometriosis were excluded from the study. Oocyte were retrieved from follicles >16 mm in diameter. Patients were normal- (7–15 oocytes)(n = 26), poor- (according to Bologna criteria)(n = 17) and hyper (>15 oocytes)(n = 17) responders according to the oocyte number. Blood samples were collected for basal FSH and AMH measurements and SNPs genotyping.

Participants/materials, setting, methods: Genotyping SNPs (AMH rs10407022, AMHR rs3741664, FSHR rs1394205, FSHR rs6166, and ESR1 rs2234693) was performed using high resolution melting (HRM) method. After follicle puncture the number of retrieved oocyte was counted and the dose of used rFSH per oocyte was calculated. Main outcomes measures were prevalence of genotypes among patient subgroups and associations between specific genotypes and clinical parameters (serum FSH and AMH, rFSH dose administered and number of oocytes retrieved).

Main results and the role of chance: AMHR rs3741664, FSHR rs1394205 and rs6166 and ESR1 rs2234693 were positively associated with serum AMH, FSH and rFSH dose used, whereas AMH rs10407022 was not. Selected SNPs do not show any association with oocyte number.

Patients with GG genotype of FSHR rs1394205 had lower measured serum AMH level, compared to women with heterozygotes and AA homozygotes in one group (AA+AG) ($p = 0.016$). No association with basal AMH level and other SNPs were detected. Patients with GG genotype of FSHR rs1394205 also required lower rFSH dose per oocyte than patients with AA+AG genotypes ($p = 0.036$). When compared genotype and allele frequencies between groups, we found higher frequency of GG genotype in poor- (76.5%) than in hyper-responders (37.5%, $p = 0.002$). Distribution of other SNPs between patient groups was not statistically significant.

Patients with AA genotype of FSHR rs6166 had higher level of measured basal serum FSH compared to those with AG+GG genotypes ($p = 0.043$). In other genotypes, hormonal levels were not different.

Women with GG genotype of AMHR rs3741664 required higher rFSH dose in COS in comparison with patients carrying genotypes AA+AG ($p = 0.028$).

Limitations, reasons for caution: Our results may be confounded by a small sample size; further investigations including more patients are needed to confirm our findings. Gene analysis on granulosa cells could confirm the impact of SNPs to the level of specific hormonal receptor expression.

Wider implications of the findings: Based on our results, genotyping of hormonal receptor SNPs from patient blood sample is not yet reliable diagnostic method for prediction of patient's ovarian response to rFSH therapy.

Trial registration number: Study was approved by Slovenian Medical Ethics Committee (012-347/2015-8). It is a part of research projects P3-0327 and J3-7177 funded by the Slovenian Research Agency.

P-709 Does the phenotype of polycystic ovary syndrome influence the ovarian response to controlled ovarian hyperstimulation for IVF?

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Study question: The aim of this study was to compare the ovarian response pattern to controlled ovarian stimulation (HOC) for IVF between different PCOS phenotypes.

Summary answer: Phenotype A seems to be the PCOS phenotype with the higher cancellation rate and the longer duration of stimulation.

What is known already: PCOS phenotypes do not have the same endocrine and metabolic profiles. But few studies have evaluated the impact of PCOS phenotype in IVF.

Study design, size, duration: This is a retrospective cohort study, in which 178 PCOS patients completed 376 IVF +/- ICSI cycles, between January 2009 and Marsh 2016, at the University Hospital of Lille.

Participants/materials, setting, methods: This PCOS population was divided into 4 phenotypes, defined by the modified Rotterdam classification, according to the presence of oligo-anovulation (OA), hyperandrogenism (HA) and polycystic ovarian morphology (PCOM) (on ultrasound and/or excessive serum AMH level). Phenotype B (OA+HA, without PCOM) was excluded because of its small size (n = 1). The response to HOC protocol was compared between the 3 phenotypes.

Main results and the role of chance: The PCOS phenotypes were defined as A (OA+HA+PCOM, n = 194 cycles), C (HA+PCOM, n = 82 cycles) and D (OA+PCOM, n = 100 cycles).

Full phenotype A had significantly higher LH and AMH levels than phenotypes C and D.

The number of canceled cycles was significantly higher in phenotype A compared to phenotypes C and D (22.7%, 13.4% and 12.0%, respectively, p = 0.038). The majority of cycle's cancellations in the phenotype A seemed to be due to a poor ovarian response, but there was no significant difference between the groups. Patients with phenotype A had significantly longer ovarian stimulation than those with phenotype D.

Furthermore, doses of gonadotropins used were not significantly different between the phenotypes.

Thus, a resistance to exogenous FSH could explain the higher cancellation rate for phenotype A, and could be associated with high AMH levels.

The hyperstimulation syndrome (OHSS) rates and clinico-biological outcomes between the 3 phenotypes were not significantly different.

Limitations, reasons for caution: The main limitation of this study is the retrospective design. However, the high number of cycles adds strong value to our study.

Wider implications of the findings: Our study suggests that patients with phenotype A seem more difficult to stimulate. A recent study showed that phenotype A may be at greater risk of OHSS. A prospective study could confirm the need to increase gonadotropin doses for phenotype A, with GnRH agonist trigger to limit OHSS.

Trial registration number: non applicable.

P-710 Multiple consecutive stimulation cycles as a method to shorten duration of infertility treatment

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Study question: Can a multiple consecutive stimulation protocol help in shortening duration of infertility treatment?

Summary answer: Multiple consecutive stimulation protocol has potential to shorten IVF treatment duration.

What is known already: In infertility treatment there are many cases when patients need to accumulate oocytes or embryos for fertility preservation, for planned preimplantation genetic testing, or due to low ovarian reserve (patients would benefit from shortening duration of their treatment).

Study design, size, duration: Our objective in the this study is to develop an effective multiple stimulation protocol (Gdansk protocol) that would allow patients to safely undergo consecutive stimulations. This would enable them to collect the desired number of oocytes or embryos in a short period of time.

Study included women aged over 37 who in previous attempts obtained few or no transferrable embryos.

Participants/materials, setting, methods: The preliminary stage of the study included 16 patients that underwent 2 (14 patients) or 4 (2 patients) consecutive stimulations. Mean age: 40.4 yo (SD 2.1) & mean basal AMH level: 1.78 ng/ml (SD 0.87 ng/ml).

Main results and the role of chance: Initial stimulation was performed with contraception priming and gonadotropin dosage was based on patients basal AMH level. As per standard procedure luteinizing hormone (LH), estradiol (E2) and progesterone (PRG) levels were monitored and on day 7 decision was made about extending the stimulation. Ovulation was triggered with triptorelin 34 hours prior to scheduled oocyte retrieval (OR).

Retrieved oocytes were fertilized using ICSI procedure and cultured for 5-6 days. Number of top quality embryos obtained was evaluated and based on that the decision to start the consecutive stimulation cycle was made.

Hormonal parameters were assessed at the start of cycle 2. Gonadotropin dosage was again based on patient's AMH level. Patients did not receive any medication to block premature LH surge due to the level of endogenous progesterone up to day 12 from the previous OR.

The reminder of the second cycle was managed as in the first one. If needed, again based on the number of embryos obtained, the decision was made to either complete the protocol or continue with another consecutive stimulation.

Mean number of blastocysts were 1.6 and 1.4 in 1st and 2nd cycle, COC – 9.0 & 8.9, MII - 6,6 & 7,1 respectively.

Limitations, reasons for caution: This is a preliminary stage of the study with a limited number of patients.

Wider implications of the findings: This appears to be a promising method of shortening duration of treatment for patients who need to accumulate embryos or face the pressure of time in their attempts to start a family. We are continuing this study and working on optimizing the protocol.

Trial registration number: Not applicable.

P-711 Effect of resveratrol and metformin on ovarian reserve and ultrastructure in PCOS: An experimental study

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Study question: Can resveratrol be used instead of metformin or combination with metformin on PCOS treatment via SIRT1 and AMPK activation?

Summary answer: Resveratrol can be used in combination with metformin to ameliorate PCOS symptoms such as impaired cell architecture, imbalanced hormone profile, oxydative stress, and inflammation.

What is known already: Polycystic ovary syndrome (PCOS) is a reproductive hormonal abnormality and a metabolic disorder. It is frequently associated with hyperandrogenism, chronic inflammation, and oxidative stress (OS), though the pathogenesis mechanism has not been well defined. Resveratrol (3,5,4-trihydroxystilbene) is a natural polyphenol synthesized by several plants as a phytoalexin. This agent may have beneficial effects such as antioxidant and anti-inflammatory actions. Metformin (1,1-dimethylbiguanide hydrochloride) is an oral antihyperglycemic drug widely used for the treatment of type 2 diabetes and can cause reductions in body weight, restore ovulation and increase the rate of pregnancy.

Study design, size, duration: 63 female Wistar albino rats (three weeks-old) were initially divided into control (n = 9) and experimental(PCOS) groups (n = 54). After DHEA administration, PCOS group (n = 54) were randomly divided into 6 groups (n = 9) as follows. Group 1 (PCOS; no treatment), Group 2 (Resveratrol; 20 mg/kg), Group 3 (Ethanol; resveratrol solvent), Group 4 (Metformin; 300 mg/kg), Group 5 (Salin; metformin solvent) and Group 6 (Metformin+Resveratrol). The daily treatments of both groups lasted for up to 28 days.

Participants/materials, setting, methods: Estrous cyclicity was monitored by vaginal smears. At the end of the treatment, body and ovary weights were measured. Right and left ovaries were processed for electron and light microscopic examination, respectively. For immunohistochemical examination, sections were performed anti-SIRT1 and AMPK antibodies. Apoptosis was evaluated by TUNEL assay. Serum FSH, LH and Testosterone levels were analysed with bio-otoanalyzer, while plazma and tissue AMH and TNF-α were determined by ELISA. Serum MDA levels were also measured.

Main results and the role of chance: Metformin and combined treatment groups reduced the body and organ weights compared to the PCOS group ($p<0.05$ and $p<0.001$, respectively). Serum testosterone levels were significantly higher in the PCOS group than in the control group($p<0.001$) and this was reduced when PCOS was treated especially with resveratrol($p<0.001$). Higher plasma AMH level in the PCOS group was decreased in the resveratrol group($p<0.05$). TNF-α levels were significantly increased in the PCOS group compared to the control group($p<0.05$) however, in all the treatment groups, TNF-α levels were significantly decreased compared to those in the PCOS group($p<0.05$). Serum MDA levels in the PCOS group significantly increased when compared to the control group($p<0.05$) but resveratrol and combined treatment reduced this oxidative stress marker levels compared to the PCOS group($p<0.05$). Primordial follicle pool was significantly diminished in the PCOS group($p<0.001$) whereas primary follicle count remained unchanged. Secondary, cystic and atretic follicles were increased in the ovarian sections of

PCOS group($p<0.001$) which were ameliorated in the treatment groups supported by light and electron microscopic findings($p<0.001$). Increased number of TUNEL(+) granulosa cells in the PCOS group were reduced significantly in the treatment groups($p<0.001$). SIRT1 and AMPK immunreactivity in the combined treatment groups were significantly increased compared to the PCOS group($p<0.001$).

Limitations, reasons for caution: Because of financial limitations, serum hormone levels were measured by otoanalyser instead of ELISA which gives more sensitive results.

Wider implications of the findings: According to our findings, resveratrol may act through its antioxidant and anti-inflammatory effects via SIRT1 and AMPK activation. However, using with metformin therapy gives better results to solve the disease. But these results should be supported by further clinical trials using different doses of these therapeutic agents.

Trial registration number: Not applicable.

POSTER VIEWING

Reproductive epidemiology, socio-cultural aspects and health economy

P-712 Fertility preservation procedures for female and male patients facing gonadotoxic treatment - a micro-costing and diagnostic related group payment comparison analysis

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Study question: What is the actual cost for fertility preservation (FP) within a large established clinical program in Sweden and its relation to Diagnosis-Related Groups (DRGs) tariffs?

Summary answer: Micro-costing analysis revealed a relative budget deficit of -27.1 % (total net value loss of €-250732) for all FP services performed in one year.

What is known already: The DRG system of payment was designed to encourage hospitals to operate efficiently and minimize overtreatment of patients. However, this may be challenging to needs for individualization and quality of services, as access to care risks being limited to selected patient groups. Despite the recognized importance of FP to cancer patients, high costs of the procedures and economic concerns are reported as main barriers limiting access to FP, especially for female patients. Until now, the actual costs of procedures involved in performing FP for both female and male patients have not yet been reported.

Study design, size, duration: A micro-costing analysis was done to accurately assess the costs of all FP-related procedures performed within one year in our clinical program, specifically oocyte cryopreservation, oocyte-embryo cryopreservation combined, ovarian tissue cryopreservation, sperm cryopreservation by semen banking and surgical sperm retrieval by testicular extraction. The costing exercise included all patients seen (n = 267) and all procedures directly related to gamete or gonadal tissue cryopreservation. Published hospital DRG prices were used for comparisons.

Participants/materials, setting, methods: Single-center study conducted at Karolinska University Hospital, which provides FP healthcare to the Stockholm region (population 2.2 million). Main health resource use categories for each procedure were identified (personnel, equipment, pharmaceuticals, laboratory related, single-use consumables and overhead charges), measured and valued using 2015 as the reference year (data for 2017 are under way). The bottom-up model included staff interviews and detailed real-life observations to accurately estimate the intensity of resource use.

Main results and the role of chance: Excluding cost of pharmaceuticals for ovarian stimulation, which are reimbursed directly to patients under the national pharmaceutical benefit scheme, the total cost of oocyte cryopreservation per patient (n = 38) was €2227, €3077 for oocyte-embryo cryopreservation (n = 50), €5990 for ovarian tissue cryopreservation (n = 28), €1356 for semen banking (n = 368), and €1981 for cryopreservation of sperm obtained by testicular biopsy (n = 10). Compared to the published DRG tariffs, the actual costs found and hence payments that the hospital received, were lower in all procedures evaluated (-14.4%, -38.0%, -8.7%, -32.1% and -27.8%, respectively). Multiplied by the number of procedures performed this translated into a net loss of €-250732 or in relative terms, a budget deficit of -27.1% for the year 2015 for FP services.

Limitations, reasons for caution: All FP procedures in Sweden are offered to the eligible population under the tax-funded health care services in academic centers only. The derived costs may not be generalizable to other FP centers.

Wider implications of the findings: An increasing demand of FP urges the complete evaluation of patient procedure costs to guide health care service for delivery and future planning. These results may increase awareness of national, regional and local health policy administrators to economic aspects of FP in planning for appropriate allocation of health services' budgets.

Trial registration number: Not applicable.

P-713 Knowledge of pregnancy and its danger signs among Indonesian women not improved by Maternal and Child Health Handbook

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Study question: What is the efficacy of the Indonesian Maternal and Child Health Handbook in improving the knowledge of various stages of pregnancy and its danger signs?

Summary answer: The Indonesian Maternal and Child Health handbook did not exert a significant influence in improving maternal knowledge around pregnancy and the associated obstetric danger signs.

What is known already: The first Maternal and Child Health handbook was introduced by Japan in the final decade of the 20th century and it was championed for empowering women in pregnancy, parturition and early childcare, as well as the pressing obstetric danger signs.

Study design, size, duration: This is a primary cross-sectional study conducted at Majalengka General District Hospital on recently delivering postpartum women between August – September 2017. 127 women were recruited, later divided into two separate groups according to their self-admission on the degree they had read the Maternal and Child Health handbook (50% and <50%).

Participants/materials, setting, methods: All the participating women were administered a pre-validated questionnaire to assess their knowledge around pregnancy and its danger signs.

Main results and the role of chance: It was discovered that our population had had high knowledge around pregnancy and its danger signs and the Maternal and Child Health handbook did not hold a significant role in effecting this finding (**p-value 0.295**). Furthermore, various socio demographic factors (age, maternal and paternal educational backgrounds, family welfare status, distance from healthcare center, parity and number of antenatal care visits) also did not exert a statistically significant influence on the level of knowledge in our population (**p-values 0.579, 0.521, 0.617, 0.908, 0.342, 0.618 and 0.939** respectively).

Limitations, reasons for caution: The relatively small sample size; selection bias as only a single population of patients (from Majalengka General District Hospital) was chosen; response bias in assessing the degree the handbook had been read and from answering the questionnaire; possible confounders, e.g. mass media and knowledge passed from relatives.

Wider implications of the findings: Our study revealed a satisfactorily high level of knowledge about pregnancy and the associated obstetric danger signs

among Indonesian women, but contrary to other studies, the use of Maternal and Child Health Handbook was not significantly responsible for such finding.

Trial registration number: not applicable.

P-714 Introducing Value Based Health Care as a new concept in treating fertility patients

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Study question: Can we increase value for fertility patients by either raising patient relevant outcomes resulting in the best possible health gain and/or by decreasing costs?

Summary answer: Value Based Health Care provides us an all-encompassing and powerful concept to provide better service for our patients against equal or even lower costs.

What is known already: Health care costs rise dramatically and society has major challenges to keep health care affordable for all. In The Netherlands the costs rose from 10% of the gross domestic product in 2000 to 14% in 2015. To turn the tide, a new concept in health care economics, Value Based Health Care (VBHC), was introduced by Michael Porter (2006). There are numerous examples where VBHC was successfully implemented (such as the UCLA Medical Center, USA, for kidney transplantation and the Martini Klinik, Germany, for the treatment of prostate cancer) but to our knowledge not yet for the treatment of fertility patients.

Study design, size, duration: Representatives of all professionals involved in fertility care were brought into a project team and during several sessions defined the necessary building blocks in close collaboration with actively involved patients. During these sessions truly relevant clinical outcome measures in relation to initial patient conditions and case mix variables were defined. Besides these, patient reported outcome measures (PROMS) and experience measures (PREMS) and the different care pathways were defined and described in detail.

Participants/materials, setting, methods: Professionals, such as physicians, clinical embryologists, nurses, technicians but also psychologists and social workers along with patients participated in defining relevant clinical outcome measures, PROMS, PREMS and the analysis of treatment pathways. Clinical outcome measures and treatment pathways are analyzed by means of a query from the electronic patient file. PROMS were linked to validated questionnaires and the PREMS to a tool measuring patient satisfaction. All these blocks are brought together into a management dashboard.

Main results and the role of chance: The most important clinical outcome measure is the cumulative live birth rate per patient journey, starting with referral to our center and ending with discharge from our center. A journey may consist of one or more treatment pathways, which in turn may consist of multiple treatment cycles. A first analysis in the pathway intrauterine insemination (IUI) with donor sperm, has revealed that a plateau is reached several treatment cycles before the maximum number of cycles that local and professional national guidelines propose. This knowledge can be shared with the patient and through shared decision making might result in more patient value. Less treatment cycles will be performed (and as a result a more rapid transition to a more invasive treatment) resulting in a more efficient treatment against lower costs and a shorter time to pregnancy.

The registration of initial patient conditions and case mix variables related to outcome measures enables us to a more individual approach, again increasing patient value.

The registration of PROMS and PREMS helps us to detect and take necessary measures for patients at risk for e.g depression and anxiety, sexual problems, but also for quality of life in general, both during treatment and in the long term.

Limitations, reasons for caution: The implementation of VBHC can be concerned as a prospective cohort study where results are systematically evaluated over a long period of time.

Currently fertility centers are paid as a fee per service. To prevent that treatments are offered which do not increase value, bundled payment arrangements should be made.

Wider implications of the findings: VBHC increases value for patients by increasing outcomes and/or by reducing costs. The implementation of an internationally accepted standard makes it possible to compare fertility centers corrected for initial patient conditions and case mix variables. Patient can then choose for the best value for their money.

Trial registration number: Not applicable.

P-715 The effect of religious background on the attitude toward sex selection

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Study question: Does the difference in religious background affect the attitude towards sex selection using pre-implantation genetic diagnosis (PGD)?

Summary answer: The study showed a negative attitude towards sex selection for non-medical reasons through PGD. Religious beliefs are the most important factors affecting such decisions.

What is known already: Surveys of the general population regarding sex selection using PGD are limited and were mainly conducted in the United States and Northern Europe. In those Western societies, surveys have shown that people's interest in using sex selection techniques is driven solely by the desire for a sexually balanced family, consisting of both sons and daughters. It is important to determine attitudes towards sex selection in a wider range of countries especially that cultural differences exists among countries.

Study design, size, duration: A questionnaire-based cross-sectional study regarding attitudes towards sex selection for non-medical reasons was designed. The population size targeted was 1500 participants. The surveys were distributed during the period extending from January 2016 to September 2017.

Participants/materials, setting, methods: 1500 participants of the reproductive age group presenting to the Women s Health Center at the American University of Beirut Medical Center (AUBMC) were offered to complete the survey. The questionnaire included demographic details, obstetric and infertility history, opinions regarding sex selection, personal interest in expanding the family, and personal interest in choosing the sex of a future child. Out of the 1500 patients approached, 1300 agreed to fill the questionnaire.

Main results and the role of chance: The calculated response rate was 86.6%. Eighty six per cent of the respondents were females and 19.4 % were males. Sixty three per cent of the respondents were Muslim, 26.2% were Christian and 8.7 % were Druze. Seventy two percent of the participants were fertile and 58.5% already had children. Nineteen per cent of the respondents considered it strictly prohibited, 38.8% considered the technique's acceptable only if medically indicated while 33.4 % believed that it should be available to all those who request it. Of those who opposed sex selection for non-medical reasons, 40 % considered the intervention as playing God while 34 % reasoned that the child is a gift that deserves to be loved irrespective of his/her sex. Only 20.1% of the respondents stated that they would use if the procedure was prohibited by their religion. When asked about the gender preference of their first child, 71.5% said they didn't care. Men and women equally stated that sex selection should be available for medically indicated conditions. Forty nine percent of Christians accepted the procedure when it was medically indicated while 35.5 % of Muslims considered it to be acceptable if medically indicated.

Limitations, reasons for caution: Despite the large sample size, all the participants were visitors of one clinic located in the center of the capital. Thus the population that we had might not actually reflect the total population living in the peripheral areas.

Wider implications of the findings: The knowledge of the factors that affect decision making in reproductive issues will improve couple counseling in this part of the world were religion plays an important role in decision making. This is imperative especially in debated situations such as sex selection for medical and non-medical reasons.

Trial registration number: not applicable.

P-716 Socioeconomic deprivation and pregnancy rates in IVF

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Study question: Are higher levels of socioeconomic deprivation associated with reduced success in IVF and ICSI cycles in terms of pregnancy and live birth rate?

Summary answer: Our study suggests that there is a statistically significant correlation between socioeconomic deprivation and successful IVF/ICSI treatment as an independent factor.

What is known already: Socioeconomic deprivation has been correlated with poorer access to health care and health outcomes and this has been particularly studied in the diabetic and mental heath care populations. There is a well reported 'postcode lottery' of access to assisted conception treatment in England but as yet there have been no studies looking into whether socioeconomic deprivation based on patient postcode is an independent variable influencing IVF /ICSI success rates.

Study design, size, duration: This was a retrospective cohort analysis. All patients undergoing a fresh IVF or ICSI cycle from the point of egg collection onwards between 1st January 2015 and 31st December 2016 were identified. All patients undergoing an elective freeze-all cycle (e.g. fertility preservation) were excluded. A total of 1661 patients were included in the study. Data was collected using both electronic and paper patient records.

Participants/materials, setting, methods: Patients undergoing IVF/ICSI at three south London hospitals were included, all using the King's College Hospital Assisted Conception Unit laboratory. Patient postcode, age at start of treatment, number of eggs retrieved and pregnancy outcomes were collected. The UK Index of Multiple Deprivation was used to assess deprivation based on patient postcode. Logistical regression analysis was performed to determine the effect of deprivation on pregnancy outcomes whilst controlling for patient age and number of eggs collected.

Main results and the role of chance: Using the UK Index of Multiple Deprivation, patients were categorised into deprivation quintiles (highest deprivation = quintile 1, lowest deprivation = quintile 5). Compared with patients undergoing IVF and ICSI in the lowest quintile of depravation, those in the highest quintile showed a statistically significant increase in both pregnancy and live birth/ongoing clinical pregnancy rates when controlled for age and number of eggs collected. The odds ratio for patients in the least deprived quintile relative to the most deprived quintile for pregnancy rates was 1.8290 (95% CI 1.251 to 2.675, $p = 0.002$). For live births and ongoing clinical pregnancy rates the odds ratio was 1.984 (95% CI 1.291 to 3.049, $p = 0.002$). There was a threshold level of deprivation (patients at deprivation quintile 3 or above) beyond which further decreases in deprivation had no further statistical impact for both pregnancy and live birth/ongoing pregnancy rates. Of note, there were increasingly more patients undergoing IVF/ICSI treatment as deprivation levels reduced (174 patients in deprivation quintile one compared to 410 in deprivation quintile 5).

Limitations, reasons for caution: This study included patients receiving treatment from three south London hospitals and, whilst this is a socioeconomically diverse part of England, it is not necessarily reflective of the wider IVF community. Inclusion of further co-variables such as duration of infertility and BMI would help improve statistical analysis.

Wider implications of the findings: By identifying socioeconomic deprivation as an independent variable affecting IVF/ICSI outcome, we may be able to provide more accurate individual predictions of treatment success. Future studies would be needed to determine which aspects of patient deprivation are most influential and whether there are any modifiable changes that can be made.

Trial registration number: not applicable.

P-717 Fertility awareness and knowledge among Indian women attending infertility clinic: A cross-sectional study

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Study question: How good is the knowledge and awareness of infertile Indian females regarding fertility in relation to age and menstrual cycle and need of assisted reproductive technique?

Summary answer: The study population had poor knowledge of fertility and reproduction and the knowledge regarding assisted reproduction varied according to socioeconomic status.

What is known already: Worldwide studies have shown that people are unaware of biological aspects of conception, have poor knowledge about most fertile period in the menstrual cycle, chances of getting pregnant in one cycle and about steep decline in fertility potential after age of 34-35 years. Also both men and women lack knowledge about effect of smoking, alcohol, job stress and other life style factors on fertility potential. There is generalized lack of fertility awareness irrespective of gender.

Study design, size, duration: Cross-sectional study of 205 infertile women seeking fertility treatment at assisted reproductive unit between March 2017 to August 2017.

Participants/materials, setting, methods: Total 205 infertile female patients in the age group of 21 to 44 years were interviewed with the help of nine item questionnaire designed after reviewing previous papers on fertility awareness and modified according to patient population and level of understanding in an Indian set up. The study population was divided into four socioeconomic classes according to modified Kuppuswamy scale. The answers were stratified according to socioeconomic status.

Main results and the role of chance: Majority of women (59%) belonged to the age group of 20-30 years indicating a young age group in need of infertility evaluation. More than half (63%) of the patients were from the middle socioeconomic strata. Even so, knowledge about fertility and reproduction was low: 85% missed the ovulatory period in the menstrual cycle, only 8% considered age more than 35 years as the most significant risk factor for infertility, approximately 60-80% were not aware when to seek treatment for infertility after trying for pregnancy. Almost 97% participants associated past intake of combined oral contraceptive pills with infertility. Participants in the upper SES scale had better knowledge (39% indicated need of donor oocytes in aged females) regarding ART treatment options. Terms like surrogacy, were significantly ($p = 0.002$) better known to participants in upper SES category (more than 50% correctly answered) than in the lower category (24%).

Limitations, reasons for caution: Studies with larger study population and a control group of fertile female patients will enable a better understanding of need of fertility education.

Wider implications of the findings: There is a significant lack of awareness regarding effect of age on fertility among women belonging to all types of social sections in India. There is need of educational interventions emphasizing on ideal age of fertility, factors affecting fertility potential and fertility options available for sub-fertile couples.

Trial registration number: Not applicable.

P-718 Randomized trial of a home pregnancy test intervention to improve cohort retention and detection of miscarriage

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Study question: In a cohort study of female pregnancy planners, does offering 12 home pregnancy tests (HPT) at enrollment improve cohort retention and detection of spontaneous abortion (SAB)?

Summary answer: Offering HPTs to female participants in a preconception cohort study appears to increase cohort retention and SAB detection.

What is known already: In prospective cohort studies of time-to-pregnancy (TTP), incorporating daily urinary human chorionic gonadotropin (hCG) testing

to detect implantation is ideal because it increases identification of early pregnancy loss, allows for examination of early vs. late pregnancy losses, and reduces misclassification of TTP. However, use of daily hCG testing is expensive and time-intensive, and is not practical for most population-based epidemiologic studies. No prospective cohort study of TTP has assessed the extent to which offering HPTs to female pregnancy planners improves cohort retention, increases detection of SAB and advances the timing of SAB detection.

Study design, size, duration: Pregnancy Study Online (PRESTO) is a web-based prospective preconception cohort study of ≥7,000 female pregnancy planners residing in the U.S.A. and Canada (2013 to present).

Participants/materials, setting, methods: Participants were aged 21-45 years, not using fertility treatment, and attempting to conceive for ≤6 cycles at study entry. Women completed baseline and bimonthly follow-up questionnaires. After enrollment, 401 U.S. participants were 1:1 randomized to receive 12 Clearblue HPTs, with guidance to test the day after a missed menstrual period (N = 198), or the standard protocol (N = 203). On follow-up questionnaires, women reported incident pregnancies, including SABs, and gestational weeks at SAB. We performed an intent-to-treat analysis.

Main results and the role of chance: After a median of 32 weeks of follow-up, cohort retention was higher among women randomized to receive HPTs (N = 166; 83.8%) relative to the standard protocol (N = 139; 68.5%) (mean difference = 15.4%, 95%CI = 7.1%-23.6%). Conception was reported by 62.7% in the HPT arm and 53.3% in the standard protocol arm. Among women who conceived, SAB was reported by 22 (21.2%) in the HPT arm (gestational weeks at SAB: median = 5.0, range: 4-10) and 13 (17.8%) in the standard protocol arm (gestational weeks at SAB: median = 5.0, range = 4-9). The number of SABs reported at <8 weeks' gestation was higher in the HPT arm (N = 18) than in the standard protocol arm (N = 11).

Limitations, reasons for caution: Numbers were small and results were imprecise. Participants were not followed for the full 12 months of follow-up, thus cohort retention will likely be higher than what we report above. Because the study relies on self-reported data, some SABs may have been missed.

Wider implications of the findings: Preconception cohort studies might benefit from offering 12 HPTs to participants at study enrollment as a means of increasing cohort retention and SAB detection.

Trial registration number: Not applicable.

P-719 The impact of night shift work on menstruation among nurses - a multi-center epidemiological investigation

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Study question: To explore the impact of night shiftonmenstruation among nurses.

Summary answer: Night shift might increase the likelihood of irregular menstrual cycle and aggravated dysmenorrhoea among nurses.Menstruation status alteration increased with the frequency of night shift increasing.

What is known already: Menstruation status was considered a symbolic of female fertility. Irregular menstrual cycle, including shorter or longer cycles were related to infertility. Some studies illuminated that stress was an important influence to menstruation,besides other factors including age,weight, tumor, imflammation or genetic factors.

Study design, size, duration: By questionnaires, a retrospective cohort study was conducted among 702 married female nurses who were at childbearing age and currently working at The First Affiliated Hospital of Sun Yat-Sen University, GuangDong Women and Children Hospital or Chinese PLA General Hospital from November 2014 to December 2014.

Participants/materials, setting, methods: By questionnaires, a retrospective cohort study was conducted among 702 married female nurses who were at childbearing age and currently working at The First Affiliated Hospital of Sun Yat-Sen University, GuangDong Women and Children Hospital or Chinese PLA General Hospital.

Main results and the role of chance: A total of702 nurses were investigated, among which 597nursesgave valid questionnaires, with an effective rate of 85.04%.Potential confounding factors were adjusted. 134nurses(22.45%) had a trend of irregular menstrual cycles, with apparent difference($P<0.05$) if they worked late night shift twice a week or more. 162nurses(27.14%) suffered aggravated dysmenorrhea, especially when nurses worked no less than thrice per week for late night shift ($P<0.05$). Corresponding OR values of late night shift were larger than those of early night shift. After adjustment for sleeping condition,OR values of both early and late night shifts on menstrual cycle and dysmenorrhea decreasedin certain degree, suggesting that sleeping condition may be the intermediaters.

Limitations, reasons for caution: Firstly, nurses without night shifts or unmarried nurses weren't included. Secondly, night shift workload and working hours weren't standardized.Thirdly, recalling bias and reporting bias was indelible in this retrospective cohort study.

Wider implications of the findings: Late night working may be the key to biological rhythm alteration. Further study on the machanism and among factors to the change of menstruastion of nurses with night-shifts

should be conducted, to better understand reproductive impairment of night shift work.

Trial registration number: not applicable

P-720 Mobile app utilization and fecundity in a north american preconception cohort

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Study question: Do women trying to conceive who utilize fertility apps have increased fecundability (shorter time to pregnancy) than women who use no apps?

Summary answer: Individuals who utilized any fertility app in comparison to no app had increased fecundability. Several apps designated *a priori* were associated with higher fecundability.

What is known already: Many women who are trying to conceive use a menstrual cycle or fertility app to identify the "fertile window," or the days of the menstrual cycle when intercourse is most likely to result in pregnancy. Prior studies have found that some apps are more accurate than others to identify the fertile window.

Study design, size, duration: Pregnancy Study Online (PRESTO) is an internet-based prospective cohort study of women trying to conceive, ongoing since 2013 in the U.S.A. and Canada. Women included in this analysis were enrolled through October 2017, were aged 21-45, in a stable relationship with a male partner, not utilizing contraception or fertility treatment, and had been attempting to conceive for < = 6 months. A total of 4345 women were included in this analysis.

Participants/materials, setting, methods: Female participants complete a baseline questionnaire providing demographic, lifestyle, medical and reproductive histories. They completed bimonthly follow-up questionnaires in English until conception or up to 12 months after study entry. Each baseline and follow-up questionnaire asked about specific menstrual cycle or fertility apps used, and any occurrence of pregnancy. A proportional probabilities model was used to estimate fecundability ratios (FR) and 95% confidence intervals (CI). Higher FR indicate higher fecundability (reduced time to pregnancy).

