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ORAL PRESENTATIONS

Monday, 06 July 2020

(*continued overleaf*)

Tuesday 07 July 2020

Wednesday 08 July 2020

- **INVITED SESSIONS**
- **SELECTED ORAL COMMUNICATION SESSIONS**

are 52 patients with grade I-III varicoceles and 52 patients without varicocele that were divided into supplementation or placebo groups.

Participants/materials, setting, methods: In accordance with the randomization schedule, subjects received 2 packets of either supplement or placebo daily for 6 months. Semen parameters were evaluated in a standard semen analysis at the beginning of the treatment (V1) and after completing 6 months of therapy (V2). Pregnancy rate was included as a secondary outcome. The present post-hoc analyses were carried out on the samples as categorized by age/BMI and presence/absence of varicocele.

Main results and the role of chance: One of the primary aims of this study was to correlate the results of the semen analysis with BMI and age. In particular, we wanted to see if aging and obesity status would decrease efficacy of the supplementary antioxidant treatment on main sperm parameters (see Tables 1-5). For BMI, a significant difference was observed in the BMI <25 group with varicocele for total sperm count (p=0.0272) and progressive motility (p=0.0159). No statistical significance was observed in the combined classes. The results were partially confirmed by carrying out the Chi-Square test on the data arranged as "Responder/Non Responder". As for the total sperm count, in both the BMI <25 and the combined varicocele group (i.e. BMI <25 and age <35) a statistical difference was observed (p=0.0066 and p=0.0078 respectively). These post-hoc analyses suggest that the nutritional supplement seems to be more effective in subjects younger than 35 years with a BMI below 25.

Looking at other parameters, patients treated with compounds obtained a statistically significant improve of sperm parameters for the following items: total count, progressive and total motility, morphology.

As a secondary outcome, 12 pregnancies occurred during the follow-up time: 10 in the supplementation group and 2 in the placebo group.

Limitations, reasons for caution: Even as a double-blind placebo-controlled study with very strict inclusion and exclusion criteria, we did not include sperm DNA fragmentation. Also, an oxidative stress measure such as ORP was not included. There also may be other factors besides aging and obesity involved, including lifestyle, associated disease and fat distribution.

Wider implications of the findings: In addition to earlier findings regarding improved sperm parameters in supplemented patients, these post-hoc analyses suggest that antioxidant supplementation seems to be more effective on improving sperm parameters in subjects aged less than 35 years old and with BMI below 25.

Trial registration number: NCT04177667

P-038 New insights into the physiopathology of teratozoospermia and its association with sperm DNA defects, apoptotic alterations and oxidative stress

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Study question: This study set out to determine the level of sperm nuclear DNA damage in patients with isolated poymorphic teratozoospermia and examining its relationship with oxidative stress and apoptosis.

Summary answer: Decreased seminal antioxidant profile may be an important factor involved in the mechanism of sperm cell death-mediated DNA breaks in teratozoospermic semen.

What is known already: Sperm morphological defects is associated with apoptosis.

Study design, size, duration: A total of 89 patients was divided into two groups, men with isolated teratozoospermia (n = 69) and men with normal semen parameters (n = 20) as controls.

Participants/materials, setting, methods: Sperm DNA breaks were evaluated by using acridine orange staining. The proportion of viable spermatozoa with mitochondrial transmembrane depolarization was detected by fluorescence microscopy through the use of MitoPT-JC-1 staining method. Bivariate Annexin V/ 6-CFDA analysis was then carried out to measure the percentage of both viable and dead spermatozoa with phosphatidylserine (PS) externalization. Seminal antioxidant profile (reduced Glutathione (GSHr); Oxidized Glutathione (GSSG); Glutathione-S-transferase (GST)), and total protein sulfhydryl (P-SH) concentrations were measured spectrophotometrically.

Main results and the role of chance: Patient with isolated teratozoospermia, when compared to fertile donors, showed significantly increased level of single sperm DNA breaks, and higher proportions of spermatozoa with phosphatidylserine externalization and mitochondrial depolarisation. Among the different studied oxidative stress seminal parameters, the rates of seminal GSHr, GST and P-SH were significantly decreased in the patient group. However, the seminal levels of GSSG and GST have decreased, but only GST didn't showed a significant difference. Interestingly, significant relationships were found between the studied apoptotic markers and the rate of atypical sperm forms with the incidences of head abnormalities. Furthermore, positive inter-correlations were found between sperm DNA defects, impaired seminal antioxidant profile and the sperm apoptotic markers.

