

Trial registration number: No clinical trials were implicated in the study.

Reproductive endocrinology

P-465 rLH supplementation to rFSH during induced ovarian stimulation in the GnRH antagonist protocol improves implantation and pregnancy rates

A. Nazzaro, A. Salerno, L. Di Iorio, G. Landino, S. Marino, and E. Pastore "Gaetano Rummo" Hospital, Physiopathology of Human Reproduction, Benevento, Italy

Study question: This study aims to compare the efficacy of mid-follicular recombinant LH (rLH) supplementation for ovarian stimulation in gonadotrophin-releasing hormone-antagonist (GnRH-a) protocol for IVF/ICSI cycles in normogonadotrophic women

Summary answer: r-LH supplementation in GnRH-a cycles improve implantation and pregnancy rates probably via an improvement in oocyte quality and/or uterine receptivity.

What is known already: The role of LH during ovarian stimulation in IVF is controversial and previous reports on this topic have revealed conflicting outcomes, mostly ought to the heterogeneity of the studies in terms of design, sample size, inclusion criteria, GnRH analogue protocol, type of LH administered (recombinant human LH (r-hLH), human menopausal gonadotrophin (HMG), dose, beginning, duration of treatment)

Study design, size, duration: 422 patients without ovulatory dysfunction, aged <40 years and at their first IVF/ICSI cycle were divided into two groups matched by age according to two ovarian stimulation schemes: Group I (n = 211): r-FSH alone and Group II (n = 211): r-FSH + r-LH. All women were synchronized with vaginal estrogenic device

Participants/materials, setting, methods: Pituitary down regulation and ovarian stimulation were carried out by the following fixed scheme: three daily administration of GnRH-a starting on day two of the menstrual cycle followed by five days of r-FSH administration (225 IU/day) alone, on day six the all women restarted daily GnRH-a administration and were randomized to receive r-FSH alone or r-FSH + r-LH (r-LH 150 IU/day) for the remainder of the stimulation. Follicular maturation was triggered with 250 µg of r-hCG. Data were analyzed using InStat version 3.0 (GraphPad Software, San Diego, California, USA). The Student's t, Mann-Whitney and χ^2 tests were utilized when appropriate. The level of significance was set at $P < 0.05$

Main results and the role of chance: The number of oocytes collected, the number of oocytes in metaphase II and fertilization rate were significantly lower in the Group I than in Group II ($P = 0.036$, $P = 0.0014$ and $P = 0.017$, respectively). The mean number of embryos produced per cycle, the mean high grade number of embryos and the mean number of frozen embryos per cycle were statistically lower ($P = 0.0092$; $P = 0.0086$; $P = 0.0008$, respectively) in Group I than in Group II. The cumulative implantation rate (fresh + thawed embryos) and clinical pregnancy rate were significantly lower ($P = 0.04$ and $P = 0.03$, respectively) in Group I than in Group II.

Limitations, reason for caution: none

Wider implications of the findings: The beneficial effect of r-hLH on implantation and pregnancy outcomes in our population could be explained by two different mechanisms. Firstly, the embryo quality seems to be superior in the rLH supplemented group; secondly, rLH supplementation may have a beneficial effect on the endometrium, which could promote embryo implantation. Although larger studies are required to further investigate these findings, r-hLH supplementation for normogonadotrophic women aged <40 years undergoing ICSI/IVF cycles is recommended as it may have a beneficial action on implantation and pregnancy rates.

Study funding/competing interest(s): None

Trial registration number: None

P-466 IVF results following transdermal testosterone in poor responders according bologna criteria

F. Fabregues, A. Iraola, G. Casals, M. Creus, S. Peralta, J. Peñarrubia, D. Manau, S. Civico, and J. Balasch

Institut Clínic de Gynaecology Obstetrics, Dept. of Gynaecology and Obstetrics, Barcelona, Spain

Study question: What is the effect of Transdermal Testosterone (TT) in IVF cycles in poor ovarian response according to the new ESHRE definition for poor ovarian responders: the Bologna criteria?