Main results and the role of chance: Among 4345 participants, 2527 pregnancies were recorded within 17,185 observed cycles (14.7% crude fecundability). Of participants, 72% used an app to record and possibly also interpret menstrual cycle data and/or fertility signs. The total number of apps used was 78, with 31% using one of a group of likely "advanced" apps specified *a priori*

because they can track multiple biomarkers of ovulation: Fertility Friend, Kindara, Glow, Clue, or Ovia. At baseline and adjusting for woman's age, gravidity, body-mass index, education, smoking, prior hormonal contraception, cycle regularity, and sleep quality, relative to using no app, the FR for individuals who used one of the *a priori* advanced apps, or any other app was 1.13 (CI: 1.03-1.23) and 1.07 (CI: 0.97-1.18), respectively. Results were similar in time-varying analyses based on responses from follow-up questionnaires, but varied somewhat when stratified based on duration of time attempting pregnancy. Strengths of the study include the prospective reporting of app use.

Limitations, reasons for caution: Fertility app use was self-reported, with no specific data on how intensively the app was used. Time to pregnancy was also self-reported. We cannot rule out bias due to differential loss-to-follow-up or residual confounding by subfertility history or attempt time at study entry.

Wider implications of the findings: A majority of women trying to conceive are likely to use a fertility or menstrual cycle app. Such app use may reduce time to pregnancy. Apps that track multiple biomarkers of fertility appear to be more effective than others to reduce time to pregnancy.

Trial registration number: NA.

P-721 Impact of patient's socioeconomic status on the outcome of IVF cycles in India

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Study question: Does patient's socioeconomic status like income, education level and occupation affect IVF outcome?

Summary answer: Patients undergoing IVF treatment in India have different success rates based on variables like income, occupation and education due to disparity in awareness and affordability.

What is known already: Apart from medical problem infertility is also a social problem affected by various factors like accessibility, availability and knowledge. In India, Infertility is not insurance covered so service seeking is comparatively difficult for lower socioeconomic class. Women working in male dominated occupational environment faces more work stress and time constraint in pursuing treatment. On the other hand, women working as teachers, dealing in public relations and housewives feel free to discuss their problems and are more comfortable in completing cycles. Education helps to counter the various negative beliefs related to Assisted Reproductive Technology.

Study design, size, duration: Longitudinal study was performed at fertility centre in Delhi, India where 800 patients were interviewed during the year January 2017 to December 2017. Data were analyzed using SPSS software version 20.

Participants/materials, setting, methods: The study included 800 patients who consented to participate. Data were analyzed using logistic regression model for the number of cycles undergone and clinical pregnancy rate on the basis of monthly income (80 Indian Rupees, INR = 1 €) {< INR 25000 (n = 256), ≥25000–100,000 (n = 400), >100,000 (n = 144)}, education {≤12thstd (n = 167), Graduate (n = 344), Postgraduate (n = 288)}, Occupation {women working in male dominated areas (n = 287), working in non male dominated areas (n = 304), housewives (n = 208)}.

Main results and the role of chance: A logistic regression was performed to ascertain the affects of income and number of cycles of IVF on the likelihood that participants achieved pregnancy. The logistic regression model was statistically significant, $\chi2(5) = 19.746$, $p < .0001$. The model explained 37.5% (Nagelkerke R2) of the variance in pregnancy and correctly classified 80.0% of cases. Patients with monthly income > INR 1,00,000 group were associated with an increased likelihood of pregnancy.

Similarly to ascertain the effects of education level on number of cycles of IVF so that participants achieved pregnancy. The logistic regression model was statistically significant, $\chi2(5) = 83.615$, $p < .0001$, model explained 30% (Nagelkerke R2) of the variance in pregnancy and correctly classified 78.0% of cases.

Logistic regression model for area of occupation was statistically significant, $\chi2(5) = 98.748$, $p < .0001$, model explained 30.7% (Nagelkerke R2) of the variance in pregnancy and correctly classified 77.0% of cases respectively.

As income, education level of patient increases and women working in non male dominant fields were associated with an increased likelihood of pregnancy. In the entire above model of logistic regression increasing number IVF cycle were associated with an increased likelihood of pregnancy although demographic features were comparable.

Limitations, reasons for caution: Although it is prospective study, it has limitation of small sample size.

Wider implications of the findings: People with low education level and limited knowledge about infertility may delay efficient interventions. Jobs that put stress on women may also negatively affect results. Income status compromises the affordability for repeated cycles of IVF. Clinicians may keep these in mind while counselling the patients and discussing about their prognosis.

Trial registration number: MCDH/2017/5

P-722 The role of ovarian hormones in generating options for social contact: Women scoring high on neuroticism show significant menstrual cycle fluctuations

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Study question: This study investigates whether the process of generating options for social decision-making is associated with ovarian hormone fluctuations during the menstrual cycle and women's personality structures.

Summary answer: The study revealed quantitative and qualitative differences in option generation between the ovulatory and the menstrual phases moderated by women's personality structures.

What is known already: Before making a decision, agents in less structured environments generate a set of options. There is a recently accumulating body of evidence on the affective, motivational, and cognitive effects of the menstrual cycle. It was argued that the pre-ovulatory rise of estrogen and dopamine may increase the quantity of options generated in everyday decision-making. There are findings on qualitative changes in the creativity of options as associated with situational effects, such as the familiarity of the situation. Individual and biological factors such as personality and endocrinology as well as the *social quality* of option generation have been neglected so far.

Study design, size, duration: An observational study with repeated measures was conducted to compare the *quantity and quality* of generated options in the ovulatory and the premenstrual phase of one menstrual cycle. In both the ovulatory and the menstrual session, naturally cycling women (N = 21) with a regular cycle (26-35 days) performed parallel versions of an *option generation task*. Session dates were individually fixed by ovulation test. The social quality of the options was independently rated by two trained raters.

Participants/materials, setting, methods: Female psychology students (mean age = 24) completed an option generation task consisting of vignettes of everyday decision making scenarios followed by the question "What could you do?" Personality was assessed using the NEO-FFI. Social option quality was rated on the dimensions *Social contact quality* (whether and what kind of person is involved) and *Social affiliative quality (*degree to which an option satisfies the human need for affiliation). We performed a multi-level analysis using SAS.

Main results and the role of chance: Concerning the quality of the generated options our analyses revealed that *Social affiliative quality* varies significantly across the cycle when Neuroticism and Extraversion are used as moderators: Significantly lower affiliative quality of generated options was found at ovulation compared to premenstrual testing in women scoring high on Neuroticism ($p<.05$). Elevated E2 levels could be associated to more self-reliance, self-sufficiency, confidence and independence, and hence less tend and befriend at ovulation. Also, women with a low extraversion score generated options with significantly higher affiliative quality at menstruation compared to ovulation ($p<.05$). This could imply that even women with low Extraversion may have increased needs for social connectedness in the pre-menstruum that is often experienced as more difficult than the ovulatory phase. *Social contact quality* never varies significantly as a function of the cycle, no matter which of the five personality scales is used as a moderator. Concerning the *quantity* of the generated options women scoring low on agreeableness as well as women scoring

low on Conscientiousness and also those scoring high on the Openness to experience dimension generated significantly more options at ovulation (p<.001), whereas women with a low openness score generated more options at menstruation (p<.01). Interrater reliability was excellent.

Limitations, reasons for caution: Limitations of the study are the rather small number of participants and no counterbalancing of order of sessions.

Wider implications of the findings: This study provides first empirical insights into the mechanisms of how ovarian hormones could be associated with social decision-making in different personalities. It also brings up more specific issues, for instance that strong social-cognitive fluctuations during the menstrual cycle may be less beneficial to relationship stability in highly neurotic women.

Trial registration number: Not applicable.

P-723 IVF outcome in Chinese versus Caucasian women

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Study question: Is there any difference between Chinese and Caucasian women concerning IVF outcomes?

Summary answer: There is a higher abortion rate in Chinese than in Caucasian women.

What is known already: Ethnicity is a common prognostic factor in medicine, but there is little data on the relationship between ethnicity and IVF outcome. It has been showed a reduced chance of live birth and clinical pregnancy rate in South Asian population compared to White women despite the similar choice of achieving pregnancy.

Study design, size, duration: In this retrospective cohort study, we investigate differences in terms of background and IVF outcomes between Chinese and Caucasian women underwent reproductive technologies in our Center from January 2014 till July 2017.

Chinese women were 3% of the total population (48 Chinese women versus 1451 Caucasian women).

Participants/materials, setting, methods: We matched some epidemiological characteristics and some outcomes of the two different groups (Chinese and Caucasian): age, infertility etiology, results of the ART in terms of pregnancy rate, miscarriage, serum estradiol values at the end of stimulation, number of eggs recovered.

We used *Chi-quadro* and *t-student* tests to match our results.

Main results and the role of chance: Differences were observed between age average, being significantly lower in Chinese than in Caucasian women (34,7 ± 4.8 *vs* 37.4 ± 4.1; P<0.01). The proportion of Tubal factor in Chinese was significantly higher in Chinese than in Caucasians (75% *vs* 18%; P<0.01), and it was found to be the most common infertility factor of Chinese women afferent to our Center.

We also observed significantly higher serum estradiol values average at triggering in Chinese than Caucasian women (2063.9 ± 1412.2 *vs* 1718.7 ± 1044.2; P<0.05) despite a similar oocyte recovery average (5.8 ± 3.3 *vs* 6.3 ± 4.6; ns)

Concerning IVF outcomes, we observe similar results in terms of pregnancy rate for transfer, but with a higher abortion rate in Chinese than in Caucasian women (27.0% *vs* 28.0% and 40.0% *vs* 18%; ns).

Limitations, reasons for caution: Further investigation and randomizations are needed in order to improve patient number and better understand the possible mechanisms involved in poorer IVF results in Chinese than in Caucasian women (systemic components, metabolic components, Other factors)

Wider implications of the findings: Our data confirm a reduced chance of live birth and clinical pregnancy rate in South Asian population compared to White women.

Trial registration number: Not applicable.

P-724 Infertility in the Middle East and North Africa region: a systematic review with meta-analysis of prevalence surveys

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Study question: What are the overall estimate of the different indicators of infertility in the Middle East and North African (MENA) region?

Summary answer: The overall clinical primary and total infertility rate in the MENA region are (3.8%, 95% CI = 1.7-8.4) and (17.2%, 95% CI = 10.6-26.7) respectively.

What is known already: Statistics on the birth rates in different regions of the world including the developing countries suggested that there is a decrease in the number of children per couple but there is limited epidemiological research on direct estimates of infertility in the developing countries.

Study design, size, duration: A systematic review with meta-analysis of infertility rates according to the clinical and demographic definitions was conducted. Relevant publications providing data from MENA region were retrieved from Science direct, Web of knowledge, Medline, Embase, Cinahl, google and WHO website with no language or date restriction on July 2017. Data of prevalence, risk factors and causes of infertility were extracted and meta-analysed to produce the overall effect sizes of the infertility estimates.

Participants/materials, setting, methods: Eight cross-sectional studies surveyed 35274 women to estimate infertility according to the clinical and epidemiological definitions, and one WHO report contained prevalence data from 4 MENA countries (35494 women between the ages of 15 to 49 years) were included in this study. Studies estimated clinical primary and total infertility were pooled in a forest plot using comprehensive meta- analysis software. In addition, the four estimates according to the demographic definition was also pooled together.

Main results and the role of chance: Research areas of studies were Iran (7 surveys) and Saudi Arabia (one survey) in the peer reviewed journal and demographic surveys on Egypt, Morocco, Jordan and Yemen which were reported by the WHO DHS program. The clinical primary infertility defined as "*.the failure to become pregnant after 12 months or more of continuous and unprotected sexual intercourse*" was estimated in 5 surveys as 3.8% (95% CI = 1.7-8.4, effect size = -7.564, p = 0.0001), with the total clinical infertility, both primary and secondary infertility, estimate was 17.2% (95% CI = 10.6-26.7, effect size = -5.5, p = 0.0001). However, this only represents studies from two countries; Iran and Saudi Arabia. *Percentage of women who have been married for the past five years, who have ever had sexual intercourse, who have not used contraception during the past five years, and who have not had any births*" was used to produce the demographic estimate of infertility in surveys by a WHO programme. Demographic primary infertility was 22.6% (95% CI = 13.4-35.5. effect size = -3.8, p = 0.0001) and demographic total infertility rate was 38.5% (95% CI = 28.8-49.2, effect size = -2.11, p = 0.035).

Limitations, reasons for caution: There was more research in Iran than on all other countries combined in MENA region which makes any overall pooling of data biased. There are also two different definitions of infertility. Infertility estimates in demographic surveys is extremely high possibly because of inclusion of many unmarried women.

Wider implications of the findings: Data suggested that infertility in the MENA region is low when it is defined clinically and high when it is defined demographically suggesting the need for standardizing infertility indicators to inform future health and social policy. There is limited information on causes of infertility. More robust epidemiological research is needed.

Trial registration number: Not Applicable.

P-725 Follicular fluid concentrations of phthalate metabolites and early reproductive outcomes among women undergoing in vitro fertilization

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Study question: Are follicular fluid (FF) concentrations of phthalate metabolites associated with impaired oocyte development and embryo quality among women from a fertility center?

Summary answer: Higher FF concentrations of certain phthalate metabolites were associated with lower mature and fertilized oocyte count, and declined embryo quality.

What is known already: Phthalates, a group of synthetic industrial chemicals, has led to widespread exposure across the world. Evidence from toxicological studies has established a role for phthalates in disrupting folliculogenesis, oocyte maturation and embryonic development. However, whether phthalates measured in the most biologically relevant matrix of the ovary (i.e. FF) can affect the developmental competence of in vivo exposed oocytes and result in impaired embryo quality is unclear.

Study design, size, duration: In this prospective cohort study, we recruited 636 women seeking infertility treatment at the Reproductive Medicine Center of Tongji Hospital, Wuhan, China, between 2014 and 2016.

Participants/materials, setting, methods: We measured eight phthalate metabolites in FF samples collected at oocyte retrieval. Data on early in vitro fertilization (IVF) outcomes, including total and mature oocyte count, number of fertilized oocytes and high-quality cleavage stage embryos, and proportion of high-quality blastocysts were obtained from electronic medical charts. Generalized linear models were performed to estimate the associations of FF phthalates and IVF outcomes. All models were adjusted for age, body mass index, smoking status and infertility diagnosis.

Main results and the role of chance: Most phthalate metabolites were highly detected in FF samples. The detection rates ranged from low (37.1%) for mono-n-octyl phthalate to high (77.2-98.6%) for the other seven metabolites. Mono(2-ethylhexyl) phthalate (MEHP) was the most abundant metabolite found in FF, with a median level of 1.65 ng/mL, followed by monobutyl phthalate (MBP) and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP). A borderline statistically significant relationship was observed for higher quartiles of FF MEHHP and lower oocyted yield (P-trend = 0.05). We found a negative dose-response association between FF monobenzyl phthalate (MBzP) and MEHHP relative to decreased mature and normal fertilized oocyte count, as well as number of cleavage embryos (all P-trend <0.05). Moreover, MEHHP was inversely associated with decreased number of high-quality cleavage stage embryos (P-trend = 0.01). Women in the second to fourth quartiles of MEHHP had a significant decrease of -11.49 (95% CI: -19.51, -2.66), -12.45 (95% CI: -20.55, -3.54) and -12.37 (95% CI: -20.31, -3.63), respectively, in high-quality Day 3 embryos compared with the first quartile. At the blastocyst stage, there was a significant trend toward lower proportion of high-quality blastocysts with increasing MBP, MBzP, MEHP, MEHHP and ∑DEHP [molar sum of three di (2-ethylhexyl) phthalate (DEHP) metabolites] quartiles (all P-trend <0.05).

Limitations, reasons for caution: The single measurement of FF may lead to misclassification of phthalate exposure owing to their short half-life and variability in individual behaviors. Our findings drawn from women undergoing IVF may not extrapolate to the general population, and the possibility of bias from uncontrolled confounding may also exist.

Wider implications of the findings: Our findings indicated that the intra-ovarian environment of women undergoing IVF has been pervasively exposed to most studied phthalates. Despite the low concentrations, certain FF metabolites were associated with adverse IVF outcomes, which raise concerns over exposure assessment at target organ and low-dose effect of phthalates on female reproduction.

Trial registration number: NA.

P-726 Donor recruitment for heterologous art in italy; still an uphill path

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Study question: Can spontaneous and altruistic donation alone enable setting up gamete donation programs for infertile couples?

Summary answer: Scarce heterologous oocyte supply in Italy four years after legalization underlines difficulties to secure local availability; establishment of sperm banks appears more easily achievable.

What is known already: In April 2014 the Italian Supreme Court legitimized heterologous ART. Gamete donation cannot be remunerated. The issue of reimbursement of expenses met by donors (e.g. absence from work, transportation) has not been clarified. As a result, a large majority of heterologous ART in Italy is based on gamete or embryo shipment from abroad.

Study design, size, duration: A total of 356 heterologous ART cycles were performed from January 2015 to January 2018. Gametes were obtained from donors recruited by our Center in Italy or shipped from abroad.

Participants/materials, setting, methods: Donors (314) were divided in 4 groups: 6 sperm internal donors (SID), 41 sperm external donors (SED), 3 oocyte internal donors (OID) and 258 oocyte external donors (OED). Number of treatments, mean donor age and number of cycles for each donor class were analyzed. Statistical significance of results was tested by Student's t-test for continuous variables, and by the χ^2 test for nominal data. $P < 0.05$ was considered statistically significant.

Main results and the role of chance: In the study period we obtained 15 local donations, 12 SID (6 excluded due to sperm or screening test abnormalities) and 3 OID. All OID were eligible. A total of 101 heterologous sperm IVF cycles were performed: 53 cycles (52.5%) with SID and 48 cycles (47.5%) with SED. A total of 275 heterologous oocyte IVF cycles were performed: 6 cycles (2.2%) with OID and 269 cycles (97.8%) with OED. The difference between the number of treatments performed with SID vs. OID was statistically significant (P<0.0001). The SID mean (±SD) age was 29.2 ± 1.5 years, whereas in SED it was 26.2 ± 4.2 (P = 0.003). The OID age was 28.3 ± 5.5 years vs. 24.7 ± 3.3 in OED (P=NS). The number of cycles for each donor group was 8.8 ± 4.8 with SID vs. 1.2 ± 0.4 with SED (P<0.05); and 2.0 ± 1.0 in OID vs. 1.1 ± 0.2 in OED (P=NS).

Limitations, reasons for caution: The number of local oocyte donors (OID) was very low, due to the logistics of donor recruitment.

Wider implications of the findings: Local sperm donor recruitment allowed to perform numerous procedures internally; the vast majority of oocyte donations required external supply. In Italy, a local donor program is possible for sperm, but still unfeasible for oocytes. It would be desirable that Italian governmental institutions provide clarifications regarding the economic treatment of donors.

Trial registration number: not applicable.

P-727 Cost-effectiveness of diagnostic investigation of infertility and subfertility: a systematic review

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Study question: Is diagnostic investigation of infertility and subfertility cost-effective?

Summary answer: Diagnostic tests shown to be cost-effective were laparoscopy, hysteroscopy and chlamydia antibody testing.

What is known already: There have been many studies on cost and cost-effectiveness of treatment for infertility and subfertility. Studies on cost and cost-effectiveness of diagnostic investigation of infertility and subfertility were quite limited and diverse in scope and methodology.

Study design, size, duration: Systematic review was conducted by two independent reviewers. English language, peer-reviewed journal articles from January 1997 to December 2016 were screened with key search terms: cost-effectiveness, infertility, subfertility and names of each diagnostic test (for both female and male infertility/subfertility). Database search was done through PubMed, Medline, Scopus and cross-references.

Participants/materials, setting, methods: Inclusion criteria

- Diagnostic tests for infertility and subfertility in male and/or in female patients.
- Cost-effectiveness analysis was performed with reporting of cost and effectiveness data.
- Published between January 1997 and December 2016
- English language.
- Exclusion criteria.
- Ongoing study.
- Review and systematic review articles.
- Therapeutic investigation.

Main results and the role of chance: 7 of 33 identified articles presented actual cost and effectiveness data.

7 studies were reported from Canada (1), Italy (1), The Netherlands (3), Turkey (1), and USA (1). None of the studies was conducted under the context of a developing country. The identified studies exclusively focused on diagnostic investigation of infertility in female patients. Investigations evaluated were laparoscopy, hysterosalpingography, hysteroscopy, sonohysterosalpingography, transvaginal hydrolaparoscopy and Chlamydia antibody testing.

5 studies used modeling models while 2 studies were a part of clinical trials. Effectiveness was reported as expected live births and probability of pregnancy. Cost was reported as mean cost per live birth, total direct procedural costs (healthcare perspective) and total or average cost of care per patient. 4 out of 7 studies reported cost-effectiveness in term of incremental cost-effectiveness ratio (ICER). Only one study used a societal perspective approach while most studies used a healthcare perspective approach.

Diagnostic tests determined to be cost-effective were laparoscopy, hysteroscopy and chlamydia antibody testing. Influential factors in sensitivity analyses were female age and dropout rate.

Limitations, reasons for caution: There were a limited number of studies on cost-effectiveness of diagnostic tests for infertility and subfertility. Measurement of cost and effectiveness varied across studies.

Wider implications of the findings: Our systematic review shows that there is a limited number of studies on cost-effectiveness of diagnostic tests for infertility and subfertility especially in developing countries or in low-resource settings. More studies should be conducted for new diagnostic tests and in low-resource settings.

Trial registration number: not applicable.

P-728 The younger the better: the case for continuing use of IUI as a first-line infertility treatment in a Ghanaian population

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Study question: In low-income countries, can performing lower technology treatments such as intrauterine insemination (IUI), offer simple and less costly fertility treatment with effective pregnancy rates?

Summary answer: IUI is an effective first-line fertility treatment for unexplained and mild male factor infertility, particularly in younger women.

What is known already: Infertility in sub-Saharan Africa can be traumatic, especially for the woman. Primary and secondary infertility is higher in younger African women (<37 years). Mostly, younger couples are in low to middle-income brackets without good economic standing. To the best of our knowledge, no African country offer medical insurance covering fertility treatments, pushing young infertile couples to seek loans at high-interest rates (over 30%) for fertility treatments. Guidance from the National Institute for Clinical Excellence in the UK advises against IUI as a first-line fertility treatment. However, in our setting, IUI offers a low-cost, low-tech option, providing effective results.

Study design, size, duration: This is a retrospective study involving 859 couples who were treated with IUI in a fertility clinic in sub-Saharan West Africa between March 2012 and December 2017. Female participants were age-stratified into three groups; ≤30 years, 31 – 36 years and ≥37 years. Sperm count and motility data were collected and presented as mean ± SEM. Pregnancy rates were reported as percentages. ANOVA was performed to determine inter-group differences.

Participants/materials, setting, methods: All participants received IUI in a stimulated cycle using clomiphene citrate for 5 days followed by FSH (150IU) every other day for 3-5 times. HCG (10,000IU) was administered when leading follicle was ≥16 mm and the first insemination performed 36 hours post-HCG administration. A second insemination was performed 60 hours post HCG (24 hours after the first insemination). Ejaculated semen was prepared using density gradient centrifugation and the prepared semen warmed to 37°C some 30 minutes prior to insemination.

Main results and the role of chance: Enrolled participants had at least one patent Fallopian tube, with IUI only taking place if ovulation matched the side of patency. The main causes of infertility were anovulation, mild male factor and unexplained infertility. Approximately half of the female participants were in the age group 31 – 36 years (49.7%), with the remaining quarters in the age groups ≤30 (25.7%) and ≥37 years (24.6%) respectively. There was no significant difference in sperm count and percentage motility across age groups; [sperm count (million/ml): 18.3 ± 1.50, 19.3 ± 1.88 and 13.6 ± 2.20, p = 0.1296], [Motility (%): 34.1 ± 2.12, 31.8 ± 1.92 and 26.1 ± 2.91, p = 0.0697] respectively. There was a significant difference in pregnancy rates across the three age categories: 14.9%, 9.1% and 4.7%, p = 0.0142 for women ≤30, 31 – 36 and ≥37 years respectively.

Limitations, reasons for caution: A poorer pregnancy rate from IUI in women ≥37 years does not preclude such women from undertaking IUI treatments.

Wider implications of the findings: In resource-limited countries where medical insurance does not cover fertility treatments, younger women (≤30 years) could perform a less costly procedure like IUI with respectable pregnancy rates.

Trial registration number: N/A.

P-729 Expert consensus for primary management of reproductive health: a Delphi study

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Study question: To identify barriers and enablers to appropriate primary management of the reproductive health by healthcare professionals.

Summary answer: A good consensus was achieved by experts to identify barriers and enablers to appropriate primary management of reproductive health.

What is known already: Infertility affects more than 80 million people worldwide. Fertility problems may be caused by genetic abnormalities, infectious or environmental agents, and certain life styles. There are modifiable factors contribute to the burden of infertility. In addition to psychosocial problems that may cause the infertility, the treatment of infertility contributes to increasing the cost of healthcare. For many causes of infertility, primary prevention is feasible. Thus, an appropriate management of reproductive health from the very beginning might reduce the prevalence of infertility and improve health and quality of life.

Study design, size, duration: This observational study was carried out from September 10, 2017 to January 20, 2018 using the modified Delphi technique. The coordinating project group selected the expert panel and defined the items of the questionnaire. The questionnaire was distributed online on November 2017 through a web page.

Participants/materials, setting, methods: 40 gynecologists from private and public assisted reproduction clinics and from different Spanish regions were selected randomly and were invited to participate as a panel member. Individual and anonymous opinions were asked by answering a 58-items questionnaire via e-mail (two rounds were done). Level of agreement was assessed using measures of central tendency and dispersion. Consensus was reached on an item when more than 65% of level agreement or disagreement was achieved.

Main results and the role of chance: Response rate: 60%. After the first round, consensus items were 42 (72.4%): of which 35 in agreement and 7 in disagreement. Eight items were modified in the questionnaire of second round according to expert requests. All the items reaching consensus by experts will be presented. Some examples of items with agreement are: 'General population have not enough information about vitrification' (100%) or 'Oncologic patients are not the only patients where vitrification could be indicated' (100%), 'General gynecologists must be more implicated in reproductive health' (100%), 'Family planning must include information about reproduction probabilities and not be centered only on anticonception' (100%).

Limitations, reasons for caution: The inherent limitations of Delphi study. A further possible limitation was the use of a structured questionnaire but free text responses were allowed in the first-round questionnaire that were incorporated into the questionnaire used in the second round.

Wider implications of the findings: Consensus was reached between assisted reproduction specialists on the most important concepts, which can provide the basis for primary management of reproductive health in Spain.

Trial registration number: Not applicable.

P-730 The association between the number of oocytes retrieved for IVF, perinatal outcomes and obstetric complications

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Study question: Is there an association between the number of oocytes retrieved for IVF and perinatal and obstetric outcomes?

Summary answer: No significant association was found between the number of oocytes retrieved and perinatal outcomes, while an association was found for placenta praevia and male gender.

What is known already: Previous studies have shown that 8-15 oocytes retrieved at oocyte pick up (OPU) are optimal for live birth in fresh cycles. We showed in a recent study that cumulative live birth rate, including fresh and all cryo cycles following one OPU, increases by the number of oocytes retrieved up to around 20 oocytes, however also with an increase in serious side effects such as severe ovarian hyperstimulation syndrome (OHSS). Few studies with contradicting results have investigated if the number of oocytes retrieved also might be associated with negative obstetric and perinatal outcomes.

Study design, size, duration: Retrospective population-based registry study including all singleton deliveries after fresh IVF cycles performed in Sweden during the years 2002-2015 (n = 27,359). Treatment characteristics from The Swedish National Quality Registries of Assisted Reproduction including the number of oocytes retrieved, fertilization method, date of embryo transfer, number of embryos transferred, embryo stage and number of sonographically visible gestational sacs at gestational weeks 7-8 were cross-linked to the Medical Birth Registry (MBR) and the National Patient Register (NPR).

Participants/materials, setting, methods: Main perinatal outcomes were preterm birth (PTB), small for gestational age (SGA), and peri/neonatal death. Main obstetric outcomes were gestational hypertension/preeclampsia and placenta praevia. Multivariate analyses.were used to explore the association between the number of oocytes retrieved and outcome variables. Adjustments were performed for maternal age, parity, smoking, BMI, cause of infertility, years of infertility, chronic diseases, paternal age, treatment period, embryo stage and vanishing twin.

Main results and the role of chance: The number of oocytes retrieved were categorized in <10, 10-14, 15-19 and >20 oocytes and 4-9 oocytes was used as a reference. SET was performed in 76.4% of the cycles. Blastocyst transfer was performed in 12.7% and cleavage stage embryo transfer was performed in 87.3% of the cycles. No significant association was observed between the number of oocytes retrieved and PTB (OR 1.004, 95% CI 0.996 to 1.013), SGA (OR 0.999, 95% CI 0.998 to 1.009) or peri/neonatal death (OR 0.992, 95% CI 0.960 to 1.025). A significant association was detected between the number of oocytes retrieved and gender distribution, with a higher rate of males after > 20 versus 4-9 oocytes (AOR 1.121, 95% CI 1.042 to 1.206). Concerning obstetric outcomes a significant association was found for placenta praevia (AOR 1.016, 95% CI 1.001 to 1.032) while no association was found for preeclampsia after adjustment for confounders.

Limitations, reasons for caution: Some of the OPUs performed in 2015 have resulted in births in 2016, thus the obstetric and perinatal outcomes are not yet available in the registry for these treatment cycles.

Wider implications of the findings: These results are assuring concerning children outcome with no indication of an adverse effect of a high number of oocytes retrieved. The association between the number of oocytes and placenta praevia was significant, however weak. The new finding of an association with gender should be interpreted with caution.

Trial registration number: 2018:0201

P-731 Dizygotic multiple pregnancy, Dutch trends over two decades from 1995 through 2015

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Study question: To identify the contribution of natural conception, in vitro fertilization (IVF), intra uterine insemination (IUI) and ovulation induction (OI) to the occurrence of dizygotic twinning over time.

Summary answer: Twinning increased until 2001 and declined thereafter, related to maternal age only in case of natural conception and not with IVF/ICSI.

What is known already: Over the past decades there has been a strong increase followed by a decline of multiples in the Netherlands, similar to many other developed countries. The increase has been associated with the introduction of artificial reproductive therapies (ART). Multiple pregnancies are often seen as a complication rather than a success, because of the association with pregnancy complications endangering mothers health as well as the foetus health. It is important to keep closely tracking trends regarding multiple pregnancy rates in relation to the various conception modes, including the natural way.

Study design, size, duration: Data from the national obstetric registration (PRN) for a period of 20 years were analysed. The data represent all hospitals in the Netherlands, as all twins are born in hospitals.

Participants/materials, setting, methods: The PRN data contains information on all primiparous and multiparous twins from an amenorrhoea of ≥16 weeks classified by mode of conception and information about gender. The number of opposite sex twins is multiplied by two to estimate the total dizygotic twins (Weinberg rule). Data of the Dutch Central Bureau for Statistics on total number of deliveries over the examined years was used in order to calculate prevalence of twin births.

Main results and the role of chance: From 1995 to 2001 there was a strong increase of twin births together with a gradually increase of the maternal age. Both naturally conceived and IVF/ICSI twins accounted for this strong increase, although the largest increase came from natural conception. Between 2001 and 2006 the number of dizygotic twin births was stable, both in the naturally conceived group as in the IVF/ICSI group. From 2006 onwards a

continuous decrease of naturally conceived dizygotic twins is seen, together with a decrease of maternal age in twin mothers.

Remarkably on average, maternal age of all delivering women over that period remained just over 31 years, whereas age of primiparous mothers continued to increase. The number of IVF/ICSI conceived dizygotic twin pregnancies declines from 2006 onwards. With a strong accelerated decline as of 2012 associated with a strong increase of the maternal age. Furthermore, since 2014 the number of dizygotic twin pregnancies after OI and IUI is larger than after IVF/ICSI in the Netherlands.

Limitations, reasons for caution: A limitation of this study that we have to rely on accurate recordkeeping in all registering hospitals. The way of recording information has not changed over the last years, therefore these data do provide accurate insight into valuable trends.

Wider implications of the findings: The decline of natural twinning from 2006 onwards in association with lower maternal age demonstrates decline of age related twinning which could reflect that family completion takes place earlier. This is supported by the ever increasing maternal age of primipara and the stable maternal age for all deliveries.

Trial registration number: not applicable.

P-732 The sex ratio in twins and the influence of gender mix in twins on gestational age, birth weight and perinatal mortality

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Study question: Is the sex ratio in twins different from singletons? And does the gender mix of a twin influence gestational age, birth weight and perinatal mortality?

Summary answer: The sex ratio in twins is lower compared to singletons. There is evidence for gender effect in twins on gestational age and perinatal mortality.

What is known already: The sex ratio in singletons is generally male-biased, and decreases in twins and triplets. This means that there are proportionally more girls in twins than in singletons.

De gender of one fetus of the twin can have an influence on perinatal factors of the other fetus of the twin, such as birth weight, gestational age and perinatal mortality. This influence is known as gender effect. For example, the gestational age in opposite gender twins is significantly longer that the gestational age in male same sex-twins.

Study design, size, duration: Our study group included 9563 twin pairs (9591 boys and 9535 girls) of the East Flanders Prospective Twin Survey (EFPTS). To study the sex ratio, the use of assisted reproductive techniques (ART), the zygosity, chorionicity and amnionicity were also taken into consideration. The sex ratio of singletons was based on the general register of East Flanders. Statistical analyses were performed using IBM SPSS Statistics 24.