Limitations, reasons for caution: Further combined analysis of oxidative stress, apoptotic markers and nuclear defects should provide complementary measurements for the evaluation of sperm quality and could contribute to provide adequate reproductive and genetic counselling for hypofertile patients with isolated polymorphic teratozoospermia.

Wider implications of the findings: Sperm DNA defects as well as apoptosis and seminal oxidative stress are interlinked in the context of teratozoospermia, and constitute a unified pathogenic molecular mechanism

Trial registration number: not applicable

P-039 In spermatozoa collected after pellet swim up, when total dna fragmentation is higher than 15%, the normal morphologically spermatozoa population shows an increased dna damage.

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Study question: We investigated the DNA Fragmentation Index (DFI) in motile normal morphologically spermatozoa comparing samples with total DFI < 15% Vs ≥ 15% collected after pellet swim up

Summary answer: In the case of DFI ≥15% the percentage of normal morphologically spermatozoa with fragmented DNA is significantly higher than the population with DFI < 15%

What is known already: Intracytoplasmic sperm injection (ICSI) is widely used in the treatment of male infertility. Only morphologically normal spermatozoa are mainly used by embryologists to fertilize an oocyte. Different papers have reported that spermatozoa with apparently normal morphology may have DNA fragmentation. These evaluations suggest that it is possible that normal-shaped spermatozoa but with DNA fragmentation could be easily selected to fertilize oocytes during ICSI. It is known that the presence of an increased proportion of normal spermatozoa with damaged DNA is negatively associated with embryo quality affecting both pregnancy and implantation outcomes after ICSI.

Study design, size, duration: We designed an observational study on 70 male patients. We speculated that the examination of DNA integrity in motile and morphologically normal sperm, collected after pellet swim up, could provide useful information concerning sperm competence, rather than the DFI evaluation in the raw seminal sample. We analyzed data from January 2019 to December 2019. The aim is to demonstrate that DFI in normal morphologically spermatozoa, could be indicated as predictive parameter of ICSI success.

Participants/materials, setting, methods: DFI and traditional semen parameters (WHO, 2010), were evaluated in all patients. DFI was calculated using *in situ* TUNEL assay in at least 250 spermatozoa. By means of NIS-Elements BR 3.10 image analyzer software (Nikon) using images of the same field (light, fluorescence and "merged") it was possible to evaluate sperm morphology associated with DNA fragmentation. Data were analyzed using the Kruskal-Wallis

test, a non-parametric ANOVA, confirmed by restrictive Bonferroni correction using the Dunn's test.

Main results and the role of chance: In this observational study we included 70 oligoasthenospermic patients undergoing ICSI. The patients were classified in 2 groups according to the sperm DFI: Group A (n=35) included those who had a DFI < 15% in the population of sperm collected after swim up. In group B (n= 35) patients with a DFI ≥ 15%. We did not find any statistical difference between the two groups in the traditional sperm parameters like density, motility and morphology.

We observed that, in Group A, the average value of the total of sperm DFI was 9.32% while in Group B was 24.71 % (p< 0.0001). When the analysis was restricted only to spermatozoa with normal morphology, it was observed that among patients of Group B the DFI value was 13.6%, while in A Group the average DFI value was 2.2%, with a strong statistical difference (p<0.0001). DFI calculated on motile, normal morphologically spermatozoa can provide an important information on the probability and risk of injecting, during ICSI procedure, a sperm with normal morphology but with fragmented DNA. This risk is higher if the sperm population collected after pellet swim up has a DFI higher than 15%.

Limitations, reasons for caution: This type of analysis only provides a prediction to select a sperm with fragmented DNA, but does not allow the selection of single spermatozoa with intact DNA to be used for ICSI. Further studies are needed to correlate these data with the clinical outcome.

Wider implications of the findings: Our results suggest that the evaluation of DFI in morphologically motile normal sperm selected after pellet swim up appears to be a more accurate strategy to evaluate the sperm competence, with the aim to improve the ICSI outcomes, than the traditional evaluation of sperm DFI in the whole seminal sample.

Trial registration number: not applicable

P-040 The impact of motility, morphology and presence of testicular spermatozoa on fertilization, embryo development and live birth rates, in fresh and frozen testicular samples

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Study question: Does cryopreservation or the quality parameters of testicular spermatozoa in fresh or frozen samples have an impact on fertilization rate, embryo development and live birth rate?

Summary answer: Although live birth rate (LBR) is not directly associated to any parameters examined, morphology and motility of testicular spermatozoa influence the number of available embryos.