Summary answer: Transdermal application of testosterone preceding gonadotrophin ovarian stimulation may improve the ovarian response in poor ovarian responders fulfilling the Bologna criteria.

What is known already: Previous studies have shown that TT pretreatment is an effective option for poor ovarian responders undergoing IVF. However, none of the trials have examined the effect of TT in poor responders according to the Bologna criteria, the newly introduced definition by the ESHRE Working Group on Poor Ovarian Response Definition. In this study, we present results obtained using this approach in such patients.

Study design, size, duration: In this retrospective cohort trial, 102 consecutive patients low responders, according to the Bologna criteria were included. Testosterone therapy was started the day when pituitary-ovarian suppression was confirmed and administered during the 5 days preceding gonadotrophin treatment.

Participants/materials, setting, methods: Patients were treated using the long-agonist protocol and recombinant FSH. The cycle was cancelled when were present < 3 follicles with diameter > 14 mm after 8-9 days of gonadotrophin therapy. The cancellation of the cycle or collection of < 3 oocytes at retrieval defined the low-responder patient.

Main results and the role of chance: All patients included in the study had a least one previous cycle with poor response (80 had cancelled cycle and 22 had poor response). The number of patients undergoing ovum retrieval was 77 (75%) and TT was associated with a significant reduction in the number of patients with poor response (100% vs 54.9%, $p < 0.05$). The number of oocytes, metaphase II oocytes and embryos on day 2 were 4.2 ± 2 , 3.05 ± 1.9 and 2.8 ± 1.6 respectively. The number of pregnancies per oocyte retrieval was 23 (29.9%). Factors associated with TT success in poor responders are analyzed.

Limitations, reason for caution: A limitation of our study is its retrospective design; however, taking into account that Bologna criteria are recent and the insufficient evidence about androgen supplementation in this topic our results are promising.

Wider implications of the findings: Our study suggest that the use of TT may be effective in poor ovarian responders fulfilling the Bologna criteria. However further studies should analyze the effectiveness in different subpopulation of poor responders.

Study funding/competing interest(s): Work supported in part by the Agència de Gestió d'Ajuts Universitaris i de Recerca-Generalitat de Catalunya (2009SGR1099). There are no competing interest to declare.

Trial registration number: Not required

P-467 Effects of FSH receptor polymorphisms

I. Lindgren and Y. Lundberg Giwercman

Lund University, Department of Clinical Sciences, Malmö, Sweden

Study question: Have previously tested doses of recombinant follicle-stimulating hormone (rFSH) been too low in experimental studies regarding the asparagine680serine polymorphism in the FSH receptor (FSHR) gene?

Summary answer: Yes, in accordance with previous *in vivo* findings, we found that the less common FSHR variant with serine in amino acid position 680 exhibited notably lower activity *in vitro* compared to the FSHR with asparagine in the same position.

What is known already: The asparagine680serine variant in the FSHR gene has previously been shown to affect reproductive function in women, so that those carrying serine in amino acid position 680 require higher doses of rFSH than those with asparagine in the same position during hormonal treatment prior to *in vitro* fertilization. However, so far *in vitro* studies on 0.03-10 mIU/mL rFSH have failed to show differences in FSHR activity between the receptor variants.

Study design, size, duration: Eukaryotic kidney cells (COS-1), lacking endogenous FSHR, were used in a reporter assay in which the capability of the wild type asparagine-containing FSHR was compared to the serine-containing FSHR with respect to stimulation of the release of cAMP, which is a downstream signaling molecule in the FSHR signaling pathway.

Participants/materials, setting, methods: Genetic variants of the *FSHR* </SSF > were cloned into the pCMV6-XL5 vector and transiently transfected into COS-1 cells. Cells were stimulated with 0–400 mIU/mL rFSH (Gonal-F) and subsequently cAMP concentration was measured with ELISA and adjusted for total protein concentration. The experiment was performed 3 times in duplicates.