Participants/materials, setting, methods: We performed statistical analyses on 9563 twin pairs of the East Flanders Prospective Twin Survey (EFPTS), which has been registering all multiple births of East Flanders since 1964. Perinatal factors were obtained from the obstetric records. To evaluate differences in sex ratio, we used Wilson score intervals and binary logistic regressions. To examine whether gender has an influence on perinatal factors, multiple linear regressions and multiple logistic regressions were used ($\alpha = 0,05$).

Main results and the role of chance: The sex ratio in twins is lower than in singletons ($p<0,05$). No significant results were found comparing the sex ratio between spontaneously conceived twins and twins conceived by ART, although there was a trend towards a decreasing sex ratio for dizygotic twins conceived through IVF+GIFT+ZIFT compared to spontaneously conceived dizygotic twins (OR = 0,929; p = 0,093). There is also a trend towards an increasing sex ratio for dizygotic twins compared to monozygotic twins (OR = 1,073; p = 0,087). We found a significant difference in sex ratio of dichorionic-diamniotic twins compared to monochorionic-monoamniotic twins (OR = 2,794; $p<0,001$), and of monochorionic-diamniotic twins compared to monochorionic-monoamniotic twins (OR = 2,760; $p<0,001$). This shows that the chance for girls is much higher in monochorionic-monoamniotic twins (in our dataset nearly 75% were girls).

There is an association between gender mix and gestational age, where the gestational age for girls with a twin brother is approximately one day shorter compared to female same-sex twins (p = 0,072), and approximately one day longer for boys with a twin sister compared to male same-sex twins (p = 0,088). No direct association between gender mix and birth weight was found. Comparing perinatal mortality, boys with a twin sister have a lower chance to die than boys with a twin brother (OR = 0,680; p = 0,022).

Limitations, reasons for caution: One important limitation of this study is that many confounding variables could have an influence on the results of gender effect (like smoking, infections during the pregnancy…), which were unknown in our database.

Wider implications of the findings: As our dataset is based on the Caucasian population in East Flanders, extrapolation to other populations is impossible. Although the effects are too small to be clinically significant, the findings do have a theoretical significance to help understand underlying mechanisms of twins.

Trial registration number: None.

P-733 Assessing the impact of obstetrics ultrasound on maternal and perinatal health outcomes in African rural or low-income settings

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Study question: Is there a direct association between obstetric ultrasound and reduction of maternal and perinatal mortality in African rural or low-income settings?

Summary answer: Use of obstetric ultrasound in African rural settings showed significant benefits with potential to reduce maternal and perinatal mortality rates.

What is known already: A disproportionate disparity exists in maternal and neonatal mortality rates between developed and developing countries of the world, with 99% of all maternal deaths occurring in developing countries.

Innovative and ground-breaking use of ultrasound in addressing disease burdens is a feature of healthcare systems in developed countries. The evidence base for routine use of ultrasound in antenatal care was mainly derived from the developed world, where other factors may account for the low maternal mortality rates.

Research done to establish a direct association between access to routine obstetric and reduction in maternal/perinatal mortality rates in African rural settings is sparse.

Study design, size, duration: Systematic review of journal articles on the use of ultrasound by appropriately trained persons, in resource-poor, African rural communities, as a diagnostic tool in obstetric care. 20 articles published between 2000-2017 which met the inclusion criteria were selected for the review.

Participants/materials, setting, methods: Electronic databases search (PubMed/Medline, Cochrane library, CINAHL, google scholar) were conducted using MeSH (Medical Subject Heading) keywords 'Ultrasonography', 'Maternal mortality and foetal mortality', 'Africa AND rural communities OR Low-income populations OR rural populations' to extract relevant articles which were screened for appropriateness and data extraction accuracy by a second reviewer. The Quality Assessment of Diagnostic Accuracy Studies -2 (QUADAS-2) tool for assessing the quality of diagnostic studies for systematic reviews was used for this review.

Main results and the role of chance: Results of this review should be interpreted with caution owing to the low quality of evidence supporting the assumptions of the authors, However, findings from the reviewed studies indicate increased rate of diagnosis of conditions associated with maternal and perinatal morbidity which has significantly informed change in clinical management decisions and early referral to specialist care. The power of ultrasound as a pull-factor to attract pregnant women and their spouses was demonstrated through increased attendance at antenatal care offered at health facilities. Opportunities for role extension through focused ultrasound training for rural healthcare workers was reported.

Though there is dearth of quality data for robust analysis to validate these observations, this review highlights the need for randomized controlled trials (RCT's)to evaluate the clinical and economic effectiveness of introducing ultrasound in African rural settings. Conceptual and methodological limitations of the currently published findings on this question should be fully considered by researchers, so that future trials will be rigorous in design and implementation to ensure conclusive findings.

Limitations, reasons for caution: Due to paucity of quality research on the study topic, the disparate nature of studies design/implementation and lack of study control groups, performing a risk of bias and applicability analysis was difficult, resulting in high likelihood of bias and limitation to the generalizability of the report's conclusions.

Wider implications of the findings: Obstetric ultrasound in African rural settings changes management of otherwise undetected high risk maternal conditions, enables early referral and increases attendance to and uptake of other beneficial interventions at health facilities. Opportunities for further research to generate the necessary evidence for use of ultrasound in antenatal care in rural Africa.

Trial registration number: not applicable

POSTER VIEWING
Reproductive surgery

P-734 Clinical analysis of gonads with ectopic adrenal tissue in disorders of sex development patients

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Study question: To summaries the characteristics, diagnosis and management of gonads with ectopic adrenal tissue in disorders of sex development(DSD) patients.

Summary answer: Removal of ectopic adrenal tissue does not affect the prognosis and may have some benefits in CAH treatment.

What is known already: The risk of dysplasia gonad with ectopic adrenal tissue in DSD patients is rare. Diagnosis relies on pathology, and hardly could be found pre-surgery.

Study design, size, duration: A total of 139 patients of DSD underwent surgery in Peking Union Medical College Hospital from Aug 2007 to Aug 2017. Among them, 5 patients were complicated with ectopic adrenal tissue and their clinical and pathology manifestation were analyzed.

Participants/materials, setting, methods: 5 patients, complicated with ectopic adrenal tissue and their clinical and pathology manifestation, were analyzed.

Main results and the role of chance: The ectopic adrenal tissue risk of DSD was 3.60% (5/139). All the 5 patients were female phenotype with Y chromosome or SRY gene. One patient was 45,XO, SRY(+) with dysgerminoma, the rest four patients were 46,XY, among which 3 were diagnosed with 17-alpha-hydroxylase(17-OHD),1 was partial gonadal dysgenesis (PGD). All the clinical manifestation and images before or in the surgery didn't show any sign but were testified of ectopic adrenal tissue in the resected gonad by pathology. 2 patients with 17-OHD received lower dosage of glucocorticoid after the surgery in the follow up. Hormone replacement therapy was given to maintain growth and development, and 2 patients obtained regular period.

Limitations, reasons for caution: Non-RCT study, only 5 patients were analyzed.

Wider implications of the findings: Current understanding of characteristics, diagnosis and management of dysplasia gonad with ectopic adrenal tissue in disorders of sex development(DSD) patients is limited. This study provided more data and evidence to learn about them.

Trial registration number: No.

P-735 Hemodynamic parameters and ICSI outcome in patients undergoing anesthesia for oocyte retrieval using alfentanil, fentanyl and remifentanil: a randomized clinical trial

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Study question: What is the effect of alfentanil, fentanyl and remifentanil on hemodynamic parameters and intracytoplasmic sperm injection (ICSI) outcome in patients undergoing general anesthesia for transvaginal oocyte retrieval?

Summary answer: All opioids lead to similar hemodynamic characteristics and pregnancy rate.

What is known already: In general anesthesia, there are some concerns about impacts of anesthetics on ICSI outcome. No study has been found to compare the effects of these three opioids in patients undergoing general anesthesia for oocyte retrieval and express the superiority of one opioid over others.

Study design, size, duration: In the present double-blinded clinical trial, 340 patients were assessed for eligibility and randomized for transvaginal oocyte retrieval following general anesthesia and 105 were lost to follow up. Data were collected from December 2014 to May 2017. Participants were randomized using block randomization method.

Participants/materials, setting, methods: Two hundred thirty five women ≤ 40 years old, with normal ovarian reserve tests and fresh embryo transfer were completed trial and divided among three groups: A (1.5 μg/kg, n = 78), F (1.5 μg/kg, n = 77) and R (15 μg/kg, n = 80). Patients with ≥ 2 unsuccessful ICSI cycle, history of cardiovascular disease, chronic opioid use and allergy to anesthetic agents did not include. All patients underwent pituitary suppression using long acting gonadotropin releasing hormone agonists.

Main results and the role of chance: There was no significant difference among groups with respect to demographic characteristics. Pre-induction and post-induction systolic and diastolic blood pressure and heart rate were not significantly different among the three groups. Time of respond to verbal command (A: 1.99 ± 1.64, F: 2.56 ± 1.72, R: 1.78 ± 1.34, P = 0.014), terminal systolic (101.61 ± 9.15, 105.29 ± 12.61, 102 ± 12.91, P = 0.01) and diastolic (A: 59.97 ± 9, F: 65.63 ± 9.13, R: 63.69 ± 11.01, P = 0.003) blood pressure and opioid induce cough (A: 21.8%, F: 11.7%, R: 31.3%, P = 0.021) was significantly different among groups.

No statistically significant difference were found among groups with respect to progesterone and estradiol level on human chorionic gonadotropin (hCG) day, total number of retrieved and metaphase II oocytes and endometrial thickness. The ratio two pronuclei (2PN) to total number of retrieved oocyte was significantly higher in remifentanil group than other groups (A: 51.6%, F: 54.4%, R: 62.2%, P = 0.018). There was no significant difference among groups with regards to implantation rate (P = 0.814), chemical (P = 0.922) and clinical (P = 0.925) pregnancy rate.

Limitations, reasons for caution: Due to high plasma level of estradiol or progesterone on hCG day in some patients and need to delay their embryo transfer, they were excluded, so sampling and data collection were taken long time.

Wider implications of the findings: Secondary endpoints revealed superiority of remifentanil due to shorter recovery time and higher ratio of 2PN to total number of retrieved oocyte compared to fentanyl and alfentanil.

Trial registration number: IRCT201410258677N4

P-736 Complicated clinical course and poor reproductive outcomes of women with tuboovarian abscess following fertility treatments

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Study question: To assess the clinical course, treatment and reproductive outcome of women with tuboovarian abscess (TOA) following fertility treatment.

Summary answer: TOA following fertility treatment has a substantial effect on the clinical course, surgical and fertility outcome.

What is known already: There is limited data regarding the clinical course, treatment and reproductive outcome of women with (TOA) following fertility treatment.

Study design, size, duration: Retrospective cohort study over 10 consecutive years.

Participants/materials, setting, methods: The study was performed in a tertiary university-affiliated hospital. Electronic medical records were used to identify patients who were diagnosed with fertility treatment associated TOA. Fertility unit charts and phone survey were used to assess patient clinical course, surgical and reproductive outcomes.

Main results and the role of chance: Thirty-seven women who were diagnosed with TOA following fertility treatments (in-vitro fertilization and intrauterine insemination) were compared with 313 women who were diagnosed with TOA not associated with fertility treatments during the same time period. Women with TOA following fertility treatments had significantly higher inflammatory markers upon admission as compared to the non-fertility treatment group (mean WBC 16.1 ×1000/mm3 (SD± 4.3) vs. 13.8 ×1000/mm3 (SD± 6.3), p = 0.001, respectively and mean CRP 149 mg/L (SD± 78.3) vs. 78.2 mg/L (SD± 68.5), p = 0.001, respectively). In addition, women with TOA following fertility treatments were associated with significantly higher surgical intervention rate and a more complicated clinical course as was shown by shorter time interval from admission to surgery (2.1 days vs. 3.2 days, p = 0.01), higher rates of antibiotic failure, higher conversion rate from laparoscopy to laparotomy (14.2% vs. 3.2%, p = 0.005), increased perioperative complications rate (25.0% vs. 3.8%, p = 0.0001) and a longer hospitalization period (7.2 days vs. 4.8 days, p = 0.01). Clinical pregnancy rate per cycle in women with TOA following fertility treatments was 9%. one case of live births was recorded.

Limitations, reasons for caution: Retrospective cohort analysis, based on medical records.

Wider implications of the findings: Prophylactic treatment and embryo transfer cancellation should be considered in patients that are at risk for infection.

Trial registration number: 'not applicable'.

P-737 Impact of laparoscopic myomectomy on reproductive and obstetric outcomes in patients with intramural myomas

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Study question: Does removal of intramural myomas improve reproductive outcomes?

Summary answer: Laparoscopic myomectomy (LM) may contribute to success infertility treatment in patients with intramural myomas.

What is known already: Uterine myomas are the most common benign tumors in women of reproductive age. Submucosal myomas have been shown to lower fertility rates, and their removal improves reproductive outcomes. However, the benefit of LM in subserosal or intramural myomas has not been clearly established.

Study design, size, duration: We performed a retrospective analysis at Shin-Yurigaoka General Hospital between 2012 and 2016. Of 766 patients who underwent LM during this time period, 81 infertile women with intramural myomas alone or both intramural and subserosal myomas were examined. Patients with submucosal myomas or those who discontinued infertility treatment at our hospital were excluded.

Participants/materials, setting, methods: Medical records were retrospectively reviewed to analyse reproductive outcomes and obstetric complications. We collected data pertaining to age, the largest myoma diameter, the number and total weight of the removed myomas, and distortion of the uterine cavity. The pregnant and non-pregnant groups were compared. The rate of pregnancy among 16 infertile patients who underwent resection of submucosal myomas by hysteroscopy during the same period was also evaluated as part of a secondary analysis.

Main results and the role of chance: Patient age in the pregnant and non-pregnant groups was 36.3 ± 4.2 and 38.2 ± 3.7years (p = 0.04), respectively. The number of intramural myomas (5.6 ± 4.6 vs 5.4 ± 3.7), the largest diameter (7.5 ± 2.4 and 6.9 ± 2.2 cm), and the total weight of myomas removed (225 ± 222 vs 169 ± 151 g) were not significantly different between the pregnant and non-pregnant groups. The rate of cavity distortion was also not significantly different between groups (55.8% vs 48.3%). The pregnancy rate among patients with intramural myomas who underwent LM was 64.2% (52/81), which is comparable to that in patients who underwent submucosal myomas resection by hysteroscopy (68.8%, 11/16). Overall, 52 patients had 63 pregnancies via ART (assisted reproductive technology) (n = 47) or non-ART (n = 16). The mean time period between LM and the first pregnancy was 12.3 months (range, 3-39 months; median, 10.5 months). The rate of miscarriages and live birth were 36.5% and 37.0%, respectively. Overall, 23 babies were born to 20 patients at our hospital by Caesarean section. All deliveries were after 37 weeks of gestation. No case of uterine rupture or placental abnormalities was observed. We did not observe any detrimental effect on reproductive and obstetric outcomes.

Limitations, reasons for caution: Our study is limited by its retrospective design and small sample size. Additionally, the lack of a control group does not allow for definitive conclusions.

Wider implications of the findings: Larger well-designed prospective randomized trials are needed to further evaluate the effect of LM on reproductive outcomes in patients with intramural myomas. LM may be offered to patients who do not become pregnant after infertility treatment. Management should be individualized considering age and infertility factors other than myomas.

Trial registration number: We did not receive a trial registration number.

P-738 Vaginoplasty and concomitant oocyte retrieval in Rokitansky syndrome

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Study question: Can invasiveness in the treatment of Mayer-Rokitansky-Küster-Hauser (MRKH) patients be minimized by performing concomitant laparoscopic oocyte retrieval and vaginoplasty?

Summary answer: We report the first three MRKH cases in which laparoscopic vaginoplasty and oocyte retrieval were efficiently combined and oocytes cryopreserved for future use.

What is known already: MRKH syndrome diagnosis is based on congenital aplasia of the upper vagina and uterus, and affects approximately 1/4,500 women. Patients experience the early distress of lack of sexual activity and infertility: thus, the main medical interests in this syndrome are to enable intercourse through vaginoplasty and genetic motherhood through oocyte retrieval and surrogacy or - more recently - uterus transplantation. Oocyte retrieval is not standardized in MRKH patients: due to reduced vaginal length or to adhesions resulting from previous surgical vaginoplasty,the routine transvaginal technique can be challenging and a laparoscopic or percutaneous approach can be needed.

Study design, size, duration: MRKH patients undergoing laparoscopic vaginoplasty between august 2017 and december 2017 at the IRCCS San

Raffaele Hospital (Milan, Italy) who expressed a desire for future motherhood were offered concomitant controlled ovarian stimulation and laparoscopic oocyte retrieval.

Participants/materials, setting, methods: After psychological and medical counselling, three patients aged 17, 17 and 21 gave informed consent and underwent controlled ovarian stimulation according to a GnRH-antagonist protocol. Final follicular maturation was induced with Gn-RH analogue to avoid the risk of ovarian hyperstimulation and combined surgery was scheduled 36 hours after. A standard 19 G ovum aspiration needle (Cook, USA) was inserted through the laparoscopic ports for oocyte retrieval and subsequent Davydov's laparoscopic vaginoplasty was performed.

Main results and the role of chance: In the first, second and third case n = 12, n = 14 and n = 18 oocytes were retrieved and cryopreserved. Subsequent steps were unchanged compared to the Davydov's laparoscopic vaginoplasty, without any additional technical difficulty. Postoperative care was also unchanged and no complications occurred. Compared to delaying oocyte retrieval, our concomitant approach abolishes the risks of ovarian aspiration in the presence of post-surgical adhesions as well as the anesthesiologic risks of a second procedure. Hence, referral to centers with expertise in vaginoplasty but also in reproductive medicine should be considered in all MRKH patients,and clinicians involved should contemplate this novel strategy for those MRKH patients with an indication to laparoscopic vaginoplasty and a desire for future motherhood.

Limitations, reasons for caution: Further evaluation of the safety of this novel approach will be of ineterest.

Wider implications of the findings: Because the doctor-patient relationship is crucial in MRKH syndrome, we envisage that simultaneous treatment of both the sexual and reproductive functions by a single team will also enhance patients' emotional ease and compliance with cares.

Trial registration number: Not applicable.

P-739 A retrospective analysis of the effect of salpingectomy for hydrosalpinx on in vitro fertilization – embryo transfer

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Study question: What is the indication of salpingectomy for hydrosalpinx in the patient undergoing in vitro fertilization and embryo transfer?

Summary answer: Salpingectomy is not effective for the pregnancy after the embryo transfer in patients older 41 years old.

What is known already: It has been demonstrated that, in patients with hydrosalpinx, the overall success of in vitro fertilization and embryo transfer (IVF-ET) is lower than expected due to implantation failure, miscarriage and ectopic pregnancy and it is generally recognized that removal of a hydrosalpinx can increase the implantation rate of IVF –ET. However, whether salpingectomy affects ovarian reserve is uncertain and the indication of salpingectomy is not clear as well.

Study design, size, duration: In this study, we compared the result of oocyte numbers and embryo genesis in 46 and 209 oocyte retrieval cycles in 124 patients after and without salpingectomy for hydrosalpinx between January 2014 and December 2016. Also, we compared the result of 52 and 189 embryo transfer cycles in 124 patients after and without salpingectomy.

Participants/materials, setting, methods: We compared the number of oocyte retrieval, fertilization rate and the rate of developing to the blastocyst in 27 patients after salpingectomy and 97 patients without salpingectomy for hydrosalpinx. Secondary, we compared the pregnancy rate, miscarriage rate and the live birth rate after the embryo transfer cycles in 29 patients after salpingectomy and 95 without salpingectomy. For the analysis of the significance, we used chi-square test and t-test and defined the significance as $P<0.05$.

Main results and the role of chance: In this study, the patients at age 23 to 45 years old were included. There was no significant difference in the number of oocyte retrieval, fertilization rate, and embryogenesis in the patients after salpingectomy and the patients without salpingectomy. Re the results after embryo transfer, the pregnancy rate was significantly higher in patients after salpingectomy than that of patients without salpingectomy (55.8% versus 39.2%, P = 0.03). The live birth rate was significantly higher in the patients after salpingectomy than that of the patients without salpingectomy (40.4% versus 18.5%, P = 0.0009).The miscarriage rate was lower in patients after salpingectomy than that of patients without salpingectomy (27.6% versus 48.6%, P = 0.07). Analysis by age showed that, in patients younger than 40 years old, the pregnancy rate and live birth rate for embryo transfer were higher in the patients after salpingectomy than that of the patients without salpingectomy. The all result in patients older than 41 years old were similar between the two groups.

Limitations, reasons for caution: The number of the patients who had salpingectomy for hydrosalpinx was smaller than that who didn't, and it means that we need to pay attention to not only the rate of the result but the numbers of oocyte retrieval and embryo transfer cycles.

Wider implications of the findings: Salpingectomy for hydrosalpinx is associated with better result in embryo transfer, but no effect on retrieval oocyte number or embryo genesis for the patients younger than 40. This means that we need to consider carefully for the suggestion of salpingectomy before the embryo transfer to the patients over 41.

Trial registration number: not applicable.

P-740 Effect of vitamin C on tissue damage and oxidative stress following tunica vaginalis flap coverage after testicular torsion

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Study question: Are the removal of compression syndrome by flap method and the use of vitamin C can lead to the reduction of testicular tissue damages induced by testicular torsion/detorsion?

Summary answer: Eliminating the pressure caused by compartment syndrome and using vitamin C decreased injuries induced by reperfusion in long-time testicular torsion.

What is known already: Tunica vaginalis flap coverage may decrease oxidative stress and tissue damage by reducing pressure on the testicles. It was found that flap coverage reduced tissue damage and oxidative stress as the duration of ischemia increased. A tunica albuginea incision with a tunica vaginalis flap can increase oxidative stress after short-term (1 h) ischemia. This may be the result of the surgical intervention; for this reason, the technique is useful only after long-term (9 h) ischemia.

Study design, size, duration: This experimental study was performed on 70 adult male Wistar rats weighing 250–300 g. Rats were randomly divided into two experimental groups: Group 1: testicular torsion for 5 h (T5) with 5 subgroups, including, TD5h, TDF5, TDVit.C5, TDFVit.C5, Sham 5h; Group 2: testicular torsion for 9 h (T9) with 5 subgroups, including, TD9h, TDF9, TDVit.C9, TDFVit.C9, Sham9.

Participants/materials, setting, methods: Torsion/detorsion, in 28 adults Wistar rats, was created by 720° counterclockwise rotation of the left testis. Also, in the same number of rats, testicular torsion was maintained for 5 or 9h followed by detorsion with tunica vaginalis flap coverage. After blood sampling, left testis was removed. Then, The Johnsen score, assessment of seminiferous tubules, and serum's oxidative stress markers were examined. The dates analyzed using SPSS 20 and ANOVA test applied for comparing the means.

Main results and the role of chance: The Johnsen score, the diameter of seminiferous tubules, and thickness of the seminiferous tubule epithelium significantly increased in the 5 h testicular torsion group receiving treatment with vitamin C and tunica vaginalis flap coverage compared with the group receiving tunica vaginalis flap alone ($p\leq0.05$). The level of testosterone decreased significantly in all groups except for the 5 h testicular torsion group receiving treatment with vitamin C and tunica vaginalis flap coverage. The MDA level also decreased in the group receiving treatment with vitamin C and tunica vaginalis flap coverage compared with the group receiving tunica vaginalis flap coverage

alone. The levels of SOD and GPx were significantly lower in the group receiving treatment with vitamin C and tunica vaginalis flap coverage than sham group.

Limitations, reasons for caution: Although this experimental study showed that flap method could prevent the reduction of spermatogenesis-related to testicular tissue damages, there are still unknown potential errors and lack of knowledge about possible side-effects of this method on this condition in the human's body; therefore, it can not use in clinical trials.

Wider implications of the findings: Testicular decompression after testicular torsion by tunica vaginalis flap technique can provide a protective effect against tissue damage and oxidative stress after prolonged ischemia and that such effects were not observed for short-term ischemia. Other studies also showed the same effect.

Trial registration number: The ethical considerations were based on the guidelines for laboratory animals from the Research and Technology Deputy of Gonabad University of Medical Sciences, Gonabad, Iran.

P-741 What is the optimal skin closure technique for 5 mm laparoscopic port-site? – a systematic review and meta-analysis

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Study question: What is the outcome differences between sutured [subcuticular (SC) or transcutaneous (TC)] and sutureless [Adhesive Skin Tape (ST) or Tissue Adhesive (TA)] 5 mm laparoscopic port-site skin closure technique?

Summary answer: Sutured and sutureless techniques have comparable cosmetic outcome. In sutured group, TC is better than SC while ST is better than TA in sutureless group.

What is known already: As laparoscopy has become the access of choice even in complex abdomino-pelvic surgery cases and more abdominal access ports have been used to assist with the surgery, the concern has now diverted into the cosmetic outcome of the skin closure. There are various techniques available, but it is mainly based on the surgeon's preference and ultimately patients' satisfaction and to date, there is no "gold standard" on the technique particularly in small 5 mm laparoscopic port-site.

Study design, size, duration: Systematic review and Meta-Analysis

Participants/materials, setting, methods: A total of 1053 papers were identified through electronic search and after screening, 5 studies (all RCT) were included for data synthesis. The PRISMA guidelines for randomised controlled trials were used to examine the quality of the studies. All suitable data were extracted and analysed using Review Manager 5.3 software.

Main results and the role of chance: We found a total of five studies comparing sutured (n = 367) with sutureless (n = 266) techniques. No studies compared closure with non-closure methods. From the studies using sutures, TC has better cosmetic outcome than SC (MD -0.79 [-1.45, -0.13], n = 104) & (OR 1.93 [1.29, 2.99], n = 118). In sutureless group, ST has comparable cosmesis with TA (OR 0.68 [0.28, 1.64], n = 88) but less reported pain (OR 5.75 [1.14, 28.88], n = 88). Compared with TC, TA has comparable cosmetic outcome (MD -0.8, [-4.44 to 2.84], n = 112) and no infection or complication such as hernia were observed in both techniques. Compared with both TC and SC, ST has comparable cosmetic outcome (MD 0.25 [-0.48, 0.98]), n = 75) and (MD -0.54 [-1.13, 0.05]), n = 75) respectively. Similarly no infection or complication were observed in these methods. Closure time were comparable between sutured group and TA (MD 8.4 [-1.27, 18.07]), n = 89) but slightly longer than ST (MD 9.7 [0.9, 18.5]), n = 89).

Limitations, reasons for caution: Despite the nature of the randomised included studies, the results of this study are still subjected to confounders relating to clinical and statistical heterogeneity. The studies reported the outcomes differently.

Wider implications of the findings: Conventional suturing – TC is still an optimal 5 mm laparoscopic skin closure technique. However, sutureless methods – TA and ST are able to eliminate risk of needle stick injury. The material cost of TA is higher than sutures or ST. Further trials should be conducted to evaluate total cost effectiveness.

Trial registration number: not applicable.

P-742 Robot-assisted myomectomy may improve pregnancy rates after surgery

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Study question: Is robot-assisted myomectomy is efficient for the management of myoma?

Summary answer: Among the patients having a desire of pregnancy, more than half obtained a pregnancy after myomectomy by surgery robot assisted, mainly spontaneously.

What is known already: Uterine myoma affects 25% of women worldwide. Conservative surgery for uterine myoma could be proposed in case of infertility, whatever the size and the number of myomas. Long terms benefits and obstetrical outcomes were not very evaluated with the robotic assistance myomectomy. The purpose of the study was to evaluate the outcomes of robot-assisted myomectomy in terms of pregnancy rates (spontaneous and with assistance reproductive technology).

Study design, size, duration: It is a monocentric retrospective study conduced between 2009 July and 2006 April in a French department of gynecology surgery and assistance reproductive center. 82 patients were referred in the center for the management of at least one uterine myoma. A robot-assisted myomectomy was realized by an experimented surgeon. An office hysteroscopy was prescribed 3 months after the surgery.

Participants/materials, setting, methods: 82 patients were included and 36 patients suffering from infertility. with a main time 2,5 years. Inclusion criteria were the indication of myomectomy (whatever the number) for patient suffering from infertility or abdominal pain. Age, BMI, time de conceive, number and size of uterin myoma were reported. The details of surgery were reported as the operative time and the surgical complications. The main objective was to evaluated the clinical pregnancy rate.

Main results and the role of chance: The mean age of patients was 36 years old (± 6). 45% of the patients suffered from an overweight.

The mean number of myoma was 2 (± 1.5) with a mean size of 65.9 mm (± 20.3) and a middleweight of 150 g (± 137) for the most voluminous myoma. The opening of the uterine cavity was noticed in 15.8% of the cases investigated.

Post operative hysteroscopy was performed 3 months after the surgery for 31 patients (85%). 2 cases of intra uterine adhesions were diagnosed and treated during the office hysteroscopy. 4 hysteroscopy resections for secondary myoma and one septum resection were also reported.

The clinical pregnancy rate was 32.8% (25 patients) which 51% of the infertile patients. The mean time to conceive was 18 months after surgery (±12.9). 8 pregnancies were obtained after assistance reproductive technology (28.6%). Among clinical pregnancy, 4 (14.3%) miscarriages were diagnosed and 2 cases of fœtal death (7,1%) and one pre term delivery (3,6%) were reported. Overall, the live birth rate was 37 % (22 cases).

Caesarean was performed in 17 cases (70.8%). No case of uterine rupture has been reported.

Limitations, reasons for caution: The main limitation was the absence of comparison between robotic-assisted and conventional laparoscopy. These encouraging results in favour of the robotic assisted myomectomy must be confirmed by randomized studies comparing to results with conventional laparoscopy.

Wider implications of the findings: Our results suggest a benefit to perform myomectomy with robotic assistance in agreement with the data of literature. The reduction of the opening uterine cavity could reduce the intra uterine adhesions and improve the pregnancy rate.

Trial registration number: This study received the agreement of the Ethics Committee of the university hospital (Institutional review board n°17.02.01)

P-743 T-shape uterus in the 21st century (post DES era) - we need to know more!

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Study question: Is T-shaped uterus prevalent in sub-fertile women in post DES era? What is the effect of hysteroscopic metroplasty on pregnancy rate, miscarriage rate and live birth rate for these women?

Summary answer: Surprisingly, the prevalence of T shaped uterus is significant even today. Hysteroscopic metroplasty is a simple procedure which can potentially improve outcomes in sub-fertile women.

What is known already: T-Shaped uterus is a rare uterine malformation, and has classically been associated with "in-utero" exposure of DES (diethylstilbestrol). It can be seen as a congenital variant or post infection synechiae rarely. The reproductive population of today's date constitute all women who were born well after the use of DES was completely stopped. The diagnosis of T shaped uterus can be made with HSG and 2D USS; and today with increasing use of 3D USS in Reproductive medicine, the detection rates have increased. The diagnostic criteria, investigation of choice, reproductive outcomes and treatment options are not well defined for these women.

Study design, size, duration: Systematic review and meta-analysis of literature.

Participants/materials, setting, methods:

- History of subfertility or poor reproductive outcomes.
- Diagnosis of T shaped uterus on HSG, USS or 3D scan.
- Hysteroscopic metroplasty done.
- Outcomes- pregnancy, miscarriage and live birth rates.

Total no of studies- 16 (7 published and 9 conference abstracts). There is scarce data on reproductive outcomes after intervention. There is no randomised controlled trial done, the only studies that are available are 9 retrospective analysis and 7 prospective case intervention studies.

Main results and the role of chance: Systematic review showed potential benefit from surgical intervention in the form of hysteroscopic metroplasty. It is reported to improve pregnancy rates and live birth rates and concurrently decrease miscarriage rates. It was difficult to derive meaningful measure of improvement due to potential differences and bias in reporting.

Meta-analysis of the data could not be done, because of following reasons:

- No randomised controlled trials.
- Heterogeneous study data – patient population, diagnostic modality and procedure details.
- Only number of pregnancies achieved mentioned in most of the studies, it is not clear if the same patient achieved multiple pregnancies subsequently.
- The interval between surgery and pregnancy is not clearly defined and it is difficult to comment if the intervention in the form of hysteroscopic surgery was the reason for improved outcome.

Limitations, reasons for caution: Scarce data and reporting bias.

Wider implications of the findings: There is a need for centralized database for registration of women with T shaped uterine anomalies which can be either a national or European registry database. This will help in defining clear diagnostic criteria, surgical indication & technique, and follow up of reproductive outcomes after the procedure.

Trial registration number: Not applicable.

P-744 Two cases of successful pregnancy after treatment for uterine cervical pregnancy by transcervical removing pregnancy contents and hysteroscopic cauterization following laparoscopic temporally uterine artery ligation

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Study question: Whether the case of uterine cervical pregnancy after treatment of our surgical methods achieve successful pregnancy and have uneventful delivery?

Summary answer: Two of the seven cases after treatment of our surgical methods for uterine cervical pregnancy were achieved pregnancy and had uneventful delivery.

What is known already: There were reports of treatment for uterine cervical pregnancy, for example MTX and/or KCL local injection, uterine artery embolization, Foley catheter balloon tamponade and hysterectomy, but our surgical methods: Transcervical removing cervical pregnancy contents and hysteroscopic cauterization following laparoscopic temporally bilateral uterine arteries ligation for uterine cervical pregnancy, has not been described.

Study design, size, duration: Study design: cohort study.

Size: Seven cases of uterine cervical pregnancy.

Duration: between 2003 and 2017.

Participants/materials, setting, methods: materials: Seven cases of uterine cervical pregnancy treated at our hospital.

methods: We examined seven cases of uterine cervical pregnancy from the medical records.

surgical methods: Transcervical removing cervical pregnancy contents and hysteroscopic cauterization following laparoscopic temporally bilateral uterine arteries ligation.