What is known already: Almost 5% of couples undergoing IVF treatments are confronted with azoospermia and are counseled towards TESE-ICSI cycles. At the same time, it has been reported that there is no influence of the use of cryopreserved testicular sperm in fertilization rate and live birth rate and in the presence of motile spermatozoa, high embryo quality and pregnancy rates are expected. Motility of the spermatozoa during the ICSI procedure has been associated to live birth, while other studies claim that motility of either fresh or frozen/warmed testicular spermatozoa is the only parameter associated to ongoing pregnancy.

Study design, size, duration: A retrospective cohort study between 01/2014 and 12/2017 was performed in Embryolab IVF Clinic, Greece, including 108 TESE-ICSI treatment cycles. Logistic regression analysis was used to explore the influence of variables (fresh-frozen/warmed testicular tissue, presence/motility/morphology of testicular spermatozoa) in fertilization rate, embryonic development and LBR. Women above 38 years old, frozen oocyte cycles, PGT cycles and couples with abnormal karyotypes were excluded from the study.

Participants/materials, setting, methods: Morphology, presence and motility were graded as: good/motile(1 grade), average/twitcher(2 grades), low/immotile(3 grades) and the sum of grades represented the total quality score for the testicular spermatozoa used for ICSI. Group A included cases with up to total grade 4, while Group B included cases with total grade 5 or higher. Embryo quality was evaluated up to day 3 (good quality: more than 5 blastomeres, less than 20% fragmentation). LBR was calculated per first transfer.

Main results and the role of chance: Fertilization rate was comparable (p>0.05) among fresh and frozen samples for both group A (fresh: n=24, 67%fertilization rate / frozen: n=33, 62% fertilization rate) and group B(fresh: n=23, 47%fertilization rate / frozen: n=38, 43% fertilization rate), with group A spermatozoa (n=27, 64%fertilization rate) performing significantly better compared to group B spermatozoa (n=91, 51%fertilization rate), p<0.05.

Group A spermatozoa produced significantly more good day 3 embryos compared to Group B (p<0.05), in particular 1,56 additional good quality embryos.

Interestingly, fresh Group B spermatozoa performed better than frozen Group B spermatozoa, resulting in significantly more good quality embryos on day 3 (p<0.05).

Furthermore, there was a significant decrease in good quality day 3 embryos, if both morphology and motility were graded as low/immotile (0.75 and 0.45 less good quality embryos respectively, p<0.05).

Although there was a trend for higher cycle cancellation rate in group B comparing to Group A, either fresh or frozen, the difference was not statistically significant.

Overall, although LBR was not affected by any parameter examined, the number of good quality embryos available for transfer was affected by both the quality of testicular spermatozoa used for ICSI and cryopreservation in low quality samples.

Limitations, reasons for caution: The evaluation of "presence" and "morphology" as good/average/bad could have a subjective complexion. However, this variation is eliminated through the grouping of samples.
Accumulative LBR was not calculated, as LBR was based only on the first transfer.

Wider implications of the findings: Since there is cryopreserved testicular tissue of good quality, there is no added value in proceeding to another surgery. However, in low quality samples, the use of fresh testicular spermatozoa could alter the final outcome, since cryopreservation affects the number of available good quality embryos.

Trial registration number: Not applicable

P-041 The effect of prolonged incubation of sperm at testis temperature (35°C) versus room temperature (26°C) on semen parameters

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Study question: Does prolonged incubation of sperm at 35° C versus room temperature (26 ° C) affect semen parameters and DNA fragmentation index (DFI)?

Summary answer: The concentration and motility of spermatozoa were significantly higher in room temperature than 35°C. However, Temperature had no effect on DFI after 24 h.

What is known already: Currently, cryopreservation is used routinely for prolonged storage of sperm even for one day, which, despite its high cost, can affect the quality of sperm samples. If long-term incubation of sperm in the laboratory environment is possible without affecting its quality, it will be possible to manage the patient's treatment with higher quality and with greater choice**.**

Study design, size, duration: In the present experimental study, sperm samples were collected from 40 participants referred to Mehr Medical Institute, Rasht, Iran, from September 2019 to December 2019.

Participants/materials, setting, methods: Each semen sample was divided into two equal parts and was subjected to swim-up procedures. One group was incubated at 35°C and the other at room temperature, in the darkness. Both groups were evaluated for number, motility (Grade A and B) and morphology at 45 min, 24 h and 48 h intervals. Statistical analysis was