Main results and the role of chance: As in previous studies, there were no differences in cAMP response between the FSHR variants, when cells were stimulated with 0–10 mIU/mL rFSH. However, in response to doses above 10 mIU/mL rFSH, the serine-containing receptor displayed approximately 5 times lower response than the wild type asparagine-containing receptor.

Limitations, reason for caution: The COS-1 cells are of primate origin. The results may therefore not be directly applicable on humans.

Wider implications of the findings: The results from this study may, at least partly, explain the need for higher rFSH doses for ovulation prior to *in vitro* fertilization in women carrying serine instead of asparagine in amino acid position 680. On the other hand, women with asparagine-containing FSHRs may be those at increased risk of developing ovarian hyperstimulation syndrome.

Study funding/competing interest(s): The study was supported by grants from the European Union, Interreg. There are no conflicts of interest.

Trial registration number: None.

P-468 Lipid features of polycystic ovary syndrome under different diagnostic criteria

E. Celik¹, I. Turkuoglu¹, B. Ata², A. Karaer¹, P. Kirici¹, and B. Berker³
¹Inonu University Turgut Ozal Medical Centre, Obstetric and Gynecology, Malatya, Turkey, ²Uludag University School of Medicine, Obstetric and Gynecology, Bursa, Turkey, ³Ankara University School of Medicine, Obstetric and Gynecology, Ankara, Turkey

Study question: Whether or not the prevalence of abnormal lipid profile of polycystic ovary syndrome (PCOS) alters in the same population according to the different diagnostic criteria?

Summary answer: The prevalence of abnormal lipid profile in PCOS under Androgen Excess and PCOS (AE-PCOS) Society criteria was higher than that of and non-hyperandrogenic PCOS (oligomenorrhea with appearance of polycystic ovary on transvaginal ultrasonography).

What is known already: The diagnosis of PCOS still remains controversial, as it is based on signs, symptoms and laboratory findings that are not unanimously recognized. It is indicated that PCOS is a multifactorial condition that is associated with dyslipidemia and insulin resistance. The current study gives the prevalence rates of insu in women with PCOS under contrasting diagnostic criteria.

Study design, size, duration: In this retrospective cohort study, the medical records of 8908 consecutive women between ages of 18 and 45 years were reviewed. The study was conducted in the gynecological outpatient department of Inonu University, Turgut Ozal Medical Centre, between 2009 and 2012. Of 1047 women with PCOS under Rotterdam criteria were included.

Participants/materials, setting, methods: The consecutive women between the ages of 18 and 45 years were included. Post-menopausal women, women with incomplete medical data, a history of hysterectomy or bilateral oophorectomy, systemic disease, taking medication and pregnant women were excluded. Totally, 7872 subjects were included and of 1047 women with PCOS were recruited for final analyses.

Main results and the role of chance: The prevalence of PCOS under Rotterdam, AE-PCOS Society criteria and non-hyperandrogenic PCOS were 13.2%, 8.7% and 4.6%, respectively. While the prevalence of high triglycerides (TG) was 21.7% in the whole study group, within the patients diagnosed as PCOS according to AE-PCOS Society criteria and non-hyperandrogenic PCOS, it was 23.5% and 14.2%, respectively ($P = 0.04$). The prevalence of low high-density lipid (HDL) in the group under AE-PCOS Society criteria was higher than that of non-hyperandrogenic PCOS (60.6% versus 40.8%, respectively; $P < 0.01$); however, the prevalence of low HDL was 56.8% in the whole study group. In terms of

prevalence of high total cholesterol and low-density lipid (LDL) parameters, there were no statistically significant differences between the groups (18.5% versus 15.0% and 14.8% versus 11.9%; $P = 0.48\%$ and $P = 0.53$, respectively).