Main results and the role of chance: Two of the seven cases after treatment for uterine cervical pregnancy were achieved pregnancy and had uneventful delivery.

Four patients had a history of previous abortion with uterine cervical dilatation and curettage, two patients had also a history of previous cesarean section. Median age was 35 years-old(range 29-30), median operative times was 69.5 min(range 54-97), median estimated blood loss was 35 ml(range 10-200) and median postoperative hospital stay was 2days(range 2-3). All patients had no conversion to hysterectomy and intra- or postoperative complications.

Limitations, reasons for caution: Limitations: Our experienced cases are too small numbers but a uterine cervical pregnancy is an extremely rare ectopic pregnancy, the incidence ranging between 1 in 8000 to 18,000 pregnancies, and uterine cervical pregnancy represents about 0.15% of all ectopic pregnancies.

Wider implications of the findings: We experienced seven cases of uterine cervical pregnancy successfully treated with our surgical methods and two of the seven cases after treatment were achieved pregnancy and had uneventful delivery. This method may be an effective option in treatment of uterine cervical pregnancy.

Trial registration number: non-clinical trials.

P-745 Clinical pregnancy outcome of secondary infertility treated by transvaginal repair of previous cesarean scar defect

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Study question: The clinical pregnancy outcome of secondary infertility treated by transvaginal repair of previous cesarean scar defect (PCSD) is still unknown.

Summary answer: Transvaginal repair of previous cesarean scar defect (PCSD) can effectively improve the clinical symptoms of PCSD and treatment of secondary infertility.

What is known already: Transvaginal repair of previous cesarean scar defect (PCSD) is simple and time-saving, however, its effect on symptoms improvement and pregnancy outcome is not totally understood.

Study design, size, duration: Observation study.

Sample size is 56.

Follow-up duration is 12 months.

Participants/materials, setting, methods: 56 cases of patients with previous cesarean scar defect in our hospital were selected for transvaginal repair from Aug, 2015 to Aug,2016. The average age was 35 ± 4.2 years old. The infertility period is 2-8 years with abnormal uterine bleeding or pubis pelvic pain. All cases were treated with standard transvaginal repair.Histological

examination were perfromed after surgery and the size, location, the thickness of the uterine myometrium were confirmed by ultrasonic examination after 3 months.

Main results and the role of chance: All the operations were successful. Histology showed fibrous scar and lymphocytic infiltration. Abnormal uterine bleeding or the pain of the pubis pelvis was obviously improved after operation. Natural pregnancy was obtained in 55 cases after 1 year follow-up, of which 49 were given live birth (89.09%). 6 cases (10.9%) were aborted, and the patients with abortion were re-examined by B-ultrasound without diverticulum.

Limitations, reasons for caution: The sample size needs further expande. In this study, infertility was observed as the only complaint and exclused other male or female-factor infertility. All cases have had a health child.

Wider implications of the findings: Transvaginal repair of previous cesarean scar defect(PCSD) is desreved to popularization for treating second infertility due to PCSD.

Trial registration number: not applicable.

P-746 Skilled reproductive surgeons may ensure better outcomes in infertile patients

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Study question: Can a skilled reproductive surgeon improve the pregnancy rate (PR) in patients who undergo laparoscopy for infertility?

Summary answer: A skilled reproductive surgeon can significantly ameliorate pregnancy rate in infertile patients undergoing surgery when a putative cause is identified and treated intraoperatively.

What is known already: Unexplained infertility (UI) affects 1/3 of subfertile couples. The use of laparoscopy in the work-up of these patients is still a subject of debate, although laparoscopy remains the gold standard for diagnosis and treatment of several pelvic pathologies. Actually, a high proportion of women with unexplained infertility or minor abnormalities treated at the time of laparoscopy, conceive spontaneously. In other fields of gynecological surgery, such as oncology, the surgeon's expertise plays a pivotal role in determining the outcomes. Few data are to date available on this matter in the field of reproductive surgery.

Study design, size, duration: This retrospective observational study included 83 infertile patients, who underwent surgery (diagnostic/operative laparoscopy and diagnostic/operative hysteroscopy) at our Institution between January 2015 and June 2017. The study population was divided in: group 1 (59 patients) operated by reproductive surgeons, and group 2 (24 patients) operated by non-specifically skilled surgeons. 9 patients were lost at follow-up. A questionnaire was administrated in January 2018, in order to establish the PR of the operated patients.

Participants/materials, setting, methods: The clinical records of women undergoing laparoscopy for infertility were retrieved from a computerized database. Exclusion criteria were: infertility male factor, overt metabolic or endocrine disorders, age >43 years. We analyzed the rate of spontaneous or ART-induced pregnancies in the two groups after dividing patients on the basis of the performed surgery (diagnostic-only vs operative). The analysis was conducted with the Mann-Whitney, Fisher-Exact and Chi-square tests. A p value ≤ 0.05 was considered statistically significant.

Main results and the role of chance: The two groups did not differ in median age (36,0 vs 37,0) and BMI (21.5 vs 23). The PR in group 1 was 40% (20/50) while in group 2 was 29.16% (7/24) with a p-value of 0.44. Afterwards, we analyzed the percentage of spontaneous and ART-induced pregnancy in each group and we found no statistical difference (12/20 vs 7/24, p-value: 0.068). Even in the subgroups of spontaneous pregnancy, the two populations did not differ in terms of age and BMI. As a secondary outcome we found out that 34/83 patients had pathologic findings at laparoscopy (40.9%): peritoneal adhesions, minimal-mild endometriosis and hydatid cysts. 4 of them were lost at FU. Patients were divided into two subgroups: patients who underwent *1*: diagnostic laparoscopy (real UI) (44/74) and *2*: operative laparoscopy (30/74). The overall PR did not differ between subgroups (p-value 0.80). Within subgroup 1, the PR was 38.6%, and no statistical difference between patients operated by skilled or non-skilled surgeon was found (34.2% vs 55.5 %, p-value 0.24). Interestingly, in subgroup 2 the PR of patients operated by a reproductive surgeon was significantly higher of one of patients operated by a non-skilled surgeon (53.3% vs 13.3%) with a p-value of 0.020

Limitations, reasons for caution: This was a retrospective observational analysis with a relatively small population. A further limitation is the use of data obtained by telephone surveys, which implies the risk of under- and misreporting.

Wider implications of the findings: Our findings suggest that skilled reproductive surgeons may ensure better outcomes in infertile patients where putative causes for infertility are identified. Based on this contention, infertile couples should be addressed to specialized Centers in order to maximize their chance to conceive. Further randomized studies are needed to confirm our hypothesis.

Trial registration number: Not applicable.

P-747 Who is at risk of endometrial cavity breach at laparoscopic myomectomy?

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Study question: To examine whether fibroid ultrasound characteristics are predictive of endometrial cavity breach at laparoscopic myomectomy.

Summary answer: The minimum distance of the fibroid edge from the endometrial cavity is the best predictor of endometrial cavity breach at laparoscopic myomectomy.

What is known already: It is accepted that submucous fibroids reduce the chance of spontaneous conception. Intramural fibroids larger than 3 cm have also recently been shown to affect the chance of conception after embryo transfer. Submucous fibroids mostly in the myometrium can be removed laparoscopically. One of the risks of surgery is breaching the endometrial cavity and associated risk of endometrial adhesions. Caesarean Section is often advised as the mode of delivery for women who have had their endometrial cavity opened at myomectomy.

Study design, size, duration: This is a retrospective cohort study of women who underwent a laparoscopic myomectomy from December 2014 to November 2017. Ultrasound images and operation notes were reviewed. The size, position and minimum distance of the fibroid from the endometrial cavity in the longitudinal plane were determined from ultrasound images (negative distance was scored when the fibroid protruded into the cavity). Endometrial cavity breaches at the time of surgery were identified from the operation notes.

Participants/materials, setting, methods: This study was carried out at a central London university teaching hospital. All women who underwent a laparoscopic myomectomy were initially included in the study. Women who did not have a pre-operative ultrasound and those who had more than two fibroids removed were excluded. Cases with incomplete ultrasound data were also excluded.

Main results and the role of chance: During the study period 74 women who had laparoscopic myomectomy and a pre-operative ultrasound were identified. The median age was 36.0 (IQR 33 – 39.8) and the median fibroid diameter was 68.2 mm (IQR 47.7 – 80.6). 10/74 (13.5%, 95% CI 5.7 – 21.3) had a breach of the endometrial cavity at laparoscopic myomectomy. Women who suffered a breach had a fibroid that was close to or within the endometrial cavity (distance from cavity -9.9 vs. 8.9 mm, p = 0.001, degree of protrusion 17% vs. 0.0% and intracavitary surface area 1463mm^2 vs. 0.0). A logistic regression model with cavity breach as independent variable and ultrasonic variables as predictors selected minimum distance from cavity as the best predictor of cavity breach (OR 0.79, 95% CI 0.73 – 0.92). 10/19 (52.6%, 95% CI 30.2 – 15.1) of women with a submucosal component to their fibroid did not have a cavity

breach. No women with a fibroid further than 5.2 mm from the endometrial cavity had a breach identified at surgery.

Limitations, reasons for caution: This is a retrospective study which could have led to selection bias. The ultrasound parameters were identified from saved ultrasound images and this confers a degree of subjectivity and bias. There is also possibility that an endometrial cavity breach may not have been identified intra-operatively.

Wider implications of the findings: There has been no previous study reporting the risk of endometrial cavity breach at laparoscopic myomectomy. Identifying patients who are at increased risk based on ultrasound facilitates appropriate pre-operative counselling regarding the risk of intrauterine adhesions and the need for Caesarean Section and more vigilant intra-operative endometrial cavity assessment.

Trial registration number: Not applicable.

POSTER VIEWING

Safety and quality of ART therapies

P-748 Fertility care: patient journey's modelling through invoice data

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Study question: The objective of this study was to build a model of the fertility care journey based solely on administrative data collected from invoices.

Summary answer: Analysis based on billing data alone is sufficient to build quick, reliable and usable model in fertility care which is a huge source of information.

What is known already: Time in fertility care is the most important prognosis factor, whether considered as the patient's age or duration of treatment. Mastering it is a way to improve the global success rate and the patient's experience. One way to tackle this issue is to take a look at the global patient journey and try to identify pitfalls that could lead to delays in care and impact the patient's experience and chances of positive outcome.

Study design, size, duration: It is a longitudinal retrospective study. Invoicing data usually used for economic purposes were collected and gathered on a 7 year period and focused on our department of reproductive medicine. Over 2.4 M patient files from January 2010 to December 2016 were studied. After extraction, analyses were conducted on data related to all activities of the center. We rebuilt each patient's billing history, gathering specific information including medical acts, medical units and date of provided care.

Participants/materials, setting, methods: Inspired by customer's modelling and decision tree, we applied 'big data" statistical approach to create a model displaying in a universally readable format the overall patient journey. We identified key steps, and calculated statistics to show time estimation and proportion of patients between each steps. In order to verify the quality and reliability of this model we conducted the same analysis, enriched with medical data extracted from MediFirst, software specialized in medically assisted reproduction field.

Main results and the role of chance: We defined a patient journey by detecting its beginning (first appointment) and its end (delivery). 45% of the patients having a first appointment pursue a treatment, 39% with intra-uterine insemination (IUI) and 61% with embryo transfer (ET). The first attempt occurs after 7 months (median) from the first appointment, faster for IUI than for ET (5 vs 6 months). We observed that 2 appointments were sufficient for 80% of the women to begin the IUI process but 3 or 4 were required for ET. Time for delivery is shorter for ET than for IUI (17 vs 22 months). After // a 3-year follow-up period the success rates are similar for IUI and ET: 64% of pregnancies and 50% of deliveries. Comparison of the two models showed that there were less than a 5% differences for each proportion tested. In conclusion, analysis based on billing data alone is sufficient to build quick, reliable and usable models in fertility care. This model is a huge source of information for the patients and the practitioners. The immediate results and concrete use of this study were a drastic reorganization in our facility practices, leading to reduced waiting time before beginning fertility treatment.

Limitations, reasons for caution: The reliability of the results depends on the quality of track keepind and encoding by medical staff.

Wider implications of the findings: The model has the potential to not only inform the patient about the overall process awaiting him, but also give a clear picture of the organization of an healthcare unit and areas to be improved. The strength of this model is to be transposable to virtually any medical unit.

Trial registration number: not applicable.

P-749 Risk evaluation for pronuclear transfer using for preventing the transmisson of mitochondrial disease: a mouse model

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Study question: Is it safe to prevent transmission of mitochondrial disease using pronuclear transfer as a mitochondrial replacement technology?

Summary answer: There is a potential risk in pronuclear transfer offspring in metabolism.

What is known already: Pathogenic mitochondria DNA (mtDNA) mutation is closely linked with many mitochondria diseases that usually lead to fatal results such as premature death. MtDNA is transmitted maternally within oocyte cytoplasm. Management of mitochondrial disease is limited due to its characteristic inheritance. Pronuclear transfer is considered to be effective in preventing the transmission of pathogenic mtDNA from mother to offspring.

Study design, size, duration: Pronuclear transfer technology were performed between zygotes of CD-1 mice to obtain constructed zygotes and offspring. We preformed 263 zygotes to assess embyoes development and 40 PNT offsprings to evaluate risk of PNT procedure.

Participants/materials, setting, methods: The constructed zygotes produced by PNT was fused by electrofusion. By testing mitochondria DNA copy number, the ratio of donor mitochondria was calculated. The cell number of balstocyst was counted and gene expression profiling was identified by NGS technique. Then PNT blastocyst were transferd to pesodu-pregnant mice. For PNT offspring, the body weight, ratio of fat/weight and energy expenditure were measured. The reproductive function was also assessed both in female and male PNT offspring.

Main results and the role of chance: The development efficiency was significantly decreased in PNT embryos compared with normal control embryos. Compared with normal fertilized embryos, total cell numbers of the blastocysts from PNT technology were decreased significantly, and a large amount of genes showed the abnormal expression levels. But there were no significant differences between neonatal body weight and plancenta weight. the ratio of donor mitochondria was no more than five percent. However, the quality of PNT blastocysts was declined. Compared with control blastocysts, implantation rate and birthrate in PNT blastocysts were all impaired.

For PNT offspring, compared to the controls, the body weight, ratio of fat/weight, energy expenditure, blood pressure and glucose tolerance showed no difference both in female and male pronuclear transfer mice. Estrous cycle, histogical analysis of ovary and fertility were normal in female pronuclear transfer mice, so were the testicular morphology, sperm concentration and fertility in male pronuclear transfer mice. However, a considerable of genes were differentially expressed between pronuclear mice and control group.In tissues where mebabolism were more vigorous like brain, heart, liver and muscle, an abundant of genes showed the abnormal expression levels. Differentially expressed genes were highly related in metabolism, which suggest a potential risk of pronuclear transfer offspring.

Limitations, reasons for caution: The ratio of donor mitochondria was not assessed in all tissues of PNT offspring during the whole life cycle. In addition, the F2 mice of PNT mice were not evaluated throughout the whole life.

Wider implications of the findings: The result not only emphasize the potential risk associated with metabolism of PNT procedure using to prevent transmission of mitochondrial disease but also facilitate a better understand of the effects of PNT procedure on development of offspring and enhancing the awareness of improving the outcome of PNT procedure.

Trial registration number: Not applicable.

P-750 To flush or not to flush: A retrospective analysis of follicle flushing during oocyte retrieval in Wales

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Study question: Is follicular flushing during transvaginal ultrasound guided eggs collection useful to improve outcomes in women undergoing IVF/ICSI treatment for sub/infertility?

Summary answer: Lower n. of oocytes, mature oocytes, fertilised oocytes in the flushing, compared to the direct aspiration group. Fertilisation, clinical pregnancy rates were not different.

What is known already: The practice of follicle flushing has been a topic of discussion since ultrasound-guided transvaginal oocyte retrieval was introduced in 1981. Theoretically, flushing would allow for the retrieval of more oocytes, and consequently result in greater fertilisation and pregnancy rates. However, many studies have found no significant increase. National Institute of Clinical Excellence (NICE) guidelines suggest that follicle flushing for women with 3 follicles prior to oocyte retrieval should not be offered, as it increases the length of the process, the pain experienced without the added benefit of greater numbers of oocytes retrieved or higher pregnancy rates.

Study design, size, duration: Data from 221 women who underwent oocyte retrieval at the University Hospital between May 2016 to April 2017 were retrospectively evaluated. 162 women were included in this study, as 59 had to be excluded due to inadequate information available. 39 women (24.1%) were noted to have undergone oocyte retrieval with direct aspiration only, while the remaining 123 (75.9%) had undergone oocyte retrieval with follicle flushing.

Participants/materials, setting, methods: Procedures at the centre were performed using 16 gauge double lumen needles. Flushing medium used was from Origio.Chi square tests performed with $P < 0.05$ considered statistically significant. To determine a cut off value for the number of follicles needed to be present at the point of HCG administration, a Receiver Operating Characteristic (ROC) curve was plotted and the Area under the Curve (AUC) was calculated.SPSS version 24 was used.

Main results and the role of chance: The total number of oocytes (13.6 ± 6.5 vs 7.7 ± 5.2), the number of mature oocytes (11.7 ± 6.4 vs 6.3 ± 4.6), the number of fertilised oocytes (7.5 ± 5.3 vs 4.4 ± 3.6), the duration of procedure (21.8 ± 7.3 mins vs 20.2 ± 5.6 mins) as well as maturation (84.7% vs 83.7%) and clinical pregnancy rates (30.8% vs 22.8%) were found to be higher in the direct aspiration only group compared to the flushing group. The chi square test [χ^2 (degree of freedom)] indicated that all of the above, except the duration of procedure and clinical pregnancy rate, were statistically significant.On the contrary, the flushing group demonstrated higher fertilisation rates (57.8% vs 54.7%) when compared to the direct aspiration only group. However, these rates were not statistically significant.The area under this curve was measured to be 0.080 with 95% confidence interval (0.000, 0.181). This area unfortunately, being <0.5, implies that the cut off value of roughly 9 follicles at the point of HCG administration, below which follicle flushing would be recommended to obtain at least 3 mature oocytes, may not be accurate.

Limitations, reasons for caution: The suggested cut off value of roughly 9 follicles at the point of HCG administration, below which follicle flushing would be recommended to obtain at least 3 mature oocytes, derived from the ROC curve may not be accurate due to a small AUC of 0.080.

Wider implications of the findings: These findings, coupled with the first principles of surgery, which dictate that shorter and simpler procedures with minimal tissue handling can result in fewer complications, form the basis of our recommendation that follicle flushing should not be routinely performed during oocyte retrieval.

Trial registration number: None.

P-751 Monozygotic twin gestations after embryo biopsy in blastocyst stage

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Study question: Check if the incidence of monozygotic pregnancies increases after the transfer of embryos biopsied at the blastocyst stage.

Summary answer: The risk of monozygotic pregnancy is double after the transfer of embryo biopsied in blastocyst stage.

What is known already: Monozygotic twin pregnancies are associated with a high number of perinatal complications. It has been demonstrated that the use of Assisted Reproduction Techniques (ART), with the manipulation of gametes, especially of the oocyte: intracytoplasmic sperm injection (ICSI), assisted hatching and blastocyst biopsy, can increase the incidence of this type of pregnancies.

However, there are no published studies on these monoclonal pregnancies after biopsied embryos in the blastocyst stage.

Study design, size, duration: Retrospective study carried out from 2014 to 2016, to collect the cases of monozygotic twin gestation after single embryo transfer with biopsy of the trophoectoderm of blastocysts, compared with a control group of pregnancies obtained with single embryo transfer cycles without biopsying the blastocysts always having carried out ICSI.

Participants/materials, setting, methods: In the study group we obtained 190 pregnancies after transfer of biopsied blastocysts, 107 were with fresh embryo transfer and 83 were after cryotransfer of thawed embryos; 102 cases were with own oocyte and 88 with donated oocyte.

In the control group we obtained 454 pregnancies after the transfer of non-biopsied blastocysts, 330 were in fresh cycle and 124 after cryotransfer; 101 cases were with own oocyte and 353 with donated oocyte.

Main results and the role of chance: There were 6 monozygotic pregnancies in the group of biopsied blastocysts, and 7 monozygotic pregnancies in the control group.

The overall analysis shows a frequency of occurrence of monozygotic twin pregnancies of 1.59% in gestation after transfer of non-biopsied embryos, compared to 3.16% after transfers of biopsied blastocysts, with an ODR: 2.08 (95% CI: 0.69-6.28) ($p = 0.18$).

In the individual analysis according to the type of treatment, an increased risk of monozygotic pregnancies is always observed, except in the case of cryotransfer with own oocyte, but without reaching any statistical significance:

– Own oocyte fresh cycle: ODR: 3.1 (95% CI: 0.27-35.32%)
– Own oocyte and cryotransfer: ODR: 0.63 (95% CI: 0.04-10.33)
– Donated oocyte fresh cycle: ODR: 1.38 (95% CI: 0.14-13.43)
– Donated oocyte and cryotransfer: ODR: 4 (95% CI: 0.53-30.08)

Limitations, reasons for caution: Although the risk of monozygotic pregnancy is double after blastocyst biopsy, this increase does not become statistically significant due to the few cases collected to date.

Wider implications of the findings: It is advisable to continue collecting cases in order to increase the total number of cases to find out if this tendency can achieve any statistically significant differences.

Trial registration number: NO.

P-752 United States rates of elective single embryo transfer tightly coincide with clinician preferences and approaches to patient education

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Study question: Do United States patients receive pressure from, or are influenced by, their clinicians to have elective Single Embryo Transfer (eSET) or elective Multiple Embryo Transfer (eMET) performed?

Summary answer: Clinician opinion, and the extent to which they educate patients, strongly coincides with how many embryos a patient receives upon first transfer.

What is known already: Patients in the United States receive eSET in a distinct minority of cases. Elective single embryo transfer is pivotal in minimizes the risks to both mother/carrier and offspring.

Study design, size, duration: Patient survey and retrospective analysis of 499 verified United States IVF patients treated after 2015.

Participants/materials, setting, methods: 499 IVF patients treated in the United States and eligible for single or multiple embryo transfer were surveyed on the ways in which they were educated by their clinician, the influence the clinician had, and the number of embryos they had transferred. Results were tabulated and characterized using multi-variate analysis using a 95% confidence interval isolating for variables pertaining to patient age when treated, year treated, use of PGS and ultimate treatment decision.

Main results and the role of chance: 91% of patients reported their doctor made a clear recommendation on the number of embryos to transfer with nearly-two thirds (65%) of patients believing their clinician had an "equal or greater say" in the decision than they did. Ultimately, patients complied with their doctor's specific recommendation to a significant degree (72% for eSET, 82% for eMET, p <.01).

24% of patients reported no discussion transpired on the risks of multiple embryo transfer to the mother and 39% reported no discussion on the risks to offspring. Those patients who were educated on the risks to mother or offspring appear more likely (p = .07) to ultimately have eSET performed.

We noted patients who underwent eSET (p=.05) and PGS (P <.01) believe they were given more time to make a decision and with each passing year more patients felt their opinion influenced the decision more than their doctor's (p = .06). We noted younger women (under age 37) were more likely to be educated by their clinician on the risks of multiple embryo transfer (p = .01) and older women on the benefit it confers (p=.02), in this case, higher rates of success upon first transfer.

Limitations, reasons for caution: Retrospective analysis without randomization.

Wider implications of the findings: eSET rates in the United States are notoriously low with clinicians claiming patients ask for this and prefer it. This data suggests otherwise and that clinician practice and educational procedures may have a meaningful influence. eSET is effective in helping avoid perinatal and post-natal medical and financial deleterious effects.

Trial registration number: Not applicable.

P-753 No differences found of Small for Gestational Age (SGA) between Fresh Embryo Transfer (ET) babies and Spontaneously Conceived (SC) ones

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Study question: Are babies born after Fresh Embryo Transfer more exposed to a risk of being SGA rather than babies born after SC as previously published?

Summary answer: The incidence of SGA in babies conceived after fresh embryo transfer (6.9%) is not higher than in babies born after a SC (6.8%).

What is known already: The literature has shown that singletons born after a fresh Embryo Transfer (ET) conceived after In Vitro Fertilization (IVF) either with or without micromanipulation IntraCytoplasmic Sperm Injection (ICSI) have an increased risk of low birth weight under 2500 g, preterm birth before 37 gestational weeks, congenital anomalies and SGA, compared to singletons born after SC. Several studies suggested that the increase for SGA in babies born after fresh ET could be due to the ovarian stimulation.

Study design, size, duration: The study design was a historical exposed and unexposed cohort of singletons conceived after Fresh ET (exposed group) matched to singletons conceived after a SC (unexposed group). The exposed group involved 1961 singletons conceived after IVF or ICSI between 1995 and 2015. The unexposed group involved 5883 singletons issued from French AUDIPOG sentinel network, and born in the same period.

Participants/materials, setting, methods: The study involved 7844 singletons (>22 weeks or >500 g).Three babies born after a SC were matched on the year of birth, maternal age, parity, and sex. SGA was defined as a birth weight below the 5th percentile. A univariate analysis using Generalized Estimating Equations (GEE) for correlated data was performed to compare maternal background, obstetric parameters and perinatal outcomes. To analyse the factors influencing the incidence of SGA a multivariate analysis using GEE were performed.

Main results and the role of chance: In univariate analyses, SGA incidence was similar between babies born after fresh ET 135 (6.9%) and babies born after a SC 399 (6.8%) (p = 0.856) while the incidence of low birth weight (8.6%) was higher in the exposed group in comparison to SC babies (7.0%) (p = 0.021).

In the multivariate analysis, the transfer of fresh embryos did not affect the incidence of SGA (aOR 1.0 [95%CI 0.8-1.3]). This result contrasts with previous reports and may be partially due to the day of embryo transfer. Indeed, in our exposed group, we have a considerable rate of transfer in D5 (47.6%). A transfer at D5 is nearest to the physiology of implantation which may favour the Single ET at this stage and increases the birth weight. In addition, selected matching criteria could be also explaining this absence of SGA risk. In fact, these factors are already known to be related to higher rates of SGA.

The significant factors influencing the SGA incidence were a low rate of multiparity (aOR 0.5 [95%CI 0.3-0.7]), an advanced maternal age (aOR 1.0 [95%CI 1.0-1.0]), maternal underweight (aOR 1.5 [95%CI 1.1-2.1]), maternal smoking behaviour (aOR 1.9 [95%CI 1.4-2.4]), and high blood pressure induced by pregnancy (aOR 2.4 [95%CI 1.8-3.4]).

Limitations, reasons for caution: The 5th percentile was used to define SGA instead of 10th percentile used by most studies. In fact, this definition varies across studies with conflicting results. We wanted to use thresholds which may represent the more possible situations involving specific medical conditions.

Wider implications of the findings: Fresh ET was often related to SGA. However, our results suggested that it is rather maternal factors which influence SGA incidence. Furthermore, a higher rate of D5 (47.6% vs 27.0% at national level) could decrease this incidence, as suggested in a recent study.

Trial registration number: not applicable.

P-754 Obstetric outcome of women with in vitro fertilization pregnancies hospitalized for ovarian hyperstimulation syndrome: a retrospective cohort study

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Study question: The aim of this study was to investigate the effects of late moderate-to-critical OHSS on pregnancy and neonatal outcomes.

Summary answer: OHSS was associated with higher rates of Venous thrombosis(VT) and Gestational diabetes mellitu (GDM).

What is known already: Earlier studies have shown that the pregnancy and abortion rates increase significantly among OHSS patients and that these patients are more likely to develop adverse pregnancy outcomes such as abortion, growth restriction, PIH, GDM and low neonatal birth weight (LBW).

Study design, size, duration: This is a retrospective cohort study of 9143 eligible patients diagnosed with clinical pregnancy after IVF/ICSI-assisted conception and fresh ET from June 2012 to July 2016. All patients were registered in our data management system, which was used to save all medical information of patients trying to conceive through ART.

Participants/materials, setting, methods: All eligible subjects were determined based on the established inclusion and exclusion criteria, then, a propensity score matching analysis was performed with matching of nine maternal baseline covariates and the number of multiple gestations. 385 patients (4.9%) were hospitalized for late moderate-to-critical OHSS and compared to a matched control group of 1540 patients. The primary outcomes were live birth rate, preterm delivery rate, miscarriage rate, gestational age at birth (weeks), obstetric complications and neonatal complications.

Main results and the role of chance: The primary strength of the present study is elimination of multiple pregnancy impact on obstetric complications and neonatal complications. Live birth and neonatal complication rates did not significantly differ. The obstetric complications of Venous thrombosis and Gestational diabetes mellitus (GDM) were significantly higher in the OHSS group (0.5% vs. 0%, OR 0.995 (95% CI 0.988-1.002; P = 0.04); 1.8% vs. 0.6%, OR 3.150 (95% CI 1.166-8.513; P = 0.017), respectively).The duration of gestation was significantly higher in the matched non-OHSS group than in the OHSS group (38.0 ± 2.2 vs. 38.4 ± 2.2 (P = 0.011), OR 0.100, 95% CI 0.6-0.08). During hospitalization, 147 (38.2%) OHSS patients underwent paracentesis and/or multifetal pregnancy reduction (MFPR), and the mean hospitalization duration was 12.7 ± 6.9 days. Hospital duration, obstetric complication rate, preterm delivery rate, and HCT and WBC values were significantly higher in the severe and critical OHSS group.

Limitations, reasons for caution: Our study has a limitation, because our center began measuring AMH from 2016, it was not included in the results of AMH.

Wider implications of the findings: OHSS was associated with higher rates of Venous thrombosis(VT) and Gestational diabetes mellitu (GDM). The surgical treatment group had increased hospitalization durations and neonatal complications and decreased obstetric complications. OHSS severity increased the incidences of obstetrical complications and preterm labor but had no effect on neonatal complications.

Trial registration number: Not applicable.

P-755 Freeze-only versus fresh embryo transfer in full-term singleton birth: a retrospective cohort study

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Study question: Investigate whether freeze-only embryo transfer is preferred than fresh embryo transfer in the full-term singleton low birth weight (LBW) and small for gestational age (SGA).

Summary answer: Our study suggested that freeze-only embryo transfer was associated with statistically significantly lower rate of LBW and SGA.

What is known already: Improvement in the technology of vitrification and increase of single embryo transfer, frozen embryo transfer(FET) cycles have rapidly increased. Meanwhile, some evidence suggested that FET may increase the pregnancy rate and perinatal outcomes were less affected by the FET. But whether freeze-only proposal is preferred than fresh embryo transfer in LBW and SGA of full-term singleton birth remains controversy.

Study design, size, duration: Retrospective cohort study of none pregnancy-related complications patients, undergoing freeze-only embryo transfer (n = 2053) and fresh embryo transfer cycles (n = 2059) resulting in full-term singletons births between July 2008 and September 2016 at the Center for Reproductive Medicine of the Third Affiliated Hospital of Zhengzhou University.

Participants/materials, setting, methods: The singleton full-term births via IVF/ICSI were included. Cycles with pregnancy-related complications, donor oocytes, PGD/PGS, vanishing twins or incomplete records were excluded. Primary outcome measures were full-term LBW (birthweight <2500 g) and SGA (<10th percentile for gestational age). Secondary outcome measures were neonatal birthweight, LGA (>90th percentile for gestational age) and macrosomia (birthweight ≥4000 g). And we used logistic regression to adjust for the baseline characteristics between two groups to minimize the potential confounding factors.

Main results and the role of chance: Neonatal birthweight in singleton born after freeze-only embryo transfer was higher compared with singleton born after fresh embryo transfer (3468.7 ± 475.3 vs. 3386.7 ± 448.1; p<0.001). The frequency of full term singleton LBW and SGA after freeze-only embryo transfer was significantly lower than that after fresh ET (1.7 % vs. 3%; 4.4 % vs. 6.7 %), for the adjusted rate ratio of 0.59(95% CI, 0.37 to 0.98; p = 0.026); 0.73(95% CI, 0.55 to 0.99; p = 0.041). While freeze-only embryo transfer resulted in a higher frequency of macrosomia (15.1% vs 10.2%) than fresh ET, for the adjusted rate ratio of 1.43(95% CI, 1.16 to 1.75; p = 0.001). Furthermore, the incidence of LGA was significantly higher in the freeze-only embryo transfer than in the fresh-embryo group (22.8% vs. 17.5%), for a rate ratio of 1.26 (95% CI, 1.07 to 1.49; P = 0.007).

Limitations, reasons for caution: The current study is a retrospective cohort study, prospective studies are required to validate the freeze-only protocol is preferred than fresh embryo transfer in LBW and SGA. Furthermore, the pathogenesis of LBW in IVF/ICSI remains unclear, this study did not explore other relevant mechanisms.

Wider implications of the findings: Our study suggested freeze-only protocol was preferred than fresh embryo transfer of LBW and SGA. FET is an important supplement for fresh embryo transfer, while whether freeze-only can be the first choice requires further clinical and fundamental research.

Trial registration number: N/A.

P-756 A survey on standardization of practice in Ultrasound in ART, gynaecology and early pregnancy

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Study question: Is there a need for standardization of practice in Ultrasound in ART, gynaecology and early pregnancy?

Summary answer: The results of this survey support the development of good practice recommendations on the use of ultrasound in ART, for gynaecological pathology and early pregnancy.

What is known already: Ultrasound practise is essential in ART, as many every day activities in the fertility units are ultrasound-based. However, there seems to be a large variation in the way ultrasound is performed, both in individual approach and between different countries. Only a small proportion of countries have national guidance on ultrasound, although are not necessarily focussed on fertility and early pregnancy. In other countries, ESHRE, RCOG, NICE, ASRM and ISUOG guidelines are used instead.

Study design, size, duration: Between November 2016 and February 2017 a survey to explore these differences was carried out. The survey included 14 questions, aiming to explore whether there is a role for collating the different recommendations or guidelines from other societies and for developing standards in ultrasound in ART and Early pregnancy that could be implemented in Europe and beyond. The survey was disseminated via mailings and social media.

