the day 3 (number of good-quality embryos on the day 3/number of oocytes retrieved) were analyzed in terms of BPR, BFR, BWR and BMR.
Main results and the role of chance: In BPR, BWR and BMR, the 2PN rates in the lower group were significantly lower than those in the higher group. In BFR, the 2PN rate in the higher group was significantly lower than that in the lower group. Secondly, the good quality embryo rate on the day 3 in the lower group was significantly lower than that in the higher group in BWR. The good quality embryo rate on the day 3 in the lower group was significantly lower than that in the intermediate group in BMR.

Table 1: 2PN rate in terms of body composition parameters.

	Lower group (A)	Intermediate group (B)	Higher group (C)	
BPR	56.7% (123/217)	64.8% (2241/3459)	85.0% (34/40)	A vs. B:$P < 0.05$, A vs. C:$P < 0.01$, B vs. C:$P < 0.01$
BFR	64.6% (464/718)	65.0% (1863/2866)	53.8% (71/132)	A vs. C:$P < 0.05$, B vs. C:$P < 0.01$
BWR	56.9% (128/225)	64.9% (2103/3241)	66.8% (167/250)	A vs. B:$P < 0.05$, A vs. C:$P < 0.05$
BMR	48.8% (41/84)	64.7% (2323/3592)	85.0% (34/40)	A vs. B:$P < 0.01$, A vs. C:$P < 0.01$, B vs. C:$P < 0.01$

Table 2: Good-quality embryo rate on the day 3 in trms of body composition parameters.

	Lower group (A)	Intermediate group (B)	Higher group (C)	
BPR	17.5% (38/217)	22.6% (783/3459)	17.5% (7/40)	N.S.
BFR	22.0% (158/718)	22.6% (648/2866)	16.7% (22/132)	N.S.
BWR	18.2% (41/225)	22.2% (719/3241)	27.2% (68/250)	A vs. C:$P < 0.05$
BMR	11.9% (10/84)	22.6% (811/3592)	17.5% (7/40)	A vs. B:$P < 0.05$

Limitations, reason for caution: After nutritional guidance, change of body composition and early embryo development didn't be carried out.
Wider implications of the findings: In terms of body composition, the 2PN rate of patients with low body protein ratio, low body water ratio or low body mineral ratio was significantly low. The 2PN rate of patients with high body fat ratio was significantly low.
Study funding/competing interest(s): Funding by hospital/clinic(s). There are no conflicts of interest in this study.
Trial registration number: N/A.

P-276 Effects of the antifreeze proteins on the vitrification of mouse oocytes: comparison of three different antifreeze proteins

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Study question: Can antifreeze proteins (AFPs) from three different sources improve efficacy of vitrification?
Summary answer: AFPs treatment during mouse oocyte vitrification showed significant improvement in oocyte development, meiotic spindle organization and intracellular ROS levels. Among the three APF-treated groups (FfIBP, pLeIBP and TYPE III AFP groups), FfIBP and LeIBP groups showed significantly higher normal meiotic spindle rates and mitochondrial activity than Type III AFP group.
What is known already: Rubinsky discovered that upon vitrification of immature oocytes and two-cell stage embryos of mice AFGPs at 40 mg/ml produced dramatic improvements in the morphological integrity of the samples and suggested that AFPs have the ability to inhibit ice formation and to stabilize the plasma membrane.
Study design, size, duration: The MII oocytes were obtained from 4-week-old BD-F1 mice. AFPs from bacteria (FfIBP), yeast (LeIBP) and fish (Type III AFP) added to vitrification and warming solutions individually. Meiotic spindle and DNA double strand breaks, intracellular ROS and mitochondrial activity were analyzed.
Participants/materials, setting, methods: Vitrification of oocyte was performed with the CryoTop (equilibration 1: 7.5% EG and 7.5% PROH for 5 min, equilibration 2: 15% EG, 15% PROH and 0.5 M sucrose for 1 min). Warming was performed in three steps with decreasing concentration of sucrose (1, 0.5 and 0.25 M sucrose).
Main results and the role of chance: AFPs treatment can improve oocyte quality and embryo development. Especially, FfIBP and LeIBP treatment may be effective in lowering DSBs and maintaining normal meiotic spindle and mitochondrial activity in vitrified-warmed murine oocytes.