Limitations, reason for caution: Even though women living at a similar environment, a potential selection bias due to undetermined differences between our study sample and the background community.

Wider implications of the findings: A diagnosis of PCOS in combination of anovulation with hyperandrogenism has the most long-term metabolic impact. Current results can be generalized to Caucasian populations and may present variations in other populations according to race and ethnicity.

Study funding/competing interest(s): No financial support. The authors have no competing interests to declare.

Trial registration number: Not applicable

P-469 Comparison of serum Anti-Müllerian hormone levels and affecting factors in polycystic ovary syndrome with and without hyperandrogenism

J. Park, J. Kim, and J. Rhee
 Keimyung University Dongsan Medical Center, Obstetrics and Gynecology, Taegu, Korea South

Study question: The aim of this study was to evaluate serum AMH levels in women with polycystic ovary syndrome (PCOS) for setting up the diagnostic cut-off value, and to investigate affecting factors to AMH levels in women with PCOS with and without hyperandrogenism.

Summary answer: Serum AMH in women with PCOS was significantly increased compared with eumenorrheic asymptomatic volunteers, however, not different between women with hyperandrogenism or not. In women with hyperandrogenism, serum AMH was well correlated with LH, T, and fasting insulin level. In women without hyperandrogenism, AMH was not correlated with any parameters.

What is known already: Serum AMH levels are elevated in women with PCOS, and proposed as a marker for diagnosis and surveillance of PCOS therapy. There is an independent effect of race and ethnicity. There were some reports the relationship of AMH with obesity, LH, hyperandrogenemia.

Study design, size, duration: This is a case control study performed from January 2012 to November 2012. Sixty eight women with PCOS, fifty five age-matched normogonadotropic regularly menstruating women were enrolled for this study.

Participants/materials, setting, methods: All women in study group had secondary amenorrhea and PCO morphology, divided into two groups depend on hyperandrogenism (HA+ and HA-). Sera were collected on the progesterone induced cycle day 2 or 3 for determining the levels of AMH, FSH, LH, E2, T, DHEA-s, TSH, prolactin, and 75g OGTT.

Main results and the role of chance: Mean serum AMH level was markedly increased in the PCOS group (12.7 ± 4.9 ng/mL) compared with control (4.8 ± 2.1 ng/mL; $P < 0.001$). Cut-off value for predicting PCOS was 8.21 ng/mL with sensitivity of 82.4%, and specificity of 94.5% by ROC curve (AUC 0.937, $P < 0.001$). Serum AMH levels were not significantly different between HA+ and HA- group. In HA+ group, AMH was well correlated with LH ($r = 0.692$), T ($r = 0.725$), and fasting insulin ($r = -0.893$), however, not correlated with BMI, waist hip ratio, fasting or 2hr blood sugar level after 75g OGTT, FSH, E2, TSH, prolactin, or DHEA-s. In HA- group, AMH was not correlated with any parameters. Serum AMH levels were not significantly different between obese and normal BMI women either.

Limitations, reason for caution:

Wider implications of the findings: The diagnostic cut-off of serum AMH was 8.21 ng/mL in this study, higher than other reports. It was possibly by ethical differences. There were inconsistent reports about the relationship of AMH with endocrinological and clinical parameters. In our study, serum AMH was not influenced by hyperandrogenemia, LH, or obesity. Only in hyperandrogenemia subgroup, AMH was well correlated with LH, T and fasting insulin. It might reflect the differences in pathophysiology between the two subgroups.