Participants/materials, setting, methods: This online survey was conducted among ESHRE members and 333 responses were collected. Most of the responders (63%) were based in Europe, 20% in Asia, and the remaining 17% in Africa, America and Australia. Regarding occupation, a majority of responders (84.9%) was medically qualified, the remaining included embryologists, nurses, midwifes, counsellors and managers. Given that experts' views were extremely valuable for drawing the conclusion, responders were felt to be with appropriate level of knowledge/experience.

Main results and the role of chance: Regarding the fertility treatment, two-thirds of the responders (68.4%), mainly from European countries, stated there is a national body regulating fertility treatments in their country of residence. Half of the responders from Asia, and approximately 80% of the South American and African, responded that they do not have guidelines for ART in their country. Interestingly, but not surprising, in countries with no national guidelines for ART, ESHRE, ASRM and NICE were the most followed ones. Regarding guidelines on Ultrasound, only a quarter of the responders stated that there is a national guideline for ART, gynaecological pathology and early pregnancy. Most of the responders (85%) stated there is a need for standardized guidelines regulating USS in ART, gynaecological pathology and early pregnancy. Notably a number of responders (181) suggested these topics to be covered in a potential guideline. Finally, a two third of the responders showed initiative to be included into a guideline development group and a half of the total number of responders provided their email addresses to be contacted.

Limitations, reasons for caution: Limitations are an absence of experts interviews and different answers from the same country of residence on national guidelines.

Wider implications of the findings: The strength of this survey results are number of responses, targeted users included and broad views on different clinical topics collected. The results of this survey will support the proposal of developing recommendations for standardised practice in USS in different aspects of ART, gynaecological pathologies and early pregnancy.

Trial registration number: none.

P-757 Comparison of pregnancy and perinatal outcomes after frozen and fresh embryo transfer

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Study question: Does frozen embryo transfer (FET) have better pregnancy and perinatal outcomes than fresh embryo transfer?

Summary answer: We found no significant improvement regarding pregnancy and perinatal outcomes in FET compared with fresh embryo transfer.

What is known already: FET has been progressively used during the past few decades and it becomes an essential part of assisted reproductive technology (ART). Extra good quality embryos are preserved in FET for women with good response to ovarian stimulation, improving cumulative pregnancy rate, increasing single embryo transfer, decreasing risk of multiple pregnancy and hyperstimulation syndrome. However, whether FET can improve pregnancy and perinatal outcomes is uncertain.

Study design, size, duration: A retrospective cohort study was performed consisting of 8370 FET cycles and 8573 fresh embryo transfer cycles between January 2008 and September 2016 at our reproductive center.

Participants/materials, setting, methods: Women who underwent FET and fresh embryo transfer are divided into two groups: FET group (8370 patients) and fresh embryo transfer group (8573 patients). To compare the rates of implantation, biochemical pregnancy, clinical pregnancy, first-trimester abortion and multiple pregnancy. The incidence of preterm delivery, live delivery,ectopic pregnancy, pregnancy-induced hypertension, gestational diabetes mellitus, mean birthweight, low birth weight and congenital malformation were evaluated.

Main results and the role of chance: After adjusting for confounding factors, no differences were observed in implantation [adjusted odd ratio (aOR) 0.98, 95% confidence interval (CI) 0.97-1.13], biochemical pregnancy (aOR 0.88 ,95%CI 0.76-1.13), clinical pregnancy (aOR 0.94, 95%CI 0.89-1.02), multiple pregnancy (aOR 1.12 ,95%CI 0.96-1.27), gestational diabetes mellitus (aOR 0.82 ,95%CI 0.62-1.12) and congenital malformation (aOR 0.94 ,95%CI 0.57-1.36) rates of the two groups. Live birth rate (aOR 1.23 ,95%CI 1.15-1.32) after FET was significantly elevated compared with fresh embryo transfer. And FET showed decreased risks of ectopic pregnancy(aOR 0.83 ,95%CI 0.60-0.98) and low birth weight (aOR 0.83 ,95%CI 0.82-0.96). Mean birthweight was higher in the FET versus the fresh embryo transfer (P<0.001). The significantly increased risks of preterm delivery(aOR 1.28 ,95%CI 1.10-1.50), first-trimester abortion(aOR 1.11 ,95%CI 1.07-1.25) and pregnancy-induced hypertension (aOR 1.63 ,95%CI 1.29-2.12) in FET compared with fresh embryo transfer were found.

Limitations, reasons for caution: As in all observational studies, the possible role of residual confounding factors and bias should be considered. In this study, we were not able to control for confounding factors, such as smoking, parity and child's sex.

Wider implications of the findings: The study suggest that FET do not present significant benefit in pregnancy and perinatal outcomes compared with fresh embryo transfer. As a consequence, a comprehensive consideration should be given when selecting a transplantation program.

Trial registration number: N/A.

P-758 The effect of the underlying type of infertility on placental-mediated adverse outcomes for patients that used IVF: a population-level study

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Study question: To investigate associations between type of infertility and placental-mediated adverse outcomes among women that used in vitro fertilization (IVF) to conceive compared to spontaneous conception.

Summary answer: There was a statistically significant increased association found among births using IVF; specifically among cases where female factor infertility was the underlying cause of infertility.

What is known already: Previous studies have found associations between fertility treatment and stillbirth, and preeclampsia. Additionally, a recent meta-analysis found an association between fertility treatment and gestational hypertension and placental abruption. Multiple pregnancies among women who used fertility treatments to conceive have been found to be associated with an increased risk of preeclampsia. However, it is important to consider the role of multiple gestations in driving the increased associated risk. Conversely, there have been studies that found no significant association between fertility treatment and preeclampsia once they adjusted for maternal age and parity.

Study design, size, duration: This was a population-level retrospective cohort study of 200,469 live births and stillbirths in Ontario, Canada from May 2013 through October 2014. This study used fertility treatment data from CARTR (Canadian Assisted Reproductive Technologies Register) Plus, and pregnancy and birth outcomes data from BORN (Better Outcomes Registry & Network) Ontario and the Canadian Institute for Health Information. This study included all documented births in Ontario, Canada during this period of time.

Participants/materials, setting, methods: The exposed group included live births and stillbirths from women that used IVF, frozen embryo transfer, frozen oocyte IVF to conceive and a control group of births from spontaneous conceptions during the same time period. Unadjusted logistic regression models were performed using generalized estimating equations, as well as models adjusted for maternal age, neighbourhood income level and smoking were generated for the composite outcome (preeclampsia (PE), placental abruption, stillbirth, small-for-gestational-age), and all individual outcomes.

Main results and the role of chance: Significant differences were found for maternal age, hospital level-of-care and pre-existing maternal health conditions for births that used IVF to conceive. However, there were no significant

differences among the infertility groups with respect to characteristics of fertility treatment except for method of insemination.

Births resulting from IVF conceptions were found to be statistically significantly associated with an increased risk of the composite outcome (RR: 1.15, 95%CI: 1.01-1.31), as well as PE (RR: 1.74, 95%CI: 1.28-2.35) and placental abruption (RR: 1.65, 95%CI: 1.12-2.44), as compared to births from spontaneous conception. Similarly, female factor infertility was significantly associated with an increased risk of the composite outcome (RR: 1.21, 95%CI: 1.01-1.46), as well as PE (RR: 2.01, 95%CI: 1.34-3.00). The results remained significant after adjusting for potential confounders.

IVF was associated with a higher risk of preterm birth (<37 weeks) and very preterm birth (<32 weeks), relative to spontaneous conception, adjusted RRs, 1.64 (95%CI: 1.40-1.93) and 1.74 (95%CI: 1.19-2.56), respectively. When type of infertility was investigated we found IVF for female factor infertility was most strongly associated with a higher risk preterm birth: adjusted RRs, 1.77 (95%CI: 1.42-2.20) and 2.40 (95%CI: 1.52-3.80) for <37 weeks and <32 weeks, respectively, compared to spontaneous conceptions.

Limitations, reasons for caution: This study used comprehensive administrative data; nevertheless this study was potentially affected by misclassification of the exposure and/or outcome variables, a data quality issue that plagues studies that use administrative data.

Wider implications of the findings: The hormonal and/or structural factors associated with female factor infertility may have affected the implantation and development of the placenta, leading to an increased associated risk of the study outcomes. The increased risk among this group should be appropriately considered and accounted for when delivering IVF conceptions.

Trial registration number: not applicable.

P-759 In Freeze-all strategy Cumulative Live Birth Rate (CLBR) is increasing according to the number of blastocysts formed in women <40 undergoing IntracytoplasmIc Sperm Injection (ICSI)

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Study question: Does the number of the formed and vitrified blastocysts affect Cumulative Live Birth Rate in women undergoing Freeze-all strategy?

Summary answer: CLBR after three frozen/ thawed embryo transfers (ETs) is increasing respectively according to the number of vitrified blastocysts.

What is known already: Elective freezing of all embryos, followed by frozen/ thawed ET cycles emerged to prevent risk of Ovarian Hyperstimulation Syndrome and to allow endometrium recovery after Controlled Ovarian Stimulation. The efficacy of embryo vitrification allowed Freeze-all policy to gain popularity. Freeze-all policy, however, should preferentially be performed on day 5 of embryo culture, in order to minimize the number of abnormal embryos and consequently to decrease failed ETs and emotional cost. Previous studies reported better In vitro Fertilization (IVF) outcomes after adopting the Freeze-all policy, instead of fresh ET. Nevertheless, efficacy in terms of CLBR has not been studied yet.

Study design, size, duration: This prospective observational cohort study was performed in 156 women undergoing ICSI with antagonist protocol, without fresh ET. CLBR was compared in three groups of women according to the number of blastocysts formed and cryopreserved; group A: 1-4 blastocysts (n = 38), group B: 5-7 blastocysts (n = 66) and group C: ≥8 blastocysts (n = 52). The CLBR was estimated separately for each group, after completing 3 cycles of frozen/thawed ETs (two women still have blastocysts frozen for transfer).

Participants/materials, setting, methods: Study was performed from January 2014 until March 2017 in Assisting Nature IVF Centre, Thessaloniki, Greece. All cases concerned couples with a female age range of 21-39 years, with fresh ejaculated sperm. Patients underwent estrogen preparation of endometrium and were divided in three groups according to the number of the blastocysts vitrified. CLBR was calculated following blastocyst Freeze-all strategy when three thawed cycles had been finalised.

Main results and the role of chance: There was no difference in the mean age of each group (34 vs 33 vs 31). The mean blastulation rate was above 50% of the number of 2 pronuclei (2PN) embryos in each group ($p>0.05$). Cumulative Live Birth Rate was 31.51% in group A (1-4 blastocysts), 75.75% in group B (5-7 blastocysts) and 84.61% in group C (≥8 blastocysts). The above difference was statistically significant ($p<0.05$) among the three groups.

Limitations, reasons for caution: No sperm quality was taken into account and no natural cycles were used for endometrium preparation.

Wider implications of the findings: Adopting Freeze-all strategy after blastocyst culture can contribute to improve delivery outcome after IVF in terms of Cumulative Live Birth Rate. The number of the total cryopreserved blastocysts produced might reflect the quality of the oocyte and can successfully predict the pregnancy outcome.

Trial registration number: not applicable.

P-760 Hospitalization before pregnancy in infertile women treated with ART, non-ART, or no fertility therapy compared with fertile women

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Study question: Do diagnoses during pre-pregnancy hospitalizations in ART-treated women, women using non-ART medically assisted reproduction(MAR), or untreated infertile women, differ from hospitalization diagnoses for fertile women?

Summary answer: Diagnoses during hospitalizations prior to pregnancy for women who have ART, MAR, or untreated infertility all differ from pre-pregnancy diagnoses for fertile women.

What is known already: Compared with fertile women, women who have been treated with ART or MAR, and those who have unassisted pregnancies but indicators of infertility, have a greater risk of adverse delivery outcomes such as low birthweight and prematurity. Prior studies have shown that infertility-related diagnoses may contribute to these adverse outcomes, but whether adverse outcomes are most attributed to underlying disease or to infertility treatment is unknown. One potential indicator of underlying disease that can shed light on this issue is hospitalization prior to pregnancy and the reasons associated with that hospitalization.

Study design, size, duration: We included 125,206 first live-birth deliveries between 2004 and 2010 to women ≥18 years of age with private health insurance from the Massachusetts Outcome Study for Assisted Reproductive Technology (MOSART) and evaluated hospital stays for the 5 years prior to pregnancy. MOSART links ART deliveries from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) to birth certificates and hospital discharges in the Pregnancy to Early Life Longitudinal (PELL) data system.

Participants/materials, setting, methods: The study included 7,080 ART and 1,834 non-ART-MAR deliveries, 1,036 deliveries to women with infertility diagnosis but no fertility treatment (infertile), and 115,256 deliveries with no indicators of infertility or treatment (fertile). Demographics, gestational age and birthweight were obtained from birth certificates. Diagnoses from PELL were evaluated for prevalence in each group and then compared among all groups. Multivariable logistic regression models with fertile group as reference were adjusted for maternal age, race and education.

Main results and the role of chance: ART, MAR and infertile deliveries differed from fertile in adjusted odds of low birthweight (<2,500 gms: adjusted odds

ratio [AOR], 95% confidence interval [CI] 2.8, 2.6-3.0, ART; 2.2, 1.9-2.6, MAR; 1.9, 1.6-2.3 infertile) and prematurity (< 37 weeks gestational age: AOR, 95% CI: 2.9, 2.7-3.2 ART; 2.1, 1.8-2.4, MAR; 1.8, 1.5-2.2 infertile). Common diagnoses at hospitalization in the ART, MAR and infertile groups included higher adjusted odds than fertile women of endometriosis (AOR, 95% CI: 12.4, 9.4-16.3 ART; 6.0, 3.5-10.6 MAR; 22.3, 14.7-33.7 Infertile), reproductive organ disease (AOR, 95% CI: 6.5, 5.5-7.8 ART; 2.1, 1.3-3.4 MAR; 8.1, 5.7-11.4 Infertile), ectopic pregnancy and miscarriage (AOR, 95% CI: 5.5, 4.4-6.8 ART; 2.8, 1.7-4.6 MAR, 4.7, 2.9-7.6 Infertile), and disorders of menstruation, ovulation, and genital tract (AOR, 95% CI: 3.8, 2.8-5.2 ART, 4.2 2.5-7.0 MAR, 7.3, 4.5-12.0 Infertile). Diagnoses of disorders of fluid and electrolyte imbalance, psychiatric disorders, and misuse of drugs, which were most common in the fertile group, did not differ between the groups. Odds of overweight and obesity diagnosed during hospitalization did not differ among the fertile, ART, and infertile groups but were significantly higher in the MAR group (AOR, 95% CI 2.2, 1.4-3.6).

Limitations, reasons for caution: The time from pre-pregnancy diagnoses to first pregnancy was not evaluated and diagnoses out of hospital are not included. ART and non-ART-MAR women may have greater access to care and thus greater likelihood of hospitalization compared to untreated infertile or fertile women. The population is from a single U.S. state.

Wider implications of the findings: Women treated with ART and non-ART-MAR and who are infertile with no treatment, all have elevated odds of pre-pregnancy hospitalizations for reproductive disorders. The data have implications for the health of deliveries to these women and suggest that underlying disease plays a role in outcome in these populations.

Trial registration number: not applicable.

P-761 Single and double embryo transfer provide similar live birth rates in frozen cycles

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Study question: Do live birth rates (LBRs) differ in frozen cycles of women who received single versus double embryo transfer?

Summary answer: LBRs are similar in women undergoing frozen embryo transfer (FET) with one or two embryos.

What is known already: FET has become an intricate part of IVF treatment and is no longer viewed as a mere supplement to fresh embryo transfer. Although the policy in many IVF centers tends towards single embryo transfer (SET) in case of a fresh embryo transfer, frozen cycles do not seem to follow the same pattern since they are still frequently considered as a means to counterbalance a previous failed fresh attempt.

Study design, size, duration: This was a large retrospective cohort study including all consecutive women who underwent their first FET in a tertiary referral University Hospital between January 2009 and December 2014. Each patient was included only once in the analysis.

Participants/materials, setting, methods: All eligible patients had vitrification as the cryopreservation method and underwent single (SET) or double (DET) FET with either cleavage or blastocyst stage embryos. Our primary outcome was LBR. Secondary endpoints included biochemical/clinical/miscarriage and multiple birth rates.

Main results and the role of chance: In total, 3601 patients were included in the analysis with 1936 (53.8%) having a SET and 1665 (46.2%) having a DET. Overall, 657/3601 (18.24%) had a live birth. LBR were similar between SET and DET either for cleavage [100/757 (13.1%) versus 153/1032 (14.8%), p = 0.33] or blastocyst stage FET [256/1179 (21.7%) versus 148/633 (23.4%), p = 0.4). Similarly, ongoing pregnancy rates were comparable between DET and SET [316/1665 (18.9%) versus 359/1936 (18.5%)]. However, biochemical pregnancy rates were significantly higher in DET, suggesting a higher risk for early pregnancy loss in case of DET compared to SET [326/1665 (19.6%) versus 308/1936 (15.9%) p = 0.004]. Multiple delivery rates were significantly higher in women with DET compared to SET [53/316 (16.7%) versus 7/359 (1.9%), p<0.001]. Multivariate logistic regression analysis allowing adjustment for relevant confounders showed that the number of embryos transferred in the frozen cycle is not related to LBR.

Limitations, reasons for caution: This is an observational study based on retrospective data collection. Despite our robust methodological approach, the presence of biases related to the retrospective design cannot be excluded.

Wider implications of the findings: This is the largest study providing evidence that both SET and DET may result in similar LBR, albeit multiple pregnancy rates are significantly lower in case of SET. Therefore, SET should be the main strategy in women undergoing FET. The higher miscarriage rates in women with DET warrant further evaluation.

Trial registration number: not applicable.

P-762 Cumulative live birth rate following freeze-only versus conventional fresh transfer cycles: a population-based cohort study

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Study question: What is the cumulative live birth rate following a 'freeze-only' strategy compared with a 'fresh transfer' strategy?

Summary answer: Compared with 'fresh transfer' strategy, 'freeze-only' strategy resulted in a similar cumulative live birth rate among high responders but significantly lower rate among normal/suboptimal responders.

What is known already: Frozen-thawed embryo transfer is associated with decreased risk of adverse obstetric and perinatal outcomes compared with fresh embryo transfer. It is unclear whether 'freeze-only' strategy should be offered to all women undergoing assisted reproductive technology (ART) treatment.

Study design, size, duration: A population-based retrospective cohort study. This study included 14331 women undergoing their first stimulated ART cycle with at least one oocyte fertilized between 1 Jul 2009 and 30 Jun 2014 in Victoria, Australia. ART treatment and resulting pregnancy and birth outcomes were recorded for the stimulated cycle and associated thaw cycles until 30 June 2016, or until a live birth was achieved, or until all embryos from the stimulated cycle had been used.

Participants/materials, setting, methods: Women were grouped by whether they had undergone the 'freeze-only' strategy (n = 1028) where all embryos were cryopreserved for future transfer, or the 'fresh transfer' strategy (n = 13303) where the best embryo(s) were transferred in the stimulated fresh cycle and remaining embryo(s) were cryopreserved for future use. A Cox proportional hazard model was used to evaluate the cumulative live birth rate following 'freeze-only' and 'fresh transfer' strategy.

Main results and the role of chance: A total of 1028 women undergoing 'freeze-only' strategy and 13303 women undergoing 'fresh transfer' strategy who had 1788 and 22334 embryo transfer cycles resulting in 452 and 5126 live births respectively. The majority of women (61.3%) in the 'freeze-only' group had more than 15 oocytes retrieved in the stimulated fresh cycle compared with less than one fifth (18.1%) of women in the 'fresh transfer' group (p<0.001). The overall cumulative live birth rate was 44.0% in the 'freeze-only' group and 38.5% in the 'fresh transfer' group. For high responders (>15 oocytes), the adjusted likelihood of live birth in the 'freeze-only' group was

similar to the 'fresh transfer' group (Adjusted hazard ratio (AHR) 0.96, 95% confidence interval (CI) 0.85–1.09). However, the likelihood of live birth was lower in the 'freeze-only' group compared with the 'fresh transfer' group among normal responders (10-15 oocytes) (AHR 0.71, 95% CI 0.54–0.93) and suboptimal responders (<10 oocytes) (AHR 0.72, 95% CI 0.49–1.04).

Limitations, reasons for caution: A limitation of this population-based study is the lack of information available on clinic-specific protocols for the 'freeze-only' strategy and the potential impact on outcomes. Data were not available on whether the 'freeze-only' strategy was used to prevent ovarian hyperstimulation syndrome.

Wider implications of the findings: This study presents population-based evidence on clinical efficacy associated with a 'freeze-only' and 'fresh transfer' strategy. The 'freeze-only' strategy may benefit some subgroups of patients, including women who are high responders and those who are at risk of OHSS, but should not be offered universally.

Trial registration number: not applicable.

P-763 Double oocyte pick-up (OPU) for the patients with diminished ovarian reserve (AHM less than 1.0 ng/ml) and showing unsynchronized follicle growth during ART treatment

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Study question: Dose double OPU result in more oocytes available for patients with diminished ovarian reserve (DOR) and who have shown unsynchronized follicular growth during ART treatment?

Summary answer: For patients with DOR, double OPU could increase the number of oocytes and usable embryos for embryo transfer in one treatment cycle.

What is known already: In order to achieve pregnancy, ART treatment can provide many oocytes in one OPU procedure, which could improve the chances of selecting the best embryo or blastocyst for embryo transfer. It is difficult, however, for DOR patients to obtain a plurality of oocytes in one OPU. On the other hand, clinicians sometimes observe unsynchronized follicle growth during ovarian stimulation. In those cases, the dominant follicle size is a crucial maturation trigger, and, subsequently, inadequately developed follicles are removed at a target of OPU, despite their value to DOR patients.

Study design, size, duration: This is an observational study of 13 women with DOR (AMH level <1.0 ng/ml) who were recruited between January and December in 2017. All patients were stimulated with clomiphene citrate (CC) with or without gonadotropins for ART treatment. When the follicles had reached ≥17 mm in diameter, this provide a maturation trigger and either human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone agonist (GnRH-a) was then administered 35 hours before OPU (first OPU).

Participants/materials, setting, methods: During the first OPU, smaller follicles (≤12 mm in diameter) were not punctured. After OPU, ovarian stimulation was restarted with CC with or without gonadotropins to develop the missed follicles. When these follicles then reached 17 mm in diameter, a maturation trigger using hCG or GnRH-a was administered, and 35 hours later a second round of OPU was performed. The number of retrieved and developed oocytes was evaluated. The cleaved embryos were cryopreserved.

Main results and the role of chance: The average age of the patients was 41.6 years, and the average AMH and FSH values on day 3 were 0.47 ng/ml and 15.1 IU/L, respectively. For the first round of OPU, the average progesterone concentration was 0.6 ng/ml with no recorded incidences of elevated progesterone concentration. During the first OPU session, at least one oocyte was collected from 12 of the patients.

During the second OPU session, all patients were stimulated by CC with or without gonadotropins. The average progesterone level at the time of the second maturation trigger was 15.6 ng/ml. During the second round of OPU, at least one oocyte was collected from 10 of the patients.

The mean numbers of collected oocytes (/patient) from the first and second rounds OPU were 1.3 and 1.2, respectively, and the total number of the collected oocytes (/patient) was 2.5. During the first and second rounds of OPU, the total numbers of usable embryos for cryopreservation were 10 and 5, respectively, and as a consequence 12 of the patients were able to obtain cryopreserved embryos. Of these 12 patients, 4 were not able to obtain cryopreserved embryos without a second round of OPU.

Limitations, reasons for caution: This study was limited by a small sample size. Moreover, only 6 patients received thawed embryo transfer, and 2 of these achieved pregnancy.

Wider implications of the findings: Progesterone elevation did not decrease the pregnancy rate when a fresh embryo transfer was avoided, and it did not affect the embryo development rate. Furthermore, a random start was reported for ovarian stimulation. This indicated that the start of ovarian stimulation was not related to the menstrual cycles.

Trial registration number: This study has no RCT status, and, therefore, a trial registration number was not assigned.

P-764 Assessment of preimplantation genetic testing for embryo aneuploidies: a SWOT analysis

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Study question: Can a SWOT analysis provide a comprehensive and objective perspective to judge preimplantation genetic testing for embryo aneuploidies (PGT-A) strategy?

Summary answer: This SWOT analysis highlights that preimplantation diagnosis has taken the slippery slope of implementation of a novel approach without adequate evaluation of threats and weaknesses.

What is known already: The acceptance by the clinical community of the PGT-A is still evolving. Currently, the use of PGT-A is steadily increasing due to the evaluation of all 24 chromosomes by comprehensive chromosome screening. Moreover, blastocyst biopsy seems to be less harmful than Day-3 biopsy. PGT-A is now actively marketed as increasing implantation rates, and consequently decreasing time to pregnancy, recurrent miscarriages and repeated implantation failures. However, current studies do not provide sufficiently robust evidence to consider PGT-A as a proven and beneficial treatment and whether it should be offered routinely to IVF patients is still at the hearth of the debate.

Study design, size, duration: The SWOT (strengths, weaknesses, opportunities, and threats) analysis represents a strategic planning tool of quality management usually used in business but also adaptable to every field of medicine. The SWOT analysis framework helps focus on the strengths, understands the weaknesses that can assist in managing and minimizing the threats, and allows to take the greatest possible advantage of the opportunities available.

Participants/materials, setting, methods: This study is based on material obtained by searching PUBMED between January 2012 and January 2018, using the following keywords: 'preimplantation genetic screening' or 'PGS' or 'PGT-A', alone or in combination with 'biopsy', 'clinical outcome', 'RCT', 'SET', 'obstetrical outcome', 'follow-up'. All pertinent articles were carefully assessed and their reference lists were evaluated to detect other studies that could be included in this SWOT analysis. Peer-reviewed, English-language journal articles were included.

Main results and the role of chance: STRENGTHS: There is a general agreement on ability of PGT-A to increase the implantation rate as confirmed by three randomized control trials (RCTs) in young and good prognosis populations and one RCT in advanced maternal age (AMA) patients. Moreover, a reduction of miscarriage rate was observed only in AMA population, with a consequent decrease of time to pregnancy.

WEAKNESSES: Only three RCTs, have been published with biopsy at the blastocyst stage, all of which have been criticized because of poor study design. The RCT in AMA patients was performed considering cleavage-stage biopsy. Cumulative live birth rate remains unchanged. A critical issue is the management of mosaicisms.

OPPORTUNITIES: PGT-A offers the possibility (i) to perform a single embryo transfer, decreasing multiple pregnancies, (ii) to reduce time to achieve a healthy live birth and (iii) to assist with patient counseling regarding expectations and outcomes.

THREATS: The high cost, the invasive nature, the poor standardization and the very limited data about obstetrical/perinatal outcomes and about long-term effects represent drawbacks and hazards when PGT-A is offered routinely to IVF patients.

Limitations, reasons for caution: It is necessary to consider that a SWOT analysis only covers issues that can definitely be considered a strength, a weakness, an opportunity or a threat. Moreover, it is not able to prioritize issues and to provide solutions offering alternative decisions.

Wider implications of the findings: These findings are alarming in regard to the effectiveness and accuracy of the procedure. They also highlight the importance of high quality relevant RCTs that are urgently needed.

Trial registration number: Not applicable.

P-765 Letrozole and risk of major congenital malformations: a systematic review and meta-analysis

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Study question: Is letrozole, used for ovulation induction (OI) or Assisted reproductive treatment (ART) associated with increased risk of major congenital malformations compared with other agents?

Summary answer: Letrozole is associated with significantly lower risk of major congenital malformations compared with clomiphene and no significant difference when compared with natural conception or gonadotrophins.

What is known already: OI agents such as clomiphene have been used in sub-fertile women with anovulatory cycles or unexplained infertility and in ART cycles. Letrozole, an aromatase inhibitor, has many advantages over clomiphene, but has not been approved for OI. It is classified as a category 'x' drug, primarily due to a conference presentation in 2005, suggesting that, it is associated with higher risk of major congenital malformations when compared with low risk naturally conceived babies, despite the fact that this paper had methodological concerns, raising questions on its validity.

Study design, size, duration: Systematic review and meta-analysis of randomised and non-randomised controlled trials that evaluated the risk of major congenital malformations in babies born with the use of Letrozole in infertile women.

Participants/materials, setting, methods: We searched MEDLINE, EMBASE, Cochrane library, Register and Meta-register for RCTs, and WHO trials' search portal without any language restrictions. Studies that evaluated infertile women undergoing fertility treatment (OI or ART cycles) and compared letrozole with natural conception or other agents and reported on risk of major congenital malformations in the newborn were included. We pooled the results using fixed effects meta-analysis and reported the findings as relative risk (RR) with 95% confidence intervals (CI).

Main results and the role of chance: We included seven studies which enrolled 2603 babies born from OI and 5 studies with 37,349 babies born from ART cycles.

Comparison with Clomiphene - Pooling the data from 8 studies which compared letrozole with clomiphene (7 studies for OI; one study for fresh ART cycle), letrozole was associated with significantly lower risk of major congenital malformations when compared with clomiphene (RR 0.62; 95% CI 0.39, 0.99; $p = 0.05$; $I^2 = 0\%$; $n = 2455$).

Comparison with Natural conception/natural cycle ART - Pooling the data from 7 studies which compared letrozole with natural conception or natural cycle ART (3 studies for natural conception; one study for fresh ART natural cycle; 3 studies for Frozen embryo transfer in natural cycle), there was no significant difference in risk of major congenital malformations between the two groups (RR 1.13; 95% CI 0.86, 1.49; $p = 0.37$; $I^2 = 0\%$; $n = 21,627$).

Comparison with gonadotrophins/HRT ART - Pooling the data from 4 studies which compared letrozole with gonadotrophins or HRT (one study for gonadotrophin OI; 3 studies for Frozen embryo transfer in HRT cycle) there was no significant risk of major congenital malformations between the two groups (RR 1.18; 95% CI 0.86, 1.61; $p = 0.31$; $I^2 = 0\%$; $n = 18168$).

Limitations, reasons for caution: Even though limited to three RCTs and nine observational cohort studies, this systematic review and meta-analysis provides safety data on large cohort of babies born following the use of letrozole for infertility treatment.

Wider implications of the findings: Letrozole appears to be safe in terms of risk of major congenital malformations in the newborn of infertile mothers undergoing fertility treatment with OI or ART. These reassuring safety data contradict undocumented concerns about the teratogenicity of letrozole and support its use as a first line OI agent.

Trial registration number: NA.

P-766 Analysing risk factors for monozygotic multiple pregnancy after single embryo transfer in the Japanese national ART registry

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Study question: To determine the prevalence and risk factors for monozygotic multiple pregnancy (MMP) after single embryo transfer (SET).

Summary answer: The prevalence of MMP after SET was 0.68%. The risk factors for MMP after SET included frozen-thawed embryo transfer, blastocyst culture and assisted hatching (AH).

What is known already: In Japan, the prevalence of multiple pregnancy after assisted reproductive technology (ART) decreased from approximately 10% in 2007 to 3% in 2014 owing to the proposed SET recommendation by the Japan Society of Obstetrics and Gynecology (JSOG) for the reduction of multiple births in 2008. Therefore, to improve the clinical pregnancy rate in Japan, increased elective SET using blastocyst transfer, frozen-thawed embryo transfer and AH had been increased. Blastocyst culture was reported to be a risk factor for monozygotic twinning after SET. However, other risk factors for MMP after SET were poorly understood.

Study design, size, duration: A retrospective observational study was performed based on registered ART data from the JSOG between 2007 and 2014. Among 937,848 SET cycles, 274,605 pregnancies were recognised. The present study was approved by the Registration and Research Subcommittee of the JSOG and our hospital's ethics committee.

Participants/materials, setting, methods: MMP was defined as one gestational sac (GS) with two or more foetuses or two GSs with three or more foetuses. To identify possible factors affecting the prevalence of MMP, multiple logistic regression analysis was performed using singleton pregnancy after SET as control. A *P* value of <0.05 was defined as statistically significant.

Main results and the role of chance: Out of 274,605 pregnancies after fresh and frozen-thawed SET, 1,863 were MMPs (prevalence rate of 0.68%), including 1,808 monozygotic twin and 55 triplet pregnancies. Statistical analysis revealed that the prevalence of MMP after frozen-thawed SET cycles [odds ratio (OR): 1.34; 95% confidence interval (CI): 1.16–1.55], blastocyst culture (OR: 1.79; 95% CI: 1.54–2.09) or AH (OR: 1.21; 95% CI: 1.08–1.35) was higher than that of singleton pregnancy after SET. In fresh ET cycles, the prevalence

rate of MMP after one blastocyst transfer was significantly higher than that after SET cycles with cleavage embryos (OR: 2.20; 95% CI: 1.83–2.66). However, no significant difference in ovarian stimulation and fertilisation methods had been observed.

Limitations, reasons for caution: In the current Japanese ART registry system, data regarding frozen-thawed ET do not include information about ovarian stimulation and fertilisation methods. Moreover, registration for AH had only been started in 2010.

Wider implications of the findings: Strict selection of good quality embryos through blastocyst culture, as well as embryo manipulation using frozen-thawed embryo transfer and AH to improve pregnancy rates, may increase the risk of MMP. However, procedures involving an oocyte, such as intracytoplasmic sperm injection, showed no such increase in risk.

Trial registration number: not applicable.

P-767 Anti-Mullerian hormone level decrease and efficacy of ART programs in HIV-infected women

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Study question: To assess the levels of anti-Mullerian hormone (AMH) and efficacy of ART programs in HIV-infected women.