The FfIBP and LeIBP groups showed significantly higher survival rates. The FfIBP group showed the highest cleavage rates. Blastocyst rates were significantly higher in FfIBP group. In blastocyst, apoptosis rates were significantly decreased in the AFPs groups. A significantly higher cell count in blastocyst was noticed in the AFPs groups. The rates of normal meiotic spindle organization and chromosome alignment were significantly higher in the FfIBP and LeIBP groups. Intracellular ROS levels significantly decreased in the AFP treated group. When compared the mitochondrial activity, the LeIBP group showed significantly higher activities. DNA double strand breaks rates were significantly lower in FfIBP and LeIBP groups than those in the control or Type III AFP group.
Limitations, reason for caution: The origins of FfIBP and LeIBP were from bacteria and yeast. Therefore, these AFPs to human oocyte and embryo should be tested before clinical applications.
Wider implications of the findings: AFP can apply to human oocyte and ovarian tissue cryopreservation to improve efficacy of vitrification.
Study funding/competing interest(s): Funding by national/international organization(s), This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea.
Trial registration number: 2.

P-277 The presence of FSH receptor polymorphism –29 G > A is associated with poor ovarian response in IVF/ICSI cycles

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Study question: The tailoring of reproductive treatments is crucial in promoting a high chance of success and a reduction in hypo- or hyper-response. Could the presence of polymorphic variants of the –29 G > A, Thr307Ala, Asn680Ser FSH receptor gene (FSHR) be used for an improved tailoring of stimulation protocols in ART?
Summary answer: The presence of GA or AA variants in –29 G > A is associated with a poor ovarian response and this knowledge could be used for an improved personalization of treatments.
What is known already: In addition to age, the most frequently used biomarkers for choosing the stimulation protocol are basal FSH, anti-Müllerian hormone (AMH) and antral follicle count (AFC). Nevertheless, these biomarkers do not permit an optimal personalization of therapies for each patient. The analysis of the polymorphisms relating to FSHR –29 G > A, Thr307Ala and Asn680Ser (the latter two in linkage disequilibrium) have been extensively studied as predictive factors for ovarian response but with contradictory results.
Study design, size, duration: An observational, prospective study, in which the analysis of FSHR polymorphisms was performed in 140 IVF/ICSI patients during 2013 for severe male or tubal factors at the ANDROS Day Surgery Clinic, Palermo, Italy. Patients with AMH >4 and <1 ng/ml, basal FSH ≥12 mUI/mL, female age ≥42 years were excluded from the study.
Participants/materials, setting, methods: 140 patients underwent a long-protocol and the primary outcome (the number of oocytes retrieved) was classified as a poor (<5 oocytes) or good response (≥5 oocytes).

The starting doses (low 75–150 IU, medium >150 <225 IU and high ≥225 IU) were determined by: age, AMH, basal FSH, AFC.
Main results and the role of chance: The frequencies were 0.21 Asn/Asn, 0.55 Ser/Asn, 0.24 Ser/Ser, for Asn680Ser; 0.04 AA, 0.34 GA, 0.62 GG, for –29 G > A.

Multivariate analysis revealed no differences on basal FSH, AFC, AMH, starting dose, total rFSH units, follicles ≥16 mm for Asn680Ser. However, the AA variant had a lower AFC than the wild type (7.6 ± 3.8 vs. 11.4 ± 6.8 respectively) for –29 G > A.

The majority of patients with a poor response (57%) presented GA or AA variants whereas most patients with a good response (67%) presented a wild type ($\chi^2 = 5.53$, df = 1, $p = 0.02$).