Summary answer: Decreased AMH level and low pregnancy rate were found in HIV-infected women in IVF programs that might be associated both with infection and HAART.

What is known already: The studies assessing ART efficacy in couples with HIV-infected women are sparse and controversial. Some of them have demonstrated that HIV infected patient show a decrease of ovarian reserve parameters and a low efficacy of ART programs. Other studies do not find any difference in ovarian reserve parameters, ovarian function stimulation and ovo- and embryogenesis in HIV-infected women as compared to healthy patients. Different evidence suggest that the frequency of developing pregnancy ranges from 6.7 to 24.1%, which is significantly lower as compared to non-HIV-infected women. The reason of low efficacy of IVF program in this patient group remains unclear.

Study design, size, duration: The prospective case-control study included 113 patients seeking in IVF. The main group included 49 HIV-infected patients; 64 patients with HIV-negative serology were included in a control group. In the main group, 111 IVF cycles were performed, including 62 treatment and 49 cryocycles; the corresponding number of cycles in control group was 68 and 33, respectively.

Participants/materials, setting, methods: Stimulation of superovulation was performed according to long IVF protocols and GnRH-antagonist-based protocols using recombinant FSH and hMG. While preparing the endometrium in cryocycles, natural estrogen- and micronized progesterone-contaning medications were used. The infectious status of the patients was assessed based on the data on the disease stage, viral load, CD4+ count and the duration of HAART.

Main results and the role of chance: AMH concentration was significantly lower (median, 1.86 vs 2.8 ng/mL; $p = 0.006$), while FSH levels were statistically significantly higher (7.9 IU/L vs 6.2 IU/L; $p = 0.004$) in HIV-infected women group as compared to HIV-negative patients. Analysis of stimulation cycle parameters did not show any significant differences in the initial or total gonadotropin doses or stimulation duration in both groups. However, a lower number of oocytes (8.5 ± 0.9 vs 12.5 ± 0.7; $p = 0.001$), mature oocytes (7.1 ± 0.7 vs 9.6 ± 0.5; $p = 0.002$), zygotes (5.9 ± 0.6 and 8.1 ± 0.4; $p = 0.001$), cleavage stage embryos (5.6 ± 0.6 vs 7.8 ± 0.4; $p = 0.001$) and blastocysts (2.7 ± 0.4 vs 4.5 ± 0.4; $p = 0.004$) were recovered in HIV-infected patients as compared to control group. Selective single embryo transfer was conducted on the culture days 3 or 5 in both groups. A lower frequency of clinical pregnancy was revealed in patients with HIV infection as compared to HIV-negative women, both in ART treatment cycles (12.8% vs 38.3%; $p = 0.007$) and cryocycles (20.4% vs 51.5%; $p = 0.002$).

Limitations, reasons for caution: small scale.

Wider implications of the findings: can be applied on a large number of patients.

Trial registration number: непригодный

P-768 Impact of vitrification of cleavage stage embryos versus slow-freezing method on neonatal outcome: a multicenter retrospective cohort study with propensity score analysis

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Study question: Does the embryo cryopreservation method have an impact on the neonatal birthweight of newborns from thawed embryo transfer (TET) cycles?

Summary answer: Singleton birthweight did not vary significantly after a vitrified embryo transfer compared with a slow-frozen cleavage embryo transfer. Transfer of vitrified Day 3 embryos did not increase the risk of low birthweight (LBW), macrosomia or other adverse neonatal outcomes.

What is known already: Vitrification has been successfully used for embryo cryopreservation and is superior to slow-freezing technique regarding the embryo survival rate and live birth rate. However, whether the vitrification method would affect fetal growth and thus the neonatal outcome is still controversial. Most studies addressed this issue by comparing the neonatal outcome after transferring vitrified embryos with fresh embryo transfer. So far, comparative data of two cryopreservation methods with respect to the singletons' birthweight outcome is limited.

Study design, size, duration: This research was a multicenter cohort study, including three large reproductive medical centers in South China. All singleton neonates born after Day 3 frozen-thawed embryo transfer cycles from January 2012 to December 2015 were analyzed. Each patient contributed only one cycle per group.

Participants/materials, setting, methods: All singletons born alive after 28th week of gestation were analyzed. Blastocyst transfer, preimplantation genetic diagnosis and intrauterine insemination cycles were excluded. The main outcome measure is neonatal birthweight outcome including average birthweight and the risk of both macrosomia (>4000 g) and LBW (<2500 g). Propensity score matching was used to select equal-sized vitrified and slow-freezed cohorts with similar characteristics. Multivariable regression analysis was then used to explore the impact of freezing methods on neonatal outcomes by adjusting potential maternal and clinical confounders.

Main results and the role of chance: A total of 1667 cases from vitrified embryo transfer and 341 babies from slow-freezed group were included. After propensity score matching, 281 pairs of newborns were finally generated for comparison. The median gestational age was both 39 (38-40) weeks in each cohort and the birthweights were comparable (3188.7 ± 479.0 g vs. 3231.0 ± 469.6 g, $p>0.05$). There were no significant differences between the two groups on the incidence of LBW (5.3% vs. 5.7%) and macrosomia (6.0% vs. 6.0%). The incidence of other outcomes including very preterm birth (0.7% vs. 0.4%), preterm birth (6.4%vs.7.4%) and sex ratio (49.3% vs 47.2%) were all comparable between the vitrified and slow freeze groups, $p>0.05$. In multivariable regression analysis, freezing methods were not found to be associated with neonatal birthweight.The odds ratio (OR) for LBW and macrosomia in the vitrified cohort is 0.93 (95%CI 0.45-1.93) and 1.00 (95% CI 0.50-2.00) compared to the slow-freezed cohort without significant difference.

Limitations, reasons for caution: As a retrospective study, our analysis depends on previously recorded data. Moreover, researches for the long-term effects of vitrification method on the health of children are essential in the near future.

Wider implications of the findings: Vitrification/warming is not inferior to slow-freezing/thawing with regard to neonatal birthweight outcome. Based on this evidence and its superiority regarding clinical outcomes, vitrification for cryopreservation will gradually take over the use of slow-freezing in the near future.

Trial registration number: The study was funded by the National Key Research and Development Program (2016YFC100205). The authors have no conflicts of interest to declare.

P-769 Key performance indicators (KPIs) based on poor prognosis patients are more sensitive on evaluating IVF laboratory staff skills: a prospective cohort study

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Study question: Is it better to follow the poor prognosis patients (PPP) compared with the gold standard patients (GSP) in IVF during new lab staff orientation?

Summary answer: The poor prognosis patients may be a more sensitive group to the new staff skills.

What is known already: It is clear that given that cryopreservation protocols, vitrification methods, evolved empirically and still depend heavily on operator skills, systems to monitor laboratorial performance gained much importance in Frozen–thawed embryo transfer practice. Consequently, KPIs are indicators deemed essential for evaluating before implementing a change, the laboratory's routine; establishing minimum standards for proficiency; monitoring ongoing performance within quality management. However, the largest variable in an IVF laboratory is the patient. For the purpose of measuring the outcome of the lab, this patient variable needs to be either factored into the analysis, or attempted to be minimized.

Study design, size, duration: This study is the prospective cohort study including 1048 infertile patients from June 2016 to December 2017. We performed a two-part prospectively study in IVF. Part A involved 548 infertile poor prognosis patients; Part B involved 500 infertile gold standard patients.

Participants/materials, setting, methods: Patients were categorized as PPPs and GSPs group based on age and functional ovarian reserve parameters. Embryos of both group patients were randomized at the time of cryopreservation to allocate to senior or junior embryologist group, and all embryos were frozen with vitrification. The primary outcome of this study was the implantation rate (IR) per embryo transferred after thawed. We then compared pregnancy rates and post-thaw outcome in terms of Morphological survival and fully intact.

Main results and the role of chance: Part A revealed in 548 poor prognosis patients, 987 embryos were thawed and Part B involved 912 embryos from gold standard patients were warmed in 524 cycles. The IR per embryo thawed by junior embryologists (16.6% (79/475)) was significantly lower after transfer than by senior ones (21.8% (112/512), $P = 0.045$; $\chi2 = 4.01$) in poor prognosis patients group. Similarly, embryos fully intact rate thawed by junior embryologists (79.2 %(376/475)) was significantly lower than by senior ones (84.6% (433/512); $P = 0.033$; $\chi2 = 4.523$). The survival rate was similar in both operators groups (97.3% (462/475) (96.7% (495/512); $P = 0.728$; $\chi2 = 0.121$) in poor prognosis patients group. In Part B, gold standard patients, no significant differences were found in implantation-, survival-, or fully intact rates after transfering the cleavage stage day 3 embryos frozen-thawed by two embryologists groups, respectively.

Limitations, reasons for caution: Culture media, temperature and gas levels were similar in the culturing and transfer, but cleavage stage day 3 embryos with poorer grades of morphology were not cryopreserved or transferred, limiting the ability to generalize our findings for grades of morphology not included in this study.

Wider implications of the findings: The poor prognosis patients may be a more sensitive group to potentially new product/procedure, even if preclinical research has been completed.

Trial registration number: not applicable.

P-770 The rising use of ICSI for non-male factor infertility without evidence of a clinical need or patient advantage

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Study question: Is ICSI being increasingly used in Clinics across Europe for non-male factor infertility and are there any benefits to using the technique when it is not clinically indicated.

Summary answer: A 16-year review of the incidence of male factor infertility in a single clinic provides no evidence for declining semen parameters and necessity for ICSI.

What is known already: Worldwide, approximately two thirds of all ART procedures performed involved ICSI. It is widely reported that across Europe, the proportion of ICSI treatment cycles has risen in recent years, regardless of the etiology of infertility. ICSI carries a number of identified risks and the benefits for use in non-male factor infertility are limited to mitigating the risk of failed fertilisation, which occurs in less than 10% of cycles.

Study design, size, duration: To address this question, we thus conducted a retrospective review of patient notes over a 16 year period for ICSI indication, usage and outcome (fertilisation and pregnancy).

Participants/materials, setting, methods: In a single clinical IVF setting, an assessment was conducted of how many patients per annum presented with semen parameters indicative of ICSI.

Main results and the role of chance: Between 2002 and 2017 a total of 5248 patients were investigated, 2032 were diagnosed as having male factor infertility on the basis of having one or more semen parameters below the WHO semen thresholds for normozoospermia. Male factor infertility accounted for 38.7% (±4.1) patient aetiologies over the study period with no evidence of rising incidence in recent years ($r = 0.001$; $p = 0.9$). Fertilisation rates were similarly comparable as were pregnancy rates for IVF and ICSI treatments on both an annual basis and for the time-course of the study ($p>0.05$).

Limitations, reasons for caution: Although statistical power has been achieved, this is a retrospective study conducted within one clinic. Over the course of the study period, reference ranges for normal semen parameters have changed, however the stringency of the applied protocols have changed in tandem to accommodate for this.

Wider implications of the findings: The data indicate that the rising use of ICSI among ART facilities within the past decade has not been as a consequence of declining semen quality, implying the technique is used for treatment of non-male factor infertility without a clear clinical indication of its superiority over IVF.

Trial registration number: not applicable.

P-771 Induced cellular lysis and increased laser exposure during trophectoderm biopsies can generate low-level mosaic profiles: A prospective study

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Study question: Can trophectoderm biopsy technique induce mosaicism using a technician-induced, manual cell lysis research model?

Summary answer: Prospective induction of cellular damage on research aneuploid embryos revealed a positive correlation to mosaicism, suggesting that mosaicism can be a laboratory created artifact.

What is known already: NextGenertion sequencing (NGS) has enhanced the reporting of mosaicism. The importance of mosaic profiles on PGS results is not well defined, but viable pregnancies have been produced. As increased mosaicism has been associated with decreased implantation potential, it is relatively unknown how accurately mosaic profiles predict the final ploidy of the fetus. It has been proposed that mosaicism may be linked to biopsy technique, but prospective studies have not been performed. Desiring to offer a transfer for all patients and encouragement to perform mosaic embryo transfers, the

reproducibility and accuracy of mosaic reports per entire embryo is not well studied.

Study design, size, duration: Nine research consented embryos were thawed as part of a sister concordance study. Upon completion of two standard biopsy procedures for the original study, the technician attempted to perform poor biopsies with emphasis on cellular lysis and increased laser ablations. A total of 52 samples were tubed, 9-original biopsy, 26-sister concordance study, 10-poor technique, 5-remaining ICM's and 2-media blanks. All samples were blindly analyzed (NGS) by two geneticists and reported according to lab standards.

Participants/materials, setting, methods: Research consented aneuploid embryos were selected based on single/dual chromosomal aneuploidies without knowledge of original NGS profiles. Vitrified blastocysts were warmed and cultured in LifeGlobal medium under tri-gas, humidified atmospheric conditions at 37°C (≤4 hours). Each blastocyst was biopsied according to standard protocols and video recorded. Samples were blindly assessed using the MiSeq (Illumia) NGS platform. Standard lab reporting procedures were applied with a threshold of 30% gain/loss being considered euploid and 30-80% considered mosaic.

Main results and the role of chance: Evaluating full gains and losses, 49 of 50 samples (98%) achieved concordance. Of the 10 samples with technician induced lysis, 3 of 10 (30%) reported the full aneuploidy and an additional mosaic profile. The video review of biopsies showed that as cellular lysis increased so did the level of genetic noise and presence of mosaicism. Of the 2 biopsies which had >30 additional zona ablations, mosaicism was not observed but unique low-level noise was observed being strikingly different from all other samples profiles from the same embryo. Most importantly, spent media from a biopsy which had significant lysis was tubed as a "blank". The latter blank tube showed amplification portraying a chaotic profile, whereas spent biopsy from a clean biopsy (second blank control) exhibited no amplification. The concordance arm of this study, including ICM analysis, showed extreme repeatability. The efforts made to induce mosaic profiles was successful. Of the 3 samples showing mosaicism, none of the other samples from the embryo, including the ICM, showed any of the same mosaic aneuploidies.

Limitations, reasons for caution: Mosaicism, and its importance on NGS calling policies, ultimately impact patient transfer decisions. Mosaic embryos graded as aneuploid can produce healthy live births, revealing a potential technical artifact. Upmost importance should focus on proper biopsy technique if we are to better understand and reduce possible sources of mosaicism.

Wider implications of the findings: Mosaic profiles can be made from known cell lines within genetic laboratories. Yet, mosaic profiles themselves are not in question, but instead the possible loss of DNA by cellular lysis as a source of low level mosaicism. Further scrutiny on laboratory procedures is needed to properly identify true mosaic embryos.

Trial registration number: none.

P-772 Post-cervical ascites at day of oocyte pick-up (OPU) and OPU+5 days and development of ovarian hyperstimulation syndrome (OHSS)

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Study question: Can we define a threshold of post-cervical ascites at day of OPU or OPU+5 days that predicts development of moderate to severe OHSS?

Summary answer: A 1 mm increase in post-cervical ascites at OPU and OPU+5 increases OHSS risk by 3.7% (AOR1.037 95%CI 1.013-1.062) and 4.6% (AOR1.046, 95%CI 1.31-1.61), respectively.

What is known already: OHSS is the most severe side effect of ovarian stimulation with gonadotrophins. Women with polycystic ovarian syndrome (PCOS) and a high ovarian reserve are at a higher risk of developing OHSS. During the ovarian stimulation cycle the number of follicles > 10-11 mm on the day of ovulation trigger is the best clinical predictor of OHSS. Ascites is part of the definition of OHSS, but it has never been investigated, whether a certain cut-off level of post-cervical ascites at day of OPU or OPU+5 days can predict OHSS and potentially guide when to use a freeze-all strategy.

Study design, size, duration: Secondary analyses of a multicenter RCT on 1050 patients randomized 1:1 to short GnRH-antagonist protocol or long GnRH-agonist protocol including women from 2009 to 2013 in Denmark. We used a fixed rFSH (Puregon®) dose of 150IU or 225IU according to age ≤36 years or >36 years. Inclusion criteria were 1. ART cycle, and exclusion criteria were uterine abnormality, age >40 years, testicular sperm extraction/aspiration and severe co-morbidity. Both normo- and anovulatory women were included.

Participants/materials, setting, methods: We pooled all patients from the two arms, who were examined at day of OPU (n = 990). Prediction of OHSS by the amount of post-cervical ascites in the anteroposterior plane was examined by receiver operator characteristics (ROC) analyses. Further, the ability of post-cervical ascites to predict development of OHSS at OPU and OPU+5 was explored with multivariate logistic regression analyses including the following explanatory parameters: Age, anovulatory infertility, GnRH-antagonist/agonist group and number of retrieved oocytes.

Main results and the role of chance: Of the 1008 patients reaching OPU, 990 patients had post-cervical ascites measured on the day of OPU and at OPU+5 days. A total of 199 (20%) patients developed moderate to severe OHSS, including both early and late onset. By ROC curve analysis post-cervical ascites at day of OPU and at OPU+5 days predicted OHSS with an area under the curve (AUC) of 0.599 (95% CI 0.559-0.638, p<0.01) and 0.737 (95% CI 0.699-0.774, p<0.01), respectively. The optimal cut-off value at day of OPU was 4.8 mm with a sensitivity of 41% and specificity of 79%. The optimal cut-off value at OPU+5 days was of 10.7 mm (sensitivity 81% and specificity 61%).

By logistic regression analysis a 1 mm increase in post-cervical ascites at day of OPU increased the odds of OHSS by 5.6% (OR 1.057, 95% CI 1.034-1.080). This association remained significant when adjusting for age, anovulatory infertility, GnRH-antagonist protocol and number of oocytes retrieved (adjusted OR 1.037 95% CI 1.013-1.062). At OPU+5 days, a 1 mm increase in post-cervical ascites increased the odds of OHSS by 6.3 % (OR 1.063, 95% CI 1.049-1.077) and by 4.6% in the adjusted analysis (adjusted OR 1.046, 95% CI 1.31-1.61).

Limitations, reasons for caution: We pooled the groups moderate and severe OHSS including both early and late onset OHSS. This may cause a mismatch as post-cervical ascites on day of OPU may be better to predict early than late onset OHSS.

Wider implications of the findings: Identification of early predictors is of importance for avoiding OHSS. Post-cervical ascites at the day of OPU and OPU+5 can predict OHSS. However, the accuracy of the test demonstrates that we cannot rely solely on the threshold of post-cervical ascites in the decision making of whether to transfer an embryo.

Trial registration number: ClinicalTrial.gov: NCT00756028

P-773 Perinatal outcome in singletons after frozen-thawed blastocyst transfer: hormone replacement treatment cycles versus natural cycles

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Study question: Are there differences in perinatal outcomes between singletons after hormone replacement cycle frozen-thawed blastocyst transfer (HRT-BT) and natural cycle frozen-thawed blastocyst transfer (NC-BT) deliveries?

Summary answer: A tendency toward more cases of heavy for date in HRT-BT was observed, but the difference was not statistically significant.

What is known already: Several studies show that the birthweights of singletons delivered after frozen-thawed embryo transfer are significantly higher than those delivered after fresh embryo transfer. A contributing factor to the increased birthweight may be hormonal environment of the endometrium before or after embryo transfer – and not the frozen-thawed procedure itself.

There are few studies that compare the mean singleton birthweights after HRT cycles versus natural cycles.

Study design, size, duration: We performed a retrospective case study of women who underwent frozen-thawed blastocyst transfer at our hospital with singleton pregnancy and a live birth after 22 weeks of gestation between 2013 and 2016. The study included 191 singletons born after blastocyst transfer (BT): HRT-BT; n = 137 and NC-BT; n = 54.

Participants/materials, setting, methods: We compared HRT-BT deliveries with NC-BT deliveries. We measured birth defects, preterm birth (PTB), hypertensive disorder of pregnancy (HDP), placenta previa, and heavy for date (HFD). The crude and adjusted odds ratios (AOR) and 95% confidence interval (CI) were calculated using a logistic regression analysis. Adjustment was made for the maternal age at BT (categorized in 35≥, 35<) and parity.

Main results and the role of chance: HFD was observed in 30 cases (21.9%) of HRT-BT and 6 cases (11.1%) of NC-BT (AOR, 2.30; 95% CI, 0.89—5.9). HDP was observed in 8 cases (5.8%) of HRT-BT and in one case (1.9%) of NC-BT (AOR, 3.20; 95% CI, 0.39—26.6). PTB was observed in 3 cases (3.2%) of HRT-BT and 1 case (3.1%) of NC-BT. Placenta previa was observed in 3 cases (2.2%) of HRT-BT and did not occur in NC-BT. Birth defects were observed in 5 cases (3.6%) of HRT-BT and 1 case (1.8%) of NC-BT. There were 3 cases of congenital heart disease, 1 case of 7q-, and 1 case of Prader-Willi syndrome in HRT-BT. There was one case of trisomy 21 in NC-BT. PTB and placenta previa were rare in this study, thus, we could not calculate the AOR. A tendency toward more cases of HFD in HRT-BT was observed, but the difference was not statistically significant.

Limitations, reasons for caution: This is a retrospective study based on a small sample size, performed by a single medical center.

Wider implications of the findings: The primary factor of increasing cases of HFD after BT may be the hormonal environment of the endometrium before or after BT. If duplicated data are included, future studies may show that HRT cycles are significantly associated with more cases of HFD.

Trial registration number: none.

P-774 Twinning in Greece in the recent 10-year period of 2004-2013; comparison of between IVF-conceived and naturally-conceived twins

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Study question: To estimate the twinning rate in Greece during 2004-2013 and possible differences on somatometric characteristics IVF-conceived and naturally-conceived live born pairs of twins at birth.

Summary answer: The 10-year twinning frequency was 2.6%. The IVF-conceived pregnancies were 26.7% showing lower birth-weight and gestational age and higher IUGR frequency compared to the naturally-conceived.

What is known already: One of the consequences of the applied medically-assisted reproduction, mainly *in vitro* fertilization (IVF) techniques for treatment of infertility is the higher rate of multiple gestations and consequently the higher number of high-risk pregnancies. The health problems of multiple pregnancies in mothers and neonates are more pronounced than the single type, presenting higher neonatal morbidity and mortality rates. Comparison of somatometric characteristics between IVF-conceived and naturally-conceived twins at birth may contribute to the evaluation of the new medically-assisted reproductive methods to neonates' health and development.

Study design, size, duration: We studied all multiple births that took place in General District Hospital Athens "Alexandra" during a recent 10-year period, 2004-2013. The "Alexandra" Hospital is the biggest public university hospital in Greece that carries out a large number of deliveries annually. For each multiple delivery, number of neonates, mode of conception, delivery and gestational age were recorded. For all pairs of twins morbidity, mortality, gender, birth weight, and intra-uterine growth retardation (IUGR) data were recorded.

Participants/materials, setting, methods: We studied all multiple births in all deliveries that took place in "Alexandra" during a recent 10-year period (2004-2013). Gender, birth weight, gestational age, mode of conception, delivery and IUGR data were recorded. Annual incidence of twinning rate was calculated. Frequency of each characteristic was calculated to show possible differences between IVF and naturally-conceived neonates. Statistical analysis for comparing the categorical data chi-square test was used. All tests were performed with p-values set to 0.05.

Main results and the role of chance: During the study period 1,235 multiple births in all 46,747 deliveries were noted; 45 (3.6%) were triple pregnancies and 1,190 (95.4%) double. The 10-year frequency of twinning rate was 2.6%, with the highest in 2010 (3.4%) and the lower in 2007 (1.6%). The *in vitro* fertilization (IVF) pregnancies were 330/1,235 multiple pregnancies (26.7%), while the rest 905/1,235 (73.3%) represent natural conception.

The mortality rate was 82/1,235 deliveries (6.6%); 40 deliveries had one dead baby and 42 both dead babies. The conception was achieved after IVF in 18/82 (22%) and 64/82 deliveries (78%) were conceived naturally. The mortality rate was similar between IVF and natural conception.

Concerning the live-born neonates, the frequency of IVF-pregnancies was 25.6% (305/1190) and naturally-conceived was 74.4% (885/1190). Male: female ratio was 0.88 for IVF-conceived and 1.02 for naturally-conceived neonates. Mean birth weight and gestational age were lower in IVF-conceived neonates than in naturally-conceived neonates; 2096.83 *g vs* 2162.31 *g* and 34^{+1} and 34^{+5} wga, respectively.

Evaluation for intrauterine growth restriction showed similar frequency of IUGR neonates between the 2 groups; 37% (113/305) in IVF (65.1% one IUGR neonate and 34.9% both IUGR neonates) and 38.4% (340/885) in naturally-conceived neonates (67% one IUGR neonate and 33% both neonates).

Limitations, reasons for caution: Limitations of the present study include lack of data concerning the medication taken by the mother during pregnancy and other pathological conditions such as chorioamnionitis and gestational diabetes. Future studies focusing on long-term outcome of IVF-conceived and naturally-conceived neonates should enable us to draw conclusions about their course and development.

Wider implications of the findings: The present study comprises an attempt to present the twinning rate and pregnancies outcome in Greece during the 10-year period 2004-2013. Mortality rates and somatometric characteristics of the IVF-conceived and naturally-conceived neonates at birth along with long-term outcome will enable more precise description of growth and developmental outcomes among twins.

Trial registration number: Not applicable.

P-775 Oocyte donation is not an independent risk factor for adverse perinatal outcomes in IVF pregnancies: results of a 5-year cohort in a UK high-risk unit

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Study question: Does oocyte donation increase the risk of adverse perinatal outcomes such as preterm birth, low birth weight, postpartum haemorrhage, stillbirth, neonatal death and low Apgar?

Summary answer: Oocyte donation is not an independent risk factor for adverse perinatal outcomes when compared with autologous IVF.

What is known already: Female age remains by far the main predictor of success in IVF, largely due to a loss of ovarian follicle reserve and oocyte quality as women get older. Oocyte donation has thus become a viable alternative in recent decades for patients with reduced egg quality or quantity. However,

there is a paucity of data on the implications of egg donation upon obstetric and neonatal outcomes for the index pregnancy. In particular, previous analyses have failed to identify predictors of adverse perinatal events, mainly due to a lack of information on confounding variables such as maternal BMI and previous uterine surgery.

Study design, size, duration: This was a retrospective cohort study conducted at the John Radcliffe Hospital in Oxford, UK, a tertiary referral centre. Demographic data and perinatal outcomes of women with singleton pregnancies resulting in live births after autologous and donor-oocyte IVF were obtained from electronic hospital records. A total of 792 singleton pregnancies, delivering between December 2012 and January 2018, were included in the analysis: 100 (12.6%) resulted from oocyte donation and 692 (87.3%) from autologous IVF.

Participants/materials, setting, methods: Univariate analysis was initially performed to identify significant differences ($p<0.05$) between the donor-oocyte and autologous IVF groups. A multivariate binary logistic regression model with backward stepwise elimination was then created to identify potential confounders (e.g. age, smoking, BMI, ethnicity, low-dose aspirin at booking, gestation at delivery, mode of birth) and determine predictors of poor outcomes (e.g. preterm birth [≥24and<37 weeks], need for operative delivery, blood loss ≥1000 mL, birth weight<10th centile [small for gestational age, SGA]).

Main results and the role of chance: Overall there were 491/792 (62%) primigravidas. Median age and BMI were lower in the autologous IVF group (35 vs 43 years,$p<0.001$; and 23.8 vs 25.4 kg/m^2,$p = 0.015$). Those with donated oocytes were more likely to take aspirin (18% vs 6.8%,$p = 0.001$). The rates of obesity (BMI≥30,$p = 0.104$), pre-pregnancy diabetes ($p = 147$), smoking ($p = 0.75$), previous uterine surgery (e.g.myomectomy/caesarean,$p = 0.318$) and chronic hypertension ($p = 0.07$) were similar in both groups.

Median gestation at delivery was 39 weeks in both groups ($p = 0.66$). There was no difference in the rates of preterm birth (9.5%,$p = 0.71$) and SGA between groups (9.6%,$p = 0.137$). Median Apgar at 5 minutes was 10 ($p = 0.49$). There were 2/792 (0.3%) stillbirths, both occurring in the autologous IVF subgroup. There were no neonatal deaths.

Women conceiving with oocyte donation were more likely to require caesarean delivery (72.4% vs 59.6%,$p = 0.015$) and sustain significant haemorrhage (26.8% vs17.1%,$p = 0.025$). Following logistic regression analysis, independent risk factors for caesarean included previous uterine surgery (OR5.5, 95%CI 2.07-15.20), primiparity (OR2.88, 95%CI 2.02-4.11), increasing age (OR1.12, 95%CI 1.09-1.16) and higher maternal BMI (OR1.061, 95%CI 1.02-1.10). Caesarean delivery was an independent risk factor for postpartum haemorrhage ≥1000 mL (OR4.18, 95%CI 2.52-6.93). Egg donation was not independently linked to risk of caesarean (OR0.94, 95%CI 0.51-1.72) or postpartum haemorrhage ≥1000 mL (OR1.23, 95%CI 0.67-2.23).

Limitations, reasons for caution: The main limitation of this study was its retrospective design. Prospectively collected data using a larger sample would be required to corroborate our results.

Wider implications of the findings: We show that oocyte donation is safe and does not increase the incidence of adverse perinatal outcomes in IVF when adjusting for confounders. The higher risk of caesarean birth and subsequent haemorrhage likely derives from the fact that women conceiving with donated eggs are older and have a larger BMI.

Trial registration number: Not applicable.

P-776 Comparison of follicular fluid candidate proteins involved in inflammation or angiogenesis and prediction of OHSS in assisted reproductive technology (ART); A pilot study

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Study question: Does the pre-ovulatory follicular fluid (FF) level of selected candidate proteins differ between women with and without OHSS and guide when to use 'freeze-all' strategy?

Summary answer: The pre-ovulatory FF level of soluble urokinase plasminogen activator receptor (SuPAR), a potential new inflammatory biomarker, was significantly lower in women developing OHSS.

What is known already: In ART, number of follicles ≥10-11 mm on trigger-day correlates to OHSS-risk. However, some women with a high number of follicles do not develop OHSS. This could reflect that not only the follicular quantity but also the quality, the intra-follicular milieu, contribute to the syndrome. Proteins involved in inflammation or angiogenesis may be involved in the development of OHSS. As increasing SuPAR plasma levels are thought to reflect increased activation state of the immune system, we hypothesized that the pre-ovulatory FF SuPAR level could be a potential new inflammatory biomarker predicting OHSS. There are no previous publications on FF SuPAR levels.

Study design, size, duration: FF was collected during a RCT including 1050 women in their first ART cycle during year 2009-2013 comparing OHSS in short versus long protocol. This sub-study is a case control study including FF measurements from subjects with ≥15 oocytes retrieved who developed severe OHSS (n = 25, Cases), subjects with ≥15 oocytes who did NOT develop moderate-severe OHSS (n = 25, Control1), and subjects with 5-8 oocytes who did NOT develop moderate-severe OHSS (n = 25, Control2).

Participants/materials, setting, methods: For each Case (n = 25), one Control1 (n = 25) and one Control2 (n = 25) was selected by matching subjects by treatment protocol (short versus long), pregnancy (+/-), age, BMI, and anovulation (+/-). FF from one large pre-ovulatory follicle was collected, centrifuged and frozen until the study-related measurements of SuPAR, placental growth factor (PlGH), vascular endothelial growth factor (VEGF), and angiopoitin I and II levels by commercially available Elisa-kits, and C-reactive protein (CRP) by a high-sensitivity immunoturbidimetric assay.

Main results and the role of chance: The FF levels of SuPAR differed significantly between the three groups (P = 0.018) with mean (SD) levels of 2.3 (0.4) ng/ml, 2.6 (0.8) ng/ml and 2.8 (0.6) ng/ml in Cases, Control1's and Control2's, respectively. When Cases (OHSS, n = 25) where compared to the combined Control groups (no moderate-severe OHSS, n = 50) the mean difference between groups was -0.40 ng/ml (95% confidence interval -0.71 to -0.09) ng/ml, (P = 0.012). Receiver operating characteristic (ROC) curve analysis demonstrated that the level of SuPAR could predict severe OHSS (AUC 0.678; 95% CI 0.553-0.803) with a sensitivity of 64% and a specificity of 66%. The level of CRP, another well-known marker of inflammation, as well as the levels of selected candidate proteins involved in angiogenesis (VEGF, PlGF and angiopoitin I and II) did not differ between the three groups or between Cases and combined Controls. Patients in the three groups were matched for anovulation (+/-) with a total of 23 subjects presenting anovulation and 52 without. The follicular level of CRP was significantly higher among subjects with anovulation; median (range) 2.0 (0.2-8.5) mg/L compared to 0.85 (0.2-6.1) mg/L in regular cycling women (P = 0.34).

Limitations, reasons for caution: Although the FF SuPAR level significantly predicted severe OHSS in the present dataset, the relatively low sensitivity and specificity currently makes a clinical application unlikely. Further, as this is the first study of SuPAR levels in relation to ART, further studies are required to determine possible predictive value of SuPAR.

Wider implications of the findings: Our findings indicate that the intra-follicular activation state of the immune system at oocyte-pickup is not increased in women developing severe OHSS. Furthermore, the FF level of the selected candidate proteins involved in angiogenesis was unchanged, especially VEGF that has been claimed to play an important role in OHSS.

Trial registration number: ClinicalTrial.gov: NCT00756028 (Primary RCT)

P-777 Achieving competency in embryo transfer and monitoring ongoing performance

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Study question: To evaluate cumulative summation (CUSUM) and mean averages in assessing the learning curve and ongoing competency of reproductive medicine trainees performing embryo.

Summary answer: Learning curve cumulative summation (LC-CUSUM) can provide a useful way of charting progress, but offers false reassurance, masking the possibility the trainee requires further training.

What is known already: Critical to successful in vitro fertilisation (IVF) is the ability to transfer embryos safely and efficiently into the uterus. When learning, it is important for the trainee to achieve competency as quickly as possible. Assessing the learning curve and ongoing competency can be achieved by plotting mean averages of pregnancy outcome. However, this is limited by over sensitivity in the initial phase and poor sensitivity as the number of procedures increases. CUSUM provides an alternative statistical model to monitor performance by detecting if a process is 'out of control' following a mathematical model which can be easily interpreted graphically.

Study design, size, duration: This prospective study included five trainees learning embryo transfer at a Nurture Fertility between November 2011 and November 2016. All trainees underwent a theoretical embryo transfer training session followed by a two-month period of observation before performing embryo transfers.

Participants/materials, setting, methods: Embryo transfer was performed in theatre with the woman in dorsal lithotomy position. Embryos were transferred using a pre-loaded flexible Wallace catheter or using an elective malleable guide prior to passing the flexible catheter within the obturator. Embryos were placed 2 cm from the fundus, with trans-abdominal ultrasound guidance. A successful outcome was defined as a positive urine pregnancy test, 14 days after embryo transfer. Charts plotting the mean average and CUSUM were prepared.

Main results and the role of chance: Five hundred embryo transfers were analysed. One hundred consecutive embryo transfers and pregnancy outcome data were available for each trainee. The overall pregnancy rates for each trainee were 44%, 61%, 53%, 57% and 44% respectively. Once competency was achieved the mean pregnancy rate for all trainees was 78%.

Plots of mean averages were unhelpful in determining when a trainee was competence, showing initial wide swings before reaching steady state.

When assessing embryo transfer competency using LC-CUSUM the five trainees achieved competency at the 5th, 5th, 8th 13th and 27th procedure respectively. Each consecutive outcome was plotted on a chart using LC-CUSUM, with a downward step for a success and upward step for failure, until the competency threshold was crossed. Graphical representation was helpful to demonstrate when competency was reached.

Subsequent monitoring of embryo transfer performance was plotted on the same chart using CUSUM to monitor their performance. Four trainees maintained their success rate within acceptable limits. The CUSUM chart for one trainee alarmed at observation 17 into the monitoring period when their CUSUM score breached the threshold. This allowed additional individualised support and training to be initiated.

Limitations, reasons for caution: Results may be affected by confounders such as the quality of embryos, number transferred, culture media, embryologist, patient characteristics and so on. CUSUM also offers false reassurance, masking the possibility the trainee requires further training. Furthermore, statistics ignore vital aspects of learning such as formative feedback, self-assessment and reflection.

Wider implications of the findings: Statistical plots using mean average or CUSUM can provide useful graphical information when learning new practical procedures such as embryo transfer as part of a wider training program. Further studies should assess CUSUM for long term quality control.

Trial registration number: not applicable.

P-778 Are course of the pregnancy and perinatal outcome any different after transfer of poor quality day 5 embryos?

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Study question: Does the morphology of embryos transferred on day 5 after oocyte retrieval affect pregnancy complications, birth complications or newborn pathology rates?

Summary answer: Patients having embryos of different quality transferred on day 5 after oocyte retrieval have similar rates of pregnancy complications, birth complications and newborn pathology.

What is known already: Questioning child health remains the concern in transfer of poor morphology embryos. Up to date, no significant differences between good and poor embryo quality have been observed in newborn congenital diseases. Lower implantation and higher abortion rates are known to be directly connected to poor embryo morphology. Adverse pregnancy and birth outcome, including low gestational age, premature birth and restricted intrauterine growth, have already been connected to the poor embryo quality indirectly, through poor reproductive history of the patients.

Study design, size, duration: Retrospective cohort study included 380 patients and 451 newborns after IVF-ET procedure performed in University Medical Centre Ljubljana between January 2011 and December 2015. According to quality of embryos transferred, maternal characteristics, course of pregnancy and perinatal outcome were observed.

Participants/materials, setting, methods: Data were collected through Perinatal information system of Slovenia. Three-hundred-nine pregnancies (309 singleton and 71 twin) were divided in three groups regarding quality of transferred embryos in the IVF procedure (group A – one or two blastocysts of best quality, group B - one good and one poor quality blastocyst, group C - one or poor blastocysts or morulae). Blastocyst morphology was assessed accordning to Gardner blastocyst score (development1-6, tropfoblast A-C and embryoblast A-C).

Main results and the role of chance: There were 239 patients in group A, 77 patients group B and 64 in group C. There were no significant differences in maternal age and number of IVF procedues. Mothers in group A had significantly more oocytes retrieved. Mothers of singletons in group C had significantly higher rate of BMI belov 18,5 (16% vs 3,5% and 1,6%; $p<0,05$, Student t-test) and significantly lower BMI overall. Comparison was also significant between individual groups (A versus C, $p = 0,03$; B versus C, $p<0,005$, Pearson chi-square test). In group C there were significantly more ceasarian sections performed (40,6% vs 35.1% vs 22,1%, $p = 0.44$). Otherwise we found no other significant differences in pregnancy complications (bleeding in each trimester, preeclampsia, multiple pregnancies, placental pathology), birth complications (ceasarian section, bleeding, premature birth) or mobidity of the newborns (birth weight, gestation, small for gestation, Apgar score, congenital disease, admission to intensive care, mortality).

Limitations, reasons for caution: Beside relatively low number of patients in groups B and C, the limitation of study was the composition of group C defined by transfer of poor quality embryos. Blastocysts with poor embryoblast/trophoblast were in the same group as normal morulae, which would be better to observe separately.

Wider implications of the findings: Reassuring results showed no significant differences in pregnancy and birth pathology or neonatal morbidity regarding quality of transferred embryos. However, we found a different insight into the pathology and lifestyle of infertile women, showing that too low BMI can affect embryo quality simiraly or even more than higher BMI.

Trial registration number: Republic Medical Ethical Comeete on 14.2.2017, number 0120-47/2017-3

P-779 Perinatal outcomes in children born after IVF using a GnRh-agonist protocol versus a GnRh antagonist protocol: a Catalan cohort study based on 4562 newborns

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Study question: To ascertain whether perinatal outcomes are affected by the type of GnRH and/or gonadotrophins used for the controlled ovarian hyperstimulation (COH) cycle.

Summary answer: Perinatal outcomes are not affected by the protocol used for the COH neither by the type of gonadotrophins.

What is known already: One of the well established parts of assisted reproductive techniques (ART) is the utilization of gonadotrophin-releasing hormones (GnRh), antagonists or agonists, and gonadotrophins for the COH. Several studies performed to compare distinct protocols of GnRh and gonadotrophins showed no significant differences between live birth rates (effectiveness) neither among ovarian hyperstimulation syndrome rates (safety). Despite this lack of differences, what is also described during COH is a hyperestrogenic environment that could lead to biochemical alterations during implantation and placentation, which may affect obstetric and perinatal results. Our study ascertain whether perinatal outcomes are affected by drugs that generate higher estradiol levels.

Study design, size, duration: We conducted a register-based cohort study using prospective data from the Department of Health of the Regional Government of Catalonia between 2008 and 2013. A total of 4562 women were included.

Participants/materials, setting, methods: The participants were women undergoing COH using autologous eggs who had a singleton pregnancy delivered from the 24th week onward. Two different groups were made for the study: women undergoing COH using a GnRh antagonist based protocol and women undergoing COH using a a GnRh-agonist based protocol. Additionally, two subgroups were made according where recombinant or urinary gonadotrophins were used.Primary outcomes were birthweight and gestational age at delivery.

Main results and the role of chance: Women undergoing COH with a GnRh antagonist protocol and women using a GnRh-agonist protocol had comparable results regarding birthweight (BW) (3,144 ± 542.5 g and 3,161.6 ± 536.18 g, respectively; P = 1.08) and gestational age at delivery (GA) (38.7 ± 2.12 and 38.1 ± 2.08 weeks, respectively; P=.87) and these outcomes persisted after adjusting for confounding factors. Furthermore, we observed no statistically significant differences in the main perinatal outcomes analyzed in the subgroups of recombinant and urinary gonadotrophins (BW: 3,158.1 ± 539.6 g and 3,140 ± 537.1 g, respectively; P = .96 and GA: 38.8 ± 2.04 and 38.6 ± 2.24 weeks, respectively; P = 1.6).

Limitations, reasons for caution: The main limitation of the study is the retrospective design. This kind of evaluation precludes an analysis of some potentially relevant data that were missing from the registry and also results in a significant loss of cases because of missing data in the main variables.

Wider implications of the findings: Perinatal outcomes are not affected by the protocol used for COH. Hence, the choice of the GnRH analog, gonadotrophins and their combinations should be based on other clinical aspects. However, prospective studies would be required to support these findings.

Trial registration number: Not applicable.

P-780 Off-label use of androgens and letrozole in infertile women – a multinational survey in Europe and Australia

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Study question: Do fertility experts often recommend off-label regimens (androgens or letrozole) in reproductive medicine, and is this based on robust scientific evidence?

Summary answer: Clinicians very often recommend off label androgens and letrozole in reproductive medicine. This is not always driven by robust evidence in favor of treatment.

What is known already: Although off-label use of drugs and supplements is common in all medical domains, the magnitude of off-label regimen prescription and the rationale behind this, remains unclear in the field of reproductive medicine. Androgens and letrozole are possibly the most widely used off–label drugs among infertile women; still, available evidence regarding their safety and efficacy appear to be fairly different. Accumulating evidence support the beneficial effect of letrozole in unovulatory women undergoing ovulation induction, while evidence regarding the use of androgens for poor ovarian responders is weak and contradictory.

Study design, size, duration: This is a cross-sectional online survey conducted in 5 different countries (Denmark, Norway, Spain, Belgium and Australia), including all private and public specialists in reproductive medicine in order to evaluate fertility specialists' attitudes towards the use of off-label medication. The survey took place between October 2016 and December 2017.

Participants/materials, setting, methods: Online survey invitations were sent to all members of the Danish, the Norwegian, the Spanish, the Australian and the Belgian Fertility Society.

Main results and the role of chance: In total, 222 fertility specialists responded among 1136 who were contacted electronically (response rate 20%).

Cumulatively, 75.5% of the physicians recommended off-label use of androgens or letrozole for their patients. Overall, letrozole was used more frequently by clinicians 65% (CI 57%-72%), as compared with testosterone 49.1% (CI 38%-59%), although the difference was not statistically significant, p = 0.59 and more frequently than DHEA 29.3% (CI 16%-41%), p<0.001. Significantly more fertility specialists recommended testosterone as compared with DHEA for poor ovarian responders p<0.001.

Almost half (42.5%) of the fertility specialists who did not recommend the use of androgens reported that their decision was based on the lack of scientific evidence, whereas the 2 main reasons for not using letrozole was either because this was not part of clinicians' guidelines (24,9%) or because the drug was not officially approved for use in ART (19%).

Limitations, reasons for caution: Fertility care providers who recommend androgens or letrozole might have been more likely to respond to the survey and this could exaggerate the use of the procedure reported here.

Wider implications of the findings: A majority of clinicians are using off-label letrozole which is supported by the accumulating evidence for treatment of anovulatory patients. However it is remarkable that almost half of the clinicians continue to use androgens in poor responders, despite the lack of evidence and the caution raised in published articles.

Trial registration number: Not applicable.

POSTER VIEWING
Stem cells

P-781 Impact of pluripotency state and Activin A supplementation on derivation of primordial germ cell-like cells from human embryonic stem cells

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Study question: Is there an effect of the pluripotency state and Activin A supplementation on differentiation of human embryonic stem cells towards the germline?

Summary answer: Germline precursors were formed in all conditions, apart from the naïve RSET condition. Activin A supplementation appeared to boost this expression.

What is known already: Gametogenesis from human embryonic stem cells (hESC) in vitro may provide new long-term perspectives for genetic parenthood for patients currently facing sterility. Recent studies have shown that human primordial germ cell-like cells (hPGCLCs) with SOX17/OCT4 expression can be obtained from hESC grown in a '4i' state, by culturing them as embryoid bodies (EBs) with BMP4, EGF, SCF, hLIF and ROCKi. Moreover, our group previously showed that primed hESC cultured as EBs in presence of BMP4 and Activin A showed increased propensity to express genes for post-migratory germ cells compared to EBs exposed to BMP4 alone.

Study design, size, duration: The germline competence of different states of pluripotency such as naive hESC and other states inhibiting one or more signalling pathways are yet to be studied in parallel and warrants further investigation.

To study this we used: two in-house derived female hESC lines were cultured in primed, primed-Wnt-inhibitor(Wnt-i), 4i (naive) and RSET (naive) conditions.

Participants/materials, setting, methods: The cells were tested for pluripotency markers using immunostaining and cultured as EBs in two groups; germ cell differentiation medium with Activin A(20ug/ml) and other without Activin A. Day 4 EBs were fixed and stained for germ cell and pluripotency markers, visualised as z-stacks on a Leica SP8 Confocal Microscope, and analysed using IMAGEJ/FIJI.

Main results and the role of chance: We found that the specific pluripotency state affects the germline induction potential of hESC. In both cell lines, EBs from primed, Wnt-i and '4i' cells expressed SOX17 and OCT4 double positive cells at varying levels after 4 days. In contrast, EBs from the naïve RSET condition did not show these markers. Positive cells also expressed Podoplanin (PDPN) on their surface, a PGC marker in embryos of cynomogolous monkeys. Therefore, the obtained hPGCLCs were positive for SOX17/OCT4 and SOX17/PDPN. EBs from Wnt-i hESCs had a similar germline competence as primed hESCs. EB's from '4i' had the highest germline competence in the standard differentiation medium. Addition of Activin A to the differentiation medium resulted in markedly increased proportion of hPGCLCs in EB's all the three conditions except RSET.

Limitations, reasons for caution: In order to present scientifically robust data, the current study, which is based on confocal imaging only should be further supported by transcriptome data of the obtained hPGCLCs, which should be compared to the profiles of in vivo harvested early human PGCs.

Wider implications of the findings: The current study aims to identify embryonic germ cell markers on hPGCLCs and to standardise the process of deriving gametes from pluripotent stem cells. Adaptation of culture conditions based on this knowledge may ultimately advance efforts to derive germ cells in vitro for patients currently facing sterility.

Trial registration number: Not Applicable.

P-782 Induction of spermatogenesis under 3-Dimentional tissue culture conditions by in vitro transplantation of human spermatogonial stem cells isolated from frozen-thawed testis tissue

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Study question: Can testis organ culture support progression of spermatogenesis after in vitro transplantation of spermatogonial stem cells under conditions of testicular tissue culture?

Summary answer: Conditions of the 3-Dimentional testicular tissue culture after spermatogonial stem cell transplantation can support the progress of spermatogenesis to produce haploid cells.

What is known already: Inducing spermatogenesis by long term preserved germ cells to produce mature and functional sperm is one of the main goals of reproductive medicine. Germ cell transplantation is one of the ways reaching to this goal. In vivo transplantation has been done successfully and frequently. But in the in vitro transplantation and organ culture method Sato et al in 2013 are first one that published protocol. They reported sperm producing from in vitro transplantation of mouse spermatogonial stem cells and organ culture.

Study design, size, duration: This study is based on 5 biopsy samples taken from different azoospermic patients with 20 to 45 years old. For each experimental group, 3 to 5 NMRI mouse were used at the age of 4 weeks.

Participants/materials, setting, methods: SSCs were obtained from patient's TESE (Testicular Sperm Extraction). To confirm identification of SSCs, PLZF protein was detected. Labeled cells by DiI were transplanted to adult mouse testis which treated by 40 mg/kg busulfan. Host testes set in organ culture condition. Another testis without transplantation set in organ culture condition as control group. Cultured testes were assessed by histomorphometric studies, tracing of DiI, immunohistochemistry and molecular (Real Time PCR) studies after 8 weeks.

Main results and the role of chance: The results of histomorphometric studies showed that the mean number of spermatogonial cells, spermatocytes and spermatids in the transplantation group was significantly more than the control group ($P<0.05$) and most of the cells responded positively to the detection of DiI. Immunohistochemical studies in host testes fragments in the experimental group express the *PLZF*, *SCP3* and *ACRBP* proteins in spermatogonial cells, spermatocyte and spermatozoa, respectively, which confirmed the human nature of these cells. Also, in molecular studies of *PLZF*, *Tekt1* and *TP1*, the results indicated that the genes were positive in the test group, while not in the control group.

Limitations, reasons for caution: Obtaining human samples were very difficult. There were few patients who gave satisfaction for researches.

Wider implications of the findings: The results of this study indicate that the in vitro transplantation system and testicular tissue culture have the ability to support the progression of spermatogenesis to obtaining haploid cells.

Trial registration number: our trial was non-clinical.

P-783 Transplantation of human menstrual blood-derived stromal cells promotes recovery of cyclophosphamide-induced premature ovarian insufficiency

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Study question: How can menstrual blood-derived stromal cells (MenSCs) take reparative effects on premature ovarian insufficiency?

Summary answer: Menstrual blood-derived stromal cells can effectively inhibit apoptosis of granulosa cells and therefore renovate ovarian function damaged by Cyclophosphamide.

What is known already: Premature ovarian insufficiency (POI) is characterized by abnormal menstruation with hormonal disorders in female aged before 40, and 1%~3% female are suffering from it whereas certain etiologies and pathophysiological mechanisms are not clearly defined. Recently, different sources of mesenchymal stem cells have been found to be therapeutic in premature ovarian insufficiency such as umbilical cord, skin, amniotic fluid, endometrial mesenchymal stem cells, etc. In these studies, paracrine fashions of mesenchymal stem cells play the principal role in the restoration of ovarian function.

Study design, size, duration: Experiments were divided into three groups (blank group without treatment, control group with cyclophosphamide injected abdominally and treatment group with MenSCs and cyclophosphamide preconditioned). In vivo, female mice were injected with cyclophosphamide in advance; and one week later, those in experiment goups were treated with MenSCs and the rest with PBS until 10 days. In vitro, KGN cells were cultured with medium or cyclophosphamide or cocultured with MenSCs for 24 hours.

Participants/materials, setting, methods: Stromal cells were isolated from menstrual blood, infected with green fluorescence protein. KGN cells line

were cultured for experiments in vitro. Female mice were divided into three groups (blank group without treatment, control group with cyclophosphamide injected abdominally and treatment group with MenSCs through tail veins with cyclophosphamide preconditioned). Then, identificating and proving the reparative effects by CCK-8 assay, FACS, in-vitro differention, H&E staining, Western, RT-PCR, immunofluorescence, TUNEL-apoptosis assay, RNA Sequencing, etc.

Main results and the role of chance: In our present study, we demonstrated that MenSCs showed properties of mesenchymal stem cells with surface markers $CD90^+$, $CD73^+$, $CD105^+$, $CD44^+$, $CD38^-$, $CD45^-$ and $CD34^-$. Osteogenic, chondrogenic and adipogenic defferentiation indicated the multipotency of MenSCs. In vivo, apoptotic cells in ovarian tissues especial granulosa cells decreased compared with control group according to TNUEL and Hoechst assays. Body weight of mice, ovarian and uterine weight and number of live births were significantly different among blank and experiment groups compared with control group. In addition, MenSCs were able to up-regulate the expression of MIS, VASA, Ki67, VEGF and LHR of ovarian tissues, modulating blood hormones with IFN-γand FSH down-regulated and E_2 and AMH up-regulated. In vitro, MenSCs reduced apoptosis of KGN cells in accordance with the downward trend of caspase molecules. RNA sequencing demonstrated that MenSCs restore ovarian function through regulating biological metabolism, angiogenesis, muticellular organismal process, cell differentiation, apoptotic process and so on with 82 related genes up-regulated and 261 relative genes down-regulated.

Limitations, reasons for caution: Our study revealed the reparative effects on POI induced by chemotherapeutic agent via inhibiting apoptosis of granulosa cells. However, the etiologies of this syndrome is not only for this reason. Thus, we need further investigation to explore the effect of MenSCs in different models such as immunogeneous POI.

Wider implications of the findings: MenSCs are capable of promoting recovery of POI by inhibiting the activation of caspase signaling in granulosa cells. It is noteworthy that related trials of MenSCs in POI treatment were mainly conducted confined to rodent animals; therefore it is necessary for us to take further investigation in mammals.

Trial registration number: not applicable.

P-784 Transcriptomic features of KRT7 positive cells derived from BMP4-treated human induced pluripotent stem cells (hiPSCs)

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Study question: Do KRT7 positive cells derived from BMP4-treated hiPSCs have trophoblast-specific features?

Summary answer: The KRT7 positive differentiated cells exhibit trophoblast features.

What is known already: Early development of human placenta remains poorly understood due to lack of proper model systems. Previous reports demonstrated that human Embryonic Stem Cells (hESCs) and hiPSCs treated with bone morphogenetic protein4 (BMP4) could differentiate into extra-embryonic tissue and they could be a useful model of the early trophoblast differentiation. However, the differentiated cells are heterogeneous, which include both trophoblast (TB) and mesoderm lineage cells.

Study design, size, duration: In order to identify the characteristics of the trophoblast lineage cells from BMP4-treated hiPSCs, cells were collected using a pan-trophoblast marker, KRT7, with flow cytometry and performed comprehensive gene expression analysis.

Participants/materials, setting, methods: Four types of hiPSCs were treated with 50 ng/ml of BMP4 in a Xeno-free medium for 10 days. After the expression of KRT7 was confirmed by immunofluorescence analysis, KRT7-positive cells were collected using flow cytometry and analyzed by DNA microarray (Agilent SurePrint G3 Human Gene Expression v3 8×60 K Microarray). The difference of the gene expression profiles between KRT7-positive cells and hiPSCs were compared, and the expressions of some genes were validated by quantitative RT-PCR.

Main results and the role of chance: All the BMP4-treated cells were stained by immunocytochemistry for KRT7. Microarray analysis demonstrated that 833 genes were commonly expressed in all the four hiPSC groups, including POU5F1, SOX2, and NANOG. 259 upregulated genes were commonly expressed in all the four BMP4 treated cell groups, including some trophectoderm-specific genes (GATA3 and GCM1) and TB-specific genes (CGA, CGB, and syncytin2). Cluster analysis and Principal Component Analysis revealed that most gene clusters were divided into two groups: hiPSCs and KRT7-positive cells. Comparing our microarray data to the large-scale analysis of human transcriptome, the features of KRT7-positive BMP-4 treated cells were most similar to placenta.

Limitations, reasons for caution: Although the KRT7 positive BMP4-treated hiPSCs are similar to the early stage of placenta in terms of the transcriptomic character, it is difficult to reproduce early placentation because there is no in vivo model to compare with this model.

Wider implications of the findings: The results suggest that some of the genes upregulated in all the BMP4-treated groups might play an important role in early placental development. Further studies are needed to confirm their functions.

Trial registration number: None.

P-785 Topical administration of human umbilical cord-derived mesenchymal stem cells on scaffolds accelerates endometrial regeneration in a rat model of Asherman's syndrome by paracrine effect

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Study question: Can collagen/hu-mscs scaffolds provide a new treatment for Asherman's syndrome?

Summary answer: Transplantation of collagen/hu-mscs scaffolds was established to be effective in promoting endometrial regeneration in a rat model of Asherman's syndrome.

What is known already: In women of reproductive age, severe injuries of endometrium are often accompanied by endometrial scar formation, and a lack of endometrial functional layer leads to infertility or miscarriage. Human umbilical cord-derived mesenchymal stem cells have been confirmed to promote tissue recovery in many organs. Collagen scaffolds have been widely applicated in regenerative medicine because of their abundant source, low immuogenicity, wonderful biocompatibility and degradability.

Study design, size, duration: Collagen/hu-mscs scaffolds were constructed. A total of 154 SD rats were involved and received different treatments. Uteri were examined at 15, 30 and 60 days after surgery. The function of regenerated endometrium was assessed at 60 days afer surgery. A non-contact co-culture system was made to explore the mechanism of hu-mscs on endometrial regeneration in vitro.

Participants/materials, setting, methods: Hu-mscs were purchased from Boyalife Group°Collagen scaffolds were obtained using dry-heat crosslinking method. Hu-mscs were seeded onto degradable collagen scaffolds. The cell-seeded scaffold was transplanted onto the inner uterine surface of a rat intrauterine adhesion model. At 15,30 and 60 days after surgery, uteri were examined by histological analysis. Fertility test was made 60 days afer surgery. The paracrine effect of hu-mscs on endometrial regeneration was established by a non-contact co-culture system in vitro.

Main results and the role of chance: Hu-mscs were mainly located to the functional layer of regenerated endometrium. At 15,30 days after collagen/hu-mscs scaffolds transplantation, the regenerated endometrium expressed higher level of VEGFa, TGF-β, PDGFbb and MMP-2 than the endometrium in collagen group or spontaneous repair group. The collagen/hu-mscs scaffold promoted endometrial regeneration and collagen degradation, induced local endometrial cells proliferation, facilitated microvasculature regeneration, and restored the endometrium's ability to receive embryos. Furthermore, we demonstrated that

hu-mscs promoted human endometrial stromal cells proliferation and inhibited apoptosis through paracrine effect in vitro.

Limitations, reasons for caution: The results of this research are inadequate to apply directly in human because human uterine and rat uterus differ in structure and function. Further large animal experiments, such as monkeys, are needed.

Wider implications of the findings: Transplantation of collagen/hu-mscs scaffold offers a reliable method in the treatment of women with Asherman's syndrome and promote fertility recovery.

Trial registration number: not applicable.

P-786 Germ-like cells generation from human menstrual blood-derived stem cells on a 3-D composite nanofibrous scaffold

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Study question: Can the fabricated 3-D nanofibrous composite scaffolds with retinoic acid trigger human menstrual blood-derived stem cells (MenSCs) to differentiate towards germ-like cells?

Summary answer: The stem cell and germ cell-specific genes expression on transcriptional in differentiated-MenSCs proofed 3-D composite scaffold-seeded MenSCs could enhance germ-like generation.

What is known already: Several factors contributing to infertility can involve men, women or both partners, and in some cases no cause can be identified. Providing a platform to generate functional germ cells in laboratories could have modified the current view on assisted reproduction technologies.

It has been proved that cells often exhibit unnatural characteristics when cultured on a two dimensional (2D) surface as a monolayer in comparison with native three-dimensional (3D) tissues. Therefore, in this study, usefulness of PLA/MWCNTs scaffolds in germ line cell culture by taking advantage of the affinity exhibited by CNTs for MenSCs differentiation to germ-like cells has been examined.

Study design, size, duration: The 3-D wet-electrospun composite scaffolds were designed to compare infiltration, proliferation, and differentiation potential of menstrual blood-derived stem cells toward germ cell lineage with 2D culture. Therefore, isolated MenSCs at a density of $1.5 10^4$ cells/cm^2 in inducing media containing DMEM/F12 supplemented with 10% FBS and 10^{-7}M retinoic acid (RA) on 2D (without scaffolds) and 3D (with fabricated PLA and PLA/MWCNTs scaffolds) culture vessels for 7 and 14 days were differentiated towards germ cells.

Participants/materials, setting, methods: After MenSCs isolation and scaffold fabrication, such assays as SEM, FTIR, Contact angel, Live/dead and MTT was used to evaluate the biocompatibility and properties of the matrix as well as morphology of differentiated-MenSCs. Reverse-transcription PCR (Oct4, C-kit, VASA, DAZL, SYCP3 and STRA8) was used to assess the germ cell development in 3-D engineered bio-scaffolds.

Main results and the role of chance: The results, showed that the porous structure of PLA and PLA/MWCNTs scaffolds provide sufficient dimensions for cell ingrowths. This was evidenced in the SEM of differentiated MenSCs showing excellent attachment, penetration, and aggregation of these cells on the scaffolds where these parameters in the PLA/MWCNTs were better than PLA. These results clearly demonstrated that scaffolds that were used not only provided a suitable support for cell homing but also enhanced proliferation of MenSCs.

The results of stem cell and germ-like cell genes expression showed that MenSCs expressed stem cell and germ-like cell genes in 3D group (PLA, PLA/MWCNTs) as well as 2D group. In this research, merging RA with carbon nanotubes in the PLA/MWCNTs group showed more effect on germ-like cells generation from MenSCs. The results from gene expression and differentiated cells morphology in all groups demonstrate that the CNTs can enhance MenSCs differentiation toward germ-like cells.

Limitations, reasons for caution: Germ-like cells produced by MenSCs in vitro on such favorable 3-D porous microenvironments could provide a continuous supply for gamete generation, but naturally, the clinical application of MenSCs-derived germ cells in humans would require great caution.

Wider implications of the findings: The previous studies showed that generation of mature germ cells in vitro may require complex niches. Due to the appropriate 3-D structure of the new fabricated scaffold, it seems be a powerful microenvironment to support the germ cells generation from MenSCs and may pave way for infertility treatment.

Trial registration number: Not applicable.

P-787 Different dynamic distribution of H3K27ac and H3K4me3 epigenetic active marks at imprinted genes during in vitro primordial germ cells differentiation

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Study question: To elucidate the chromatin dynamics of histone modifications at imprinted genes during primordial germ cell (PGCs) differentiation in mouse.

Summary answer: Imprinted genes present differences in the dynamic distribution of H3K27ac and H3K4me3 histone modification marks compared to whole genome distribution pattern during PGC differentiation.

What is known already: Epigenetic reprogramming plays an important role in germ cell differentiation to guarantee the normal development of epigenetic information transmitted across generations. In fact, it has been pointed out the importance of a global genome demethylation and an optimal chromatin configuration for an efficient reprogramming, among other epigenetic mechanisms. However, some genes, such as imprinted genes, can escape the reprogramming process and still be partially methylated at the end of reprogramming, suggesting the presence of a potential mechanisms important for transgenerational epigenetic inheritance. In spite of that, the chromatin distribution of imprinted genes during PGC differentiation is completely unknown.

Study design, size, duration: Chip-Sequencing analysis of histone active marks (H3K27ac and H3K4me3) and histone repressive mark (H3K27me3) during *in vitro* PGC differentiation. Induction of *in vitro* PGC was carried out form mouse embryonic stem cells (ESCs), to epiblast-like cells (EpiLCs) and finally into primordial germ cell-like cells (PGCLCs) at day 2 and 6 (d2PGCLCs and d6PGCLCs respectively).

Participants/materials, setting, methods: Bioinformatic approaches from Next-Generation Sequencing (NGS). NCBI database (GEO accession number: GSE60204).

Main results and the role of chance: First, we have evaluated the normal pattern of chromatin state dynamics in both active marks and the repressive mark. H3K27ac and H3K4me3 levels showed a reduction from ESCs to EpiLCs and d2PGCLCs, followed by a recovery in d6PGCLCs. In return, the transitions of H3K27me3 during in vitro PGC specification exhibited a small drop from ESCs to EpiLCs, and reached the highest level in d2PGCLCs, to finally recover the low level in d6PGCLCs.

Next, we have analyzed the transitions of these three epigenetic marks more deeply focusing on imprinted genes. As a result, we have found out that, maternally and paternally imprinted genes have different distribution in both active marks, H3K27ac and H3K4me3, but not in the repressive mark, H3K27me3 that seems to maintain the same pattern as the general.

Finally, we have studied the chromatin dynamics of the repetitive elements during PGC differentiation, as these elements also have been described to escape from the demethylation process. Unlike the previous case, we have observed that there is no change in the distribution of these three histone marks in repetitive elements, compared to the general pattern.

Limitations, reasons for caution: To perform the in vitro analysis.

Wider implications of the findings: Maternally and paternally imprinted genes seem to have a different chromatine remodeling pattern in H3K27ac and H3K4me3 active marks, during PGC differentiation. This fact reaffirms imprinted genes as potential candidates to escape the reprogramming process and sheds light on the complex mechanism of epigenetic inheritance.

Trial registration number: Not applicable' for non-clinical trials.

P-788 The synergetic effects of multiwall carbon nanotubes/poly lactic acid 3-D composite nanofibrous scaffold containing naringenin on spermatogenesis

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Study question: Can the fabricated 3-D nanofibrous composite scaffolds with naringenin induct spermatogenesis in the spermatogonial stem cells (SSCs)?

Summary answer: The stem cell and germ cell-specific markers expression on transcriptional and protein in the SSCs proofed successful spermatogenesis induction 3-D composite scaffold-seeded SSCs.

What is known already: The efficiency of spermatogenesis concerns the presence of the Sertoli cell–SSCs niche. This connection between proliferating SSCs and surrounding testicular environment enables them to receive a variety of signals and factors necessary for SSC self-renewal and differentiation. Fortunately, new developments in engineered three-dimensional cultures have helped mimic the structure as well as function of the natural extra cellular matrix to improve cellular behavior of SSCs in vitro. Therefore, in this study, we have incorporated MWCNTs into PLA, with hypothesis that this carbon-based scaffold combined with an antioxidant, naringening, may have enhanced proliferation and differentiation of SSCs compared to 2D-medium.

Study design, size, duration: The 3-D wet-electrospun composite scaffolds were designed to compare proliferation, and differentiation SSCs with 2D culture. Therefore, isolated SSCs at a density of 10^4 cells/cm^2 in inducing media containing DMEM/F12 supplemented with 10% FBS, 50 ng/ml BMP4 and 20 ng/ml GDNF on 2D (without scaffolds and 10 μM naringenin) and 3D (with fabricated PLA/ MWCNTs scaffolds and 10 μM naringenin) culture vessels for 7 and 14 days were differentiated towards germ cells.

Participants/materials, setting, methods: After SSCs isolation and scaffold fabrication, such assays as SEM, FTIR and MTT was used to evaluate the biocompatibility and properties of the matrix as well as morphology of differentiated-SSCs. Reverse-transcription PCR (PLZF, C-kit, ID4 and SYCP3) and immunocytochemistry (PLZF, C-kit and ID4) were used to assess the germ cell development in 3-D engineered bio-scaffolds. Also, reactive oxygen species (ROS) was used to asses ROS amount in 2D and 3D groups.