Considering only those who had started with low and medium doses, patients with a poor response presented GA or AA variants in a higher percentage than patients with a good response (65 vs. 35%, $\chi^2 = 3.89$, df = 1, $p < 0.05$).

Limitations, reason for caution: These results should be validated in further, larger studies.

Wider implications of the findings: The results of this study show that the presence of FSHR −29 G > A polymorphism is associated with a poor outcome during IVF/ICSI. This knowledge could be introduced into routine clinical practice for providing specific counselling, for choosing the best stimulation protocol and for optimizing the starting dose of gonadotropins.

Study funding/competing interest(s): Funding by hospital/clinic(s), ANDROS Day Surgery Clinic, Palermo, Italy.

Trial registration number: None.

P-278 Correlation between antral follicle counts and the number of oocytes retrieved

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Study question: How well do stimulation day 1 (congruent to spontaneous cycle day 2 or 3) antral follicle counts (AFC) predict the number of oocytes retrieved (NOR) following ovarian stimulation in a gonadotropin-releasing hormone (GnRH) antagonist protocol?

Summary answer: AFC was confirmed to be a statistically significant predictor of the number of oocytes retrieved during ovarian stimulation in a GnRH antagonist protocol, but on average explains only 20% of the variation in the number of oocytes.

What is known already: AFCs are commonly used to predict the ovarian response during *in vitro* fertilization (IVF) cycles. They are used to help determine patients at risk for ovarian hyperstimulation, as well as for prediction of those at risk of a poor ovarian response.

Study design, size, duration: This is a pooled analysis by study comparing AFC on stimulation day 1 vs. NOR for subjects of 3 randomized controlled trials: Pursue ($N = 1388$), Engage ($N = 1476$), and Ensure ($N = 395$). All women underwent controlled ovarian stimulation in a GnRH antagonist protocol followed by hCG trigger prior to IVF/intracytoplasmic sperm injection.

Participants/materials, setting, methods: Women in Pursue (35–42 years, ≥50 kg) received 150 µg corifollitropin alfa (CFA) or 300 IU daily recombinant FSH (rFSH), in Engage (18–36 years, >60 kg) received 150 µg CFA vs. 200 IU rFSH, and in Ensure (18–36 years, ≤60 kg) received 100 µg CFA or 150 IU rFSH.

Main results and the role of chance: Per study, Pearson correlation coefficients (ρ) were calculated and linear regression performed for NOR with covariates AFC, treatment, and their interaction. Mean AFC was 10.7, 12.4, and 11.2 in Pursue, Engage, and Ensure, respectively. Correlation between AFC and NOR was stronger in Pursue ($\rho = 0.53$; $P < 0.0001$) than in Engage and Ensure ($\rho = 0.34$ and $\rho = 0.27$, respectively; $P < 0.0001$). In Pursue and Ensure, correlation tended to be stronger in the CFA than the rFSH group ($\rho = 0.57$ vs. $\rho = 0.48$ and $\rho = 0.33$ vs. $\rho = 0.17$, respectively). In Engage, correlations were similar in the 2 groups ($\rho = 0.34$ and $\rho = 0.36$, respectively). Multiple correlation coefficients ranged from 11% in Ensure to 28% in Pursue. AFC explained approximately 20% of the variation in NOR. Individual prediction errors ranged between −4.3 and +3.2 oocytes (25th, 75th percentiles).

Limitations, reason for caution: This was a retrospective analysis. Equipment used to measure AFC was not standardized across centers.

Wider implications of the findings: Use of AFC to predict the number of oocytes retrieved for individual patients should be done with caution as prediction errors are typically between −4 and +3 oocytes.

Study funding/competing interest(s): Funding by commercial/corporate company(ies), Financial support for this study was provided by Merck & Co., Inc., Whitehouse Station, NJ, USA.