Main results and the role of chance: The results showed that the porous structure PLA/MWCNTs scaffolds provide sufficient dimensions for cell ingrowths. This was evidenced in the SEM of differentiated SSCs showing excellent attachment, penetration, and aggregation of these cells on the scaffolds. These results clearly demonstrated that scaffolds that were used not only provided a suitable support for cell homing but also enhanced proliferation and differentiation of SSCs.

The results of stem cell and germ cell markers expression showed that SSCs expressed stem cell and germ cell genes in 3D group (PLA/MWCNTs) as well as 2D group. In this research, merging naringenin with carbon nanotubes in the PLA/MWCNTs group showed more effect on germ cells generation from SSCs. The results from gene and protein expression and differentiated cells morphology in all groups demonstrate that the CNTs can enhance SSCs differentiation toward germ cells. Also, ROS level was less in 3D group in comparison with 2D group.

Limitations, reasons for caution: Germ cells produced by SSCs in vitro on such favorable 3-D porous microenvironments could provide a continuous supply for gamete generation but the functionality of germ cells needs to be investigated by other methods such ART.

Wider implications of the findings: The previous studies showed that generation of mature germ cells in vitro may require complex niches. Due to the appropriate 3-D structure of the new fabricated scaffold, it seems be a powerful microenvironment to support the germ cells generation from SSCs and may pave way for infertility treatment.

Trial registration number: Not applicable.

P-789 Imprinting status of human parthenogenetic embryonic stem cells: analysis of 63 imprinted genes expression levels in two undifferentiated and early differentiated stages

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Study question: What is imprinted genes expressive status of human pathenogenetic embryonic stem cells (hPESCs)?

Summary answer: Human parthenogentic embryonic stem cells show a substantial degree of epigenetic stability with respect to imprinted genes.

What is known already: Human parthenogenetic embryonic stem cells represent a source of histocompatible tissues for transplantation and thus their use avoids immune rejection and ethical obstacles. Human parthenogenetic embryonic stem cells carry two copies of the maternal genome and lack a paternal genome. Given that the disruption of imprinting is associated with human diseases, there are main concerns about imprinted genes expressive status of human parthenogenetic stem cells and the influence of aberrant imprinted genes on stem cell derivative.

Study design, size, duration: The expression levels of imprinted genes were compared between human parthenogentic embryonic stem cells and human biparental embryonic stem cells. Two undifferentiated human parthenogentic embryonic stem cell lines were cultured in vitro and digested for passaging every 5 days. For early differentiation and collection of embryonic body, human parthenogetic embryonic stem cells and human biparental embryonic stem cells were cultured in the medium with RA for two weeks.

Participants/materials, setting, methods: We investigated the expression profile of all 63 genes that are known to be human imprinted (27 maternally and 36 paternally expressed) in two undifferentiated human parthenogentic embryonic stem cell lines by quantitative real Time reverse transcription-PCR and the change of imprinted genes' expression were compared with the result of two human embryonic stem cell lines (hESCs). Four DMRs of different expressive imprinted genes were detected in undifferentiated hPESCs and hESCs by bisulfite sequencing.

Main results and the role of chance: All the maternally expressed genes were expressed at similar levels in both human parthenogentic embryonic stem cell lines and in human embryonic stem cell line except ZNF264 and ATP10A. 21 detected paternally expressed genes were expressed at the same levels in two human parthenogentic embryonic stem cell lines as in the human embryonic stem cell line, whereas 15 paternally expressed genes were significantly downregulated or inactivated. During prolonged passage, the expression levels of the majority of imprinted genes remained stable in two human parthenogentic embryonic stem cell lines. PEG3/ZIM2 DMRs, SNURF/SNRPN DMRs and KVDMR1 DMRs are highly methylated in two early passages (p27) undifferentiated human parthenogentic embryonic stem cells and its embryonic bodies, whereas DMRs in early passages (p27) undifferentiated human embryonic stem cells and its EBs are half methylated, and clones are almost completely methylated or demethylated. Comparing with human embryonic stem cells, two human parthenogentic embryonic stem cell lines showed the same imprinted genes expressive trending in early differentiation stage. The expression levels of H19, IGF2, SLC22A2, SLC22A3/SLC22A18 and CPA4 were both significantly up-regulated in early differentiation process of human parthenogentic embryonic stem cells and human embryonic stem cells.

Limitations, reasons for caution: The limitation of this study is imprinted gene expression levels were only detected in undifferentiated and early differentiated hPESCs. The aberrant imprinted gene status was not detected in the

derivative of hPESCs and their influence on hPESCs differentiation and derivate cells function were still unknown.

Wider implications of the findings: This study quantitatively analyzed the expression patterns of imprinted genes of undifferentiated and early differentiated stage hESCs from parthenogenetic embryos. To our knowledge, this is by far the most wide-range study on survey imprinting profile of hPESCs and evaluating epigenetic status of hPESCs from the aspect of imprinting genes expression.

Trial registration number: not applicable.

P-790 human embryonic stem cells carrying an unbalanced translocation demonstrate impaired differentiation capacity

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Study question: whether human embryonic stem cells carrying unbalanced translocation can be used as a model to analyze the development potential of unbalanced translocation embryos?

Summary answer: HESC lines carrying unbalanced translocation after preimplantation genetic diagnosis (PGD) are excellent in vitro model of partial monosomy and/or trisomy for early embryonic development.

What is known already: Human embryonic stem cells (hESCs) have the ability of self-renewal and multiple differentiation potential. Unbalanced translocation was known as one of the common structural chromosomal defects involved in abnormal embryogenesis. Differentiated translocated hESCs were reported that impaired expression of the trophoblastic genes may be associated with implantation failure.

Therefore, Unbalanced hESCs can serve as a tool for studying chromosome partial segments abnormalities linked to early stages of embryonic development.

Study design, size, duration: Six HESC lines were established from PGD embryos carrying unbalanced translocation. Subsequently, we aimed to indentify the biological characteristics and genetic stability of such embryonic stem cells. We further analyzed the consequences of copy number variation involved in embryonic developmental potential.

Participants/materials, setting, methods: A total of nine hESC lines were used, including three controls. HESC lines exhibit hallmarks of pluripotency, pluripotency-associated gene expression was evaluated by RT-PCR and pluripotency markers were assessed by immunocytochemistry. The differentiation capability was demonstrated by teratoma formation in vivo and embryoid body (EB) formation in vitro. Genetic stability of hESC lines was evaluated using karyotype analysis, fluorescence in situ hybridization(FISH) and Next Generation Sequencing(NGS). RNA-seq was used to compare differentially expressed gene profiles.

Main results and the role of chance: Six abnormal hESC lines were established from eleven blastocysts carried an unbalanced chromosomal defect. All hESC lines expressed the pluripotency-related genes such as POU5F1, SOX2, NANOG, KLF4, TERF1 and THY1 and stained positive for pluripotency markers POU5F1, NANOG, TRA-1-60 and TRA-1-81. Next Generation Sequencing confirmed that genome exactly matched between embryo and hESCs. Moreover, these cell lines maintained a stable karyotype of the donors during long-term culture. RNA-seq results identified that the expression level of gene was significant changes in chromosome imbalances hESC lines compared to normal hESCs. The genes expression level of partial chromosome trisomy or monosomy was upregulated or downregulated. Only three of hESC lines could differentiate into all three germ lineages. For teratoma formation and analysis, normal hESCs generated teratomas in a shorter time than unbalanced hESCs. During homogenous embryoid body differentiation, the efficiency of unbalanced hESCs aggregate formation was lower compared to normal hESCs. In conclusion, affected differentiation capacities and cell apoptosis in differentiating embryoid bodies may be caused by gene expression dosage imbalance.

Limitations, reasons for caution: Although we revealed that the embryonic development potential was impaired by CNV, the dosage imbalances of chromosomes and reduce of key gene expression may lead to lethality at early stages of human embryonic development. The underlying mechanism for early embryonic arrest or miscarriages has not been fully investigated.

Wider implications of the findings: Current research models for determining the cause of implantation failure are still very limited. These cell lines will help us to explore developmental several aspects of translocated embryos. Moreover, the effects of partial monosomy or trisomy on human development and birth need to further search.

Trial registration number: No application.

P-791 Homing of mouse spermatogonial stem cells on acellular testis following in vitro transplantation

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Study question: Could acellular testis support homing of mouse spermatogonial stem cells after in vitro transplantation?Could acellular testis support homing of mouse spermatogonial stem cells after in vitro transplantation?

Summary answer: In vitro transplantation of mouse spermatogonial stem cells in to acellular testis and two week organ culture could initiate in vitro spermatogenesis.

What is known already: Vermeulen et al reported culturing of Sertoli cells with testicular scaffold could increase the proliferation rate of the cells.

Baert et al reported that primary adult and prepubertal human testicular cells can assemble to testicular organoid with and without support of natural testicular scaffold.

Study design, size, duration: In this study we had two groups. Control group: without transplantation of Spermatogonial stem cells. Experimental group: with transplantation of Spermatogonial stem cells. For each group, 5 NMRI male mice were used at the age of 8 weeks for decellularization. Spermatogonial stem cells isolated from 6 NMRI neonate male mice, then injected to acellular testis and cultured for 2 weeks.

Participants/materials, setting, methods: Testes decellularized by 0.05% SDS for 24 h+0.05% Triton X-100 for 24 h, then were washed with 1× PBS for 24 h, and disinfected using 0.1% peracetic acid in 4% ethanol for 2 h, rinsed three times with sterile 1× PBS for 4 hours each. Spermatogonial stem cells isolated from neonate testis with twice enzymatic treatment, injectod to acellular testes and cultured on agarose gel for two weeks. Finally, immunohistochemistry and molecular studies were done.

Main results and the role of chance: Immunohistochemically studies in the control and experimental group express *PLZF* proteins in spermatogonial cells. Also, in molecular studies of *PLZF*, the results indicated that this genes was positive in the experimental group, while not in the control group.

Limitations, reasons for caution: If we want to use the result of this research in clinic, we need to be able to decellularize human whole testis for injection of spermatogonial stem cells. Decellularization human whole testis is difficult and need set up new protocol.

Wider implications of the findings: Our result showed that natural testicular scaffold could support of spermatogonial stem cells during 2 week culturing. These results in agreement with literature that reported testicular scaffold could increase proliferation of Sertoli cells and support of auto assembly testicular organoid.

Trial registration number: 0

P-792 Are we losing valuable reserves by discarding post-TESE (testicular sperm extraction) material? Analysis of the quantity and distribution of spermatogonial stem cells (SSCs) after TESE

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Study question: Assuming the relationship between IVF success and the quantity of SSCs in discarded-TESE material, should we consider post-TESE tubules as future sources of SSCs?

Summary answer: SSCs are found in post-TESE tubules which is routinely discarded. There is a statistically significant relationship between SSEA4 positivity and pregnancy rate.

What is known already: SSCs differentiate to mature spermatozoa by a process called spermatogenesis, during which stage-specific proteins play roles and can be used to evaluate the differentiation mechanisms. Spermatozoa from azoospermia patients can be obtained via surgical methods like TESE. Following TESE, not only operated region of testis heals with fibrosis causing a loss in sperm production but also remaining parts of the isolated seminiferous tubules, which may harbor SSCs, are discarded after collection of spermatozoa. The questions that what we lose after TESE in terms of SSCs and if there is a correlation between amount of SSCs and IVF success still stand.

Study design, size, duration: After ethical board approval, materials were obtained from 45 non-obstructive azoospermia patients who applied for a governmental IVF-center between 2015 and 2017. After TESE operation and the collection of spermatozoa for IVF treatment, remaining seminiferous tubules to be discarded were collected for this study. Outcomes of IVF including, fertilization, embryo development and pregnancy rates were obtained from the IVF center to evaluate the relationship between IVF success and analyzed parameters.

Participants/materials, setting, methods: Obtained tissue materials were stained with antibodies that were produced against DDX4, MAGEA4, SSEA4 and Oct4 proteins which are used to determine various differentiation steps of spermatogenesis and considered as SSCs markers. The distribution and intensity of those markers were evaluated by routinely used histological scoring techinquetechnique (H-SCORE). The correlation between IVF outcomes and H-SCORE analysis were evaluated by appropriate statistical tests. Values were presented as mean±S.D.

Main results and the role of chance: Cytoplasmic MAGEA4 and SSEA4 expressions at the basal compartments of seminiferous tubules indicated the presence of spermatogonia, while the strong juxta-luminal and mid-basal expression of DDX4 demonstrated the presence of spermatocytes and spermatids. Cytoplasmic expression of Oct4 was also detected in spermatogonia and spermatocytes but no evidence of expected nuclear expression was encountered. Since the IVF center indicated that spermatozoa were found from 27 of total 45 patients for ICSI, hereupon these groups were considered as Sp(+) and others Sp(-). First, H-SCORE outcomes of Sp(+) patients (n = 27) were compared with Sp(-) (n = 18) ones, and found that albeit outcomes of DDX4 staining in Sp(+) parameter reach to significant levels. Considering the pregnancy rates, 9 of the Sp(+) patients resulted with pregnancy, Pr(+) and only SSEA4 outcomes reach to statistically significant levels (121 ± 51) compared to Pr(-) (n = 16) ones (74 ± 49), p = 0.048. The expression pattern of germ cell markers suggested the possible chance of spermatogenesis failure at different stages of spermatogenesis within the seminiferous tubules of Sp(-) samples, whereas the H-Score values showed no significant difference of germ cell presence among the groups.

Limitations, reasons for caution: A comparison between obstructive and non-obstructive cases would be informative to understand the SSCs reserve in spermatogenesis and possible relationship with IVF success. Since seminiferous tubules show high variations in terms of positivity of SSCs markers, similar studies with higher case numbers will decrease the role of chance factor.

Wider implications of the findings: This study highlights the requirement of future studies aiming the isolation, freezing and also in-vitro maturation of SSCs particularly in oligo/astheno/azoo-spermia patients who may lose their SSCs reserve by a surgical application, TESE, and also concludes that testicular reserve needs more attention for IVF treatments like ovarian reserve.

Trial registration number: N/A.

P-793 Characterization of human granulosa progenitor cells as a potential source for the treatment of chemotherapy-induced ovarian damage

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Study question: Can human granulosa progenitor cells which derived from human mural granulosa iPSCs (GiPSCs) restore chemotherapy-induced ovarian damage and what's the underlying molecular mechanism?

Summary answer: Human granulosa progenitor cells which derived from human mural granulosa iPSCs, may restore damaged ovarian function through the Paracrine pathway.

What is known already: Young female patients who received chemotherapy frequently face premature ovarian failure (POF), for which there were currently no ideal treatments or medications. Furthermore, apoptosis of ovarian granulosa cells were an important mechanism underlying the decline in ovarian reserve and function which can be effectively alleviated through some kinds of stem cell transplantion.

Study design, size, duration: Mice ovaries were injured with cyclophosphamide to create a damaged ovary mice model. Transplanted GiPS-derived granulosa progenitor cells were injected into the tail vein of sterilized mice (n = 20), or cell culture medium was injected into the sterilized mice via the tail vein as cell-media group (n = 20). Nonsterilized mice were untreated controls (n = 20). After 1 month,ovarian tissue weight,plasma E2 level,and the number of follicles was compared in all groups.

Participants/materials, setting, methods: Human mural granulosa iPSCs were reprogrammed from mural granulosa cells collected from egg follicles retrieved from women undergoing infertility treatment using Sendai viral vectors.Then we used two step approaches comprising in vitro treatments with cocktails of growth factors (Wnt 3a,ActivinA,BMP4,ect) for 12days.After passaged 3 times,we obtained the granulosa progenitor cells(GPCs). Expression of granulosa cell, ES markers and metabolic culture medium were analyzed.These cells and media were injected into the tail vein of sterilized mice.

Main results and the role of chance: GiPS-derived granulosa progenitor cells expressed OCT4, but not GC lineage markers. Also are capable of clonogenic self-renewal and extensive proliferation *in vitro* and could differentiate into neural or mesenchymal cell lineages, as well as GCs, with the ability to produce E2 from testosterone, under defned conditions. After transplanted into the POF models 1 month,the Ovarian tissue weight,plasma E2 level,and the number of follicles were all significantly higher in cell treated group compared with chemoablated group,but approached the same effect between cell and cell media group. After using human cytokines array to analyze the cell media,revealed that many key cytokines were in high level, participating in a variety of biological processes including apoptosis, angiogenesis, cell cycle and immune response. These results suggested that GiPS-derived granulosa progenitor cells may not only effectively enhance granulosa cells growth and repair damaged ovarian tissue, but regulating the ovarian tissue niche through the Paracrine pathway.

Limitations, reasons for caution: Although GiPSCs-derived granulosa progenitor cells did not induce teratoma formation in vivo in the POF mice,the undifferentiated iPSCs are still potential risk in clinical trial in future.

Wider implications of the findings: Our research results showed that GiPSCs-derived granulosa progenitor cells can repair ovarian injury, stimulate regeneration, and improve ovarian function. GiPSCs-derived granulosa progenitor cells transplantation may provide an effective and novel method for treating POF.

Trial registration number: not applicable.

P-794 Feasibility of CRISPR-Cas9 on the human sperm cell

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Study question: What are the optimal transfection parameters to ensure viability of spermatozoa in a CRISPR-Cas9 protocol?

Summary answer: Among all different parameters tested, 1100 V, 20 ms, 1 was the optimal set to successfully transfect CRISPR-Cas9 plasmid in human spermatozoa retaining their motility.

What is known already: The CRISPR-Cas9 system recently made headway in reproductive medicine by a group of researchers that used viable human embryos to correct a mutation that causes hypertrophic cardiomyopathy, a severe heart condition in humans. Through intracytoplasmic sperm injection (ICSI), the researchers injected CRISPR-Cas9 solution and spermatozoa

simultaneously into an egg. However, there is currently no research done on using the CRISPR-Cas9 tool directly on human sperm. Importantly, to date, no known transfection parameter optimal for human sperm cells has been described, especially when compared to various known transfection parameters utilized for somatic cells.

Study design, size, duration: Our aim is to find the optimal transfection parameters for human spermatozoa that can ensure the highest viability and motility of cells after exposing them to high voltage pulses. To meet this goal, we designed a unique 24-well optimization protocol, in which 24 different sets of transfection parameters were tested. We plan to use CRISPR-Cas9 on sperm cells to knockout LAMA1, a gene that is found to be upregulated in male infertility patients.

Participants/materials, setting, methods: As a preliminary experiment, we transfected (Neon Transfection System, Invitrogen) embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) grown in vitro with Oct3/4 CRISPR-Cas9 knockout plasmid utilizing a GFP reporter gene. Successful transfection was the absence of GFP expression. We then began our first attempt using human sperm cells and evaluated different sets of parameters ranging from 800 V, 27 ms, 5 to 1700 V, 20 ms, 1.

Main results and the role of chance: The results of our preliminary experiment using ESCs and iPSCs were highly successful as they matched the threshold of 79% transfection efficiency found in prior research with DNA transfection of stem cells. Oct3/4 expression was remarkably decreased by 81 ± 2% after transfection when compared to the initial expression.

As for our experiment with human spermatozoa, we used a donor sperm sample with initial concentration of 70 ± 6×10^6/ml and a motility of 40.3 ± 4%. Following density gradient, concentration became 22 ± 11×10^6/ml and motility 86.2 ± 3%, respectively. The highest motility after transfection was only 5.9 ± 3%. Moreover, because spermatozoa were further diluted after transfection, the concentration decreased to 0.03 ± 7×10^6/ml.

Going forward, we plan to use semen samples with higher concentration and motility to obtain a higher motile fraction after density gradient. After transfection, we intend to isolate the motile portion from the immotile by microfluidics to avoid the decaying cells' affect on viability of the motile sibling spermatozoa due to leakage of acrosomal enzyme and production of reactive oxygen species (ROS). In this way, we can allow more time for plasmids to execute the double-stranded break and for the DNA to repair itself while retaining cell viability.

Limitations, reasons for caution: The execution of transfection experiments on spermatozoa dealt with the structural peculiarity and the innate kinetic characteristics of this cell, which may have affected the accuracy and reproducibility of our analysis. However, utilization of CRISPR-Cas9 on human spermatozoa may ease qualms related to the direct genetic manipulation of embryos.

Wider implications of the findings: Our results of finding motile spermatozoa after exposing them to high voltage pulses through electroporation gives hope that correcting mutations in human sperm cells is possible. Genetic manipulation on the male gamete may prove more reliable in granting the desired effect on the resulting conceptus.

Trial registration number: N/A.

P-796 Intrauterine infusion of autologous peripheral blood mononuclear cells in frozen blastocyst transfer improves clinical outcome and metabolomic profile in repeated implantation failure- A pilot study

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Study question: Do autologous peripheral blood mononuclear cells (PBMC) improve endometrial receptivity and clinical pregnancy in frozen embryo transfer (FET) in women with repeated implantation failure (RIF)?

Summary answer: Infusion of PBMC improves clinical pregnancy and live birth rate in RIF, which could be associated with down-regulation of metabolites like tryptophan, valine and lysine.

What is known already: Despite advancement of IVF protocols and assessment of embryo morphokinetics, methodologies continue to strive to optimize IVF success in management of RIF. Autologous PBMC is a convenient source of adult stem cell; usage being to increase implantation potential of embryos and increase in endometrial receptivity. Recent studies document metabolic profiling as a promising platform for assessment of endometrial receptivity. Furthermore, infusion of stem cells into spiral arterioles of patients with refractory Asherman's syndrome has shown improvement in implantation rate. In view of these facts, use of PBMC in the management of RIF holds a promising tool for the future.

Study design, size, duration: Prospective study from January 2016 to January 2017; 160 patients with ≥ 3embryo transfer (ET) failure (fresh and frozen) undergoing day5 FET were divided into Group A (n = 73; intrauterine infusion of PBMC) and B (n = 87; without PBMC infusion). Mononuclear cells were used as a source of stem cell, isolated from patient's peripheral blood by density gradient centrifugation using commercially available lymphoprep. Main outcome measures were: implantation rate, clinical pregnancy rate and live birth rate.

Participants/materials, setting, methods: All patients received estradiol valerate 2 mg thrice daily from d2 onwards. Intra-uterine infusion of PBMC was done on D5 of cycle in Group A. Once endometrial thickness reached ≥7 mm, progesterone was administered for five days. Blood samples were collected on day of embryo transfer. Samples were analyzed using 700-MHz NMR spectrometer and acquired spectra were subjected to chemometric and statistical analysis. $p< 0.05$ was considered to be significant as evaluated by chi-square and t-test.

Main results and the role of chance: Baseline clinical parameters and number of embryos transferred were found to be comparable in both groups. A significant increase ($p< 0.01$) in implantation rate was observed in group A (23.97%) as compared to group B (9.2%). Clinical pregnancy rate was found to be significantly higher ($p <0.05$) in group A (38.36%) versus group B (17.24%). Simultaneously, live birth rate in group A (24.66%) was significantly higher than Group B (11.49%). Miscarriage rate was higher in group A, difference being non significant. Valine, tryptophan, and l-lysine, were found to be significantly down-regulated ($p< 0.03$, $p<0.01$; $p<0.02$) with fold change values of 0.81, 0.76, 0.74 respectively in women with RIF after PBMC infusion when compared with those without administration of PBMC. However, when metabolomic markers were evaluated between pregnant and non-pregnant subjects in either group the difference was not statistically significant. Since the interplay between these molecules in RIF is complex, study holds merit for further exploration.

Limitations, reasons for caution: Owing to application of PBMC in FET cycle, we cannot evaluate the potential receptivity markers in endometrium to ascertain the immunomodulatory role of PBMC for successful blastocyst implantation. In-depth studies of the arginine metabolic pathway in endometrial tissues seem necessary to validate our findings.

Wider implications of the findings: Intra-uterine PBMC infusion can be relatively inexpensive and minimally invasive treatment for patients with RIF; simultaneously it may be beneficial in recurrent pregnancy loss aiming to improve endometrial receptivity. We speculate that serum metabolomics can provide an improved understanding of increased clinical pregnancy after PBMC infusion in women with RIF.

Trial registration number: Not applicable.

P-797 Proteomic profiling of the exosomes released by endometrial mesenchymal stem cells: possible relations with embryo development and implantation

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Study question: Is the proteomic profile of exosomes derived from human endometrial mesenchymal stem cells (exo-endMSCs) associated with embryo development and implantation?

Summary answer: The bioinformatics analyses of exo-endMSCs have identified exosomal proteins that are involved in the maternal-embryo cross-talk influencing the embryo's developmental competence and subsequent implantation.

What is known already: Implantation failure is the most common cause of recurrent pregnancy loss in humans who resort to assisted reproduction technologies (ARTs). Among others, hormonal or metabolic disorders, advanced age, immunological factors, infections or uterine abnormalities can affect endometrial receptivity. In addition, diminished embryo developmental competence due to any of these conditions increases the likelihood of implantation failure. The cross-talk between the endometrium and the embryo is mediated by paracrine factors delivered by the exo-endMSCs, but a complete description of the proteomic profile of these microvesicles has not been reported yet.

Study design, size, duration: Endometrial Mesenchymal Stem Cells (endMSCs) were isolated from menstrual blood of healthy women (n = 2). Supernatants from these cells were collected every 72 hours. The collected supernatants were enriched and purified to isolate the released exosomes.

Participants/materials, setting, methods: The endMSCs were *in vitro* expanded and characterized by flow cytometry. The exo-endMSCs were isolated and characterized by nanoparticle tracking analysis and flow cytometry. Then, protein extracts from exo-endMSCs were processed by high-resolution liquid chromatography coupled to mass spectrometry-based proteomic analyses (HPLC-MS). Finally, the functional enrichment analysis was performed by using the DAVID functional annotation database (https://david.ncifcrf.gov/).

Main results and the role of chance: EndMSCs analysis did not demonstrate positive staining for CD34, CD45 or HLA-DR while they expressed the CD29, CD56, CD44, CD73, CD90, CD105 stemness markers. The mean size of the isolated exo-endMSCs was 153.5 ± 63.05 nm (mean ± SD) and showed a positive expression of the exosome-related proteins CD9 and CD63. A total of 714 proteins (with more than two peptides per protein at 1% FDR) were identified in the exo-endMSCs of both cell lines.

Among the 714 selected proteins, 517 of them were associated to the Gene Ontology term *extracellular exosome* (GO:0070062, $p < 0.01$, 5% FDR) and 60 of them were present in the 100 top identified proteins in ExoCarta database.

Moreover, exo-endMSCs proteins were connected to several biological processes and molecular functions related to embryo developmental competence and implantation, such as *cell-cell adhesion* (n = 55 in GO:0098609), *extracellular matrix organization* (n = 57 in GO:0030198), *cadherin binding involved in cell-cell adhesion* (n = 65 in GO:0098641), and also to the Reactome pathway *ECM proteoglycans* (n = 35 in R-HSA-3000178).

Finally, it is worth mentioning that 11 exosomal proteins have been previously identified in endometrial cells extracts from women during the peri-implantation phase.

Limitations, reasons for caution: The microvesicles used were obtained from 2 donors showing differing protein profiles. Increasing the number of endMSC lines will help to fully identify the proteome of exo-endMSCs. Further studies involving *in vitro* implantation assays and embryo transfers coupled to exo-endMSCs are required to clarify their importance as ARTs coadjutants.

Wider implications of the findings: This study demonstrated that exo-endMSCs may be involved in the embryo-maternal cross-talk. Several proteins from exo-endMSCs are also released by endometrial cells and involved in embryo implantation and development. These proteins could be considered predictive biomarkers for implantation and exo-endMSCS could be used as coadjutants during *in vitro* embryo culture.

Trial registration number: Not applicable.

P-798 Differentiating mouse induced pluripotent stem cells into male germ cells through embryoid bodies

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Study question: We assess the effectiveness of the hanging drop method with specific growth factors to differentiate induced pluripotent stem cells into male germ cells.

Summary answer: Embryoid bodies (EB) supplemented and maintained in hanging droplets (HD) containing specific differentiating factors generated a high proportion of mitotic germ cells.

What is known already: Many methods have been proposed to coax stem cells into male germ cells. One simple method to induce germ cell differentiation utilizes the formation of EBs, three-dimensional structures that create microtissues which simulate *in vivo* conditions. While EBs can spontaneously differentiate into various cell types without external influence, specific growth factors can be added in order to reproduce *in vivo* cell-signaling processes and ensure differentiation of a higher proportion of stem cells into germ cells.

Study design, size, duration: Over a four-month period, 100 EBs were formed from mouse induced pluripotent stem cells (*mi*PSC) using the hanging drop method. EBs were maintained in HDs after formation and differentiated in a 2-step culture system, each with a specific medium composition. Expression of PGC markers was assessed by immunofluorescence staining.

Participants/materials, setting, methods: Prior to differentiation, *mi*PSCs were maintained on mouse embryonic fibroblast (MEF) cells. Once confluent, colonies were trypsinized. HDs were formed from 25uL droplets containing *mi*PSCs at a concentration of 1×10^5 cells/ml. Step one medium contained Activin A, bFGF, and KSR. After 3 days, EBs were transferred to HDs containing step two medium composed of LIF, BMP4, BMP8b, SCF, and EGF. On day 8, EBs were treated with collagenase and stained for OCT4, VASA, and DAZL.

Main results and the role of chance: After the development of 100 EBs in their respective HDs, EBs were re-plated on day 3 to corresponding HDs containing step two medium. EBs were disaggregated through the usage of collagenase IV in order to isolate cells for immunofluorescence staining. In order to identify the stage of *in vitro* spermatogenesis achieved, OCT4, VASA, and DAZL expression was compared between EBs cultured in the 2-stage growth factor-conditioned medium system and control EBs maintained in standard culture medium. Of the isolated cells, 86% were strongly positive for OCT4 compared to 54% of cells isolated from control EBs cultured without differentiating factors. Additionally, EBs cultured with differentiating factors yielded 31% VASA-positive cells, whereas control EBs only yielded 1% VASA-positive cells. Furthermore, EBs cultured with differentiating factors yielded 4% DAZL-positive cells, whereas control EBs only yielded 1% DAZL-positive cells. The retained expression of OCT4, along with the presence of VASA-positive and DAZL-positive cells, strongly indicate that the cells reached the spermatogonia/spermatocyte cell stage. Cells that were positive for VASA and DAZL without OCT4 expression also suggest that a fraction of cells were able to progress to the advanced meiotic stages of *in vitro* spermatogenesis.

Limitations, reasons for caution: This preliminary study only attempts to achieve early stages of differentiation in *mi*PSC. One possible direction can be to use retinoic acid as a third-step culture medium to coax PGCs into meiosis. Furthermore, for this to be applicable for clinical use, trials need to be confirmed in human iPSC.

Wider implications of the findings: Artificial gametogenesis is a developing technology that will ultimately allow azoospermic men to develop spermatozoa from their stem cells. Mouse iPSC provide a step towards raising genotyped cells suitable for clinical treatment for patients with spermatogenic arrest or even germ cell aplasia.

Trial registration number: N/A.

P-799 In vitro generation of germ cells from human induced pluripotent stem cells

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Study question: Can we differentiate human induced pluripotent stem cells (hiPSC) into male germ cells using the hanging drop (HD) culture system?

Summary answer: The culture of hiPSC within HDs containing several growth factors generated male germ cells of different stages of development.

What is known already: Several monolayer culture systems have been suggested for differentiating stem cells into male germ cells. However, the natural three-dimensional (3D) condition for the cells within a tissue allows them to establish important connections among each other. The culture of stem cells within embryoid bodies (EBs) in HDs has been proposed as a simple method to simulate the in vivo 3D environment. In fact, we have previously described the use of EBs within HDs containing specific factors to successfully differentiate mouse embryonic stem cells and mouse iPSC into male germ cells.

Study design, size, duration: Over a three-month period, 50 EBs were formed from hiPSC using the hanging drop method. EBs were maintained in HDs after formation and differentiated in a 2-step culture system, each with a specific medium composition. Expression of germ cell markers was assessed by immunofluorescence staining.

Participants/materials, setting, methods: HiPSCs were originally maintained undifferentiated on fibronectin in the presence of growth factors and ROCK inhibitor. Colonies were trypsinized and HDs were formed from 25uL droplets containing hiPSCs (1×10^5 cells/ml). In the first three days, the medium was composed of Activin A, bFGF, and KSR. Then, the medium of HDs was modified to contain LIF, BMP4, BMP8b, SCF, and EGF. On day 12, cells were stained for OCT4, VASA, DAZL, and BOULE.

Main results and the role of chance: On day 12, and after culturing 50 EBs for 3 days in step-1 medium followed by 9 days in step-2 medium, collagenase IV was used to disaggregate EBs. Cells were isolated and stained for OCT4, VASA, DAZL, and BOULE to determine the stage of in vitro spermatogenesis. The staining of EBs was compared to those of undifferentiated hiPSC, which were 84% positive for OCT-4 but negative for VASA, DAZL, and BOULE. EBs cultured with differentiating factors yielded 75% OCT-4-positive cells, 60% VASA-positive cells, 40% DAZL-positive cells, and 5% BOULE-positive cells. The detection of VASA–positive and DAZL–positive cells that preserved the expression of OCT-4 is a strong evidence of the differentiation of hiPSC to spermatogonia/spermatocyste cell stage. In addition, the presence of cells expressing VASA, DAZL, and/or BOULE while they were negative for OCT-4 suggests an advanced differentiation of hiPSC reaching meiotic stages.

Limitations, reasons for caution: Despite the encouraging preliminary data, this study did not confirm the completion of in vitro spermatogenesis and attainment of post-meiotic stages. A potential strategy to overcome this limitation would be the addition of retinoic acid as a third-step culture medium to coax primordial germ cells into meiosis.

Wider implications of the findings: In vitro gametogenesis is an important technology that provides an option for azoospermic men to develop spermatozoa from either their stem cells or skin fibroblasts. HiPSCs could potentially help azoospermic men who fail testicular sperm extraction due to spermatogenic arrest or germ cell aplasia to have biological children.

Trial registration number: N/A.

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