Trial registration number: NCT01144416, NCT00696800, NCT00702845.

P-279 Ovarian reserve and thyroid autoimmunity. A cross-sectional analysis using age-specific AMH levels

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Study question: Is there a significant association between low ovarian reserve and hypothyroidism and/or thyroid autoimmunity.

Summary answer: Low ovarian reserve is not related with hypothyroidism or thyroid autoimmunity.

What is known already: One retrospective study supported a potential association between diminished ovarian reserve (DOR) and increased TSH levels. Nonetheless, according to this study, DOR was defined as high basal FSH (14 UI/L), low antral follicular count (AFC <5) and prior poor ovarian response to stimulation, without taking into account one of the most accurate markers of ovarian reserve, anti-Mullerian hormone (AMH).

Study design, size, duration: A cross-sectional study including 4690 women between 2009 and 2012.

Participants/materials, setting, methods: Overall 4690 women were included. Patients were eligible for inclusion if they:

1. Had their AMH, FT4, TSH and TPO-Ab levels tested on the same day and
2. Did not have any risk factor that could potentially compromise their ovarian reserve (e.g., ovariectomy, ovarian surgery for endometriosis or gonadotoxic chemotherapy).

AMH levels were plotted in relation to age for the whole patients' cohort and age-specific AMH values (per year) were considered in order to categorize women according to the level of ovarian reserve.

In this regard, patients were categorized as women with: 1. Low ovarian reserve (women with age-specific AMH below the 10th percentile of the values), 2. Normal ovarian reserve (women with age-specific AMH between the 10th and 90th percentile of the values), and 3. High ovarian reserve (women with age-specific AMH above the 90th percentile of the values).

Kruskal-Wallis test was performed to compare TSH and FT4 between different ovarian reserve categories.

Chi-square test was performed in order to examine differences in the number of patients with positive TPO-Ab (>34 0kU/l) and the number of patients with hypothyroidism (clinical or subclinical).

All analyses were performed in SPSS 22 statistical software.

Main results and the role of chance: Overall, 462 patients were women with low ovarian reserve, 3756 demonstrated normal reserve and 472 demonstrated high reserve. Age and BMI did not significantly differ between patients' groups.

Mean (SD) serum FT4 levels were comparable between groups: low ovarian reserve, 11.98 (1.91), normal reserve 12.03 (1.93) and high reserve, 12.05 (1.62), $p = 0.524$, whereas, in accordance, TSH levels did not demonstrate any difference in women with low [median (IQR), 1.56 (1.14–2.18)], normal [1.54 (1.12–2.17)] and high ovarian reserve [1.51 (1.05–2.31)], $p = 0.841$.

Four hundred and eighty-three (483) patients (10.4%) had positive TPO-Ab. The percentage of patients with positive TPO-Ab did not significantly differ between low (11.7%), normal (10.2%) and high (10.0%) in ovarian reserve patients, $p = 0.594$). Finally, no differences were observed between groups in the incidence of clinical or subclinical hypothyroidism as defined by serum FT4 and TSH levels (3.2% in low, 3.6% in normal and 2.5% in high ovarian reserve patients $p = 0.447$).

Limitations, reason for caution: Due to the retrospective design of this study we cannot exclude the presence of biases related to retrospective data collection. Thus, results should be interpreted with caution. However, cross-sectional analysis is considered by most the optimal study design to assess prevalence of conditions (such as hypothyroidism).

Wider implications of the findings: This cross-sectional analysis failed to demonstrate an association between thyroid autoimmune disease and ovarian reserve. The major strength of this study is that it includes a very large group of patients in whom serum AMH, FT4, TSH and TPO-Ab were measured on the same day for the whole patients' cohort. In addition we clearly defined ovarian reserve based on age-specific AMH values from the same cohort of patients and not based on other variables (such as FSH levels alone, antral follicle count or response to previous treatment) by using specific threshold which may indeed vary between different age groups.