Study funding/competing interest(s): NO

Trial registration number: NO

P-470 The role of ethnicity and body weight in determination of AMH levels in women diagnosed with subfertility

M. Krishnan¹, O. Rustamov², R. Russel³, C. Fitzgerald¹, and S. Roberts⁴
¹Central Manchester University Hospitals NHS Foundation Trust, Department of Reproductive Medicine, Manchester, United Kingdom, ²Central Manchester University Hospitals NHS Foundation Trust, Manchester Royal Infirmary, Manchester, United Kingdom, ³Liverpool Women's Hospital, Hewitt Fertility Centre, Liverpool, United Kingdom, ⁴University of Manchester, Centre for Biostatistics Institute of Population Health, Manchester, United Kingdom

Study question: What are the roles of the ethnicity and the body weight in determination of AMH levels in infertile women?

Summary answer: It appears that ethnicity does not play significant role in determination of AMH levels in subfertile women. However obese women appear to have higher AMH levels compared to their lean counterparts, which remained statistically significant following controlling for age, PCOS status, ethnicity and the causes of infertility.

What is known already: Female ovarian reserve is largely determined by genetic factors and therefore it is believed that female ethnicity may play a role in determining AMH levels. However data based on large cohort of subjects is currently not available. Similarly, effect of body weight on AMH is unknown.

Study design, size, duration: This is first observational study that has evaluated effect of ethnicity and body weight on AMH levels using large cohort of subjects. ANOVA with quadratic adjustment for age (and diagnosis of PCOS, causes of infertility and history of reproductive surgery) was used to estimate effect size and statistical significance.

Participants/materials, setting, methods: All women (20-45 years) referred for management of infertility (01.10.2008-18.10.2010) and had AMH measurements using DSL ELISA were included (n = 3488). Distribution of ethnicity was as follows: 1973 White British, 150 Other White, 106 Black, 174 Asian Indian, 322 Asian Pakistani, 32 Chinese. 731 women did not report their ethnicity.

Main results and the role of chance: When compared to White British there were no significant differences in AMH levels of Other White, Asian Indian, Asian Pakistani and Chinese women following adjustment for age and PCOS status whilst Black women had significantly higher hormone levels (p = 0.008). However this significance was lost following adjustment for BMI (p = 0.26).

Obese women (BMI 30-40) had significantly higher AMH levels compared to lean women (BMI 20-25), which remained statistically significant following adjustment for age, PCOS status and ethnicity (p = 0.007). The analysis of the interaction of these two confounding factors suggests that the effect of ethnicity on AMH appears to be the consequence of obesity, whilst the effect of obesity is independent of the ethnicity.

Limitations, reason for caution: This data is based on heterogeneous infertile population; which includes 'healthy' sub-population (n = 330) consisting of women with no history of reproductive pathology and whose partner/husbands diagnosed with azoospermia. The data for all possible confounding factors (causes of infertility, reproductive surgery) were collected and the analysis included adjustment for these factors.

Wider implications of the findings: Currently women's age and diagnosis of PCOS are only known factors which affects AMH levels. This study suggests that BMI is independent factor that affects AMH levels and therefore future research studies should take this into account when controlling the trials and/or in adjustment for the confounding factors.

Study funding/competing interest(s): Study funding:

Competing interest: None

Trial registration number: Non-applicable (observational study)

Ethics Reference: 10/H1015/22, North West NHS Research Ethics Committee.

P-471 Current practice of assessment of ovarian reserve in the UK

S. Hapuarachi, B.K. Tan, and R.S. Mathur
 Cambridge IVF, Reproductive Medicine and Surgery, Cambridge, United Kingdom

Study question: We sought to investigate the current practice of assessment of ovarian reserve in United Kingdom (UK) reproductive medicine centres.

Summary answer: Anti-Müllerian hormone (AMH) appears to be the most widely used marker of ovarian reserve in tertiary practice in the UK.

What is known already: Traditionally, patients' age and early follicular phase Follicle Stimulating Hormone levels have been widely used in the UK as

markers of ovarian reserve. Recent evidence suggests that AMH and antral follicle count (AFC) are superior to FSH measurements in this regard.

Study design, size, duration: Between October 2012 and January 2013, we undertook a national postal questionnaire involving 155 reproductive medicine centres in the UK.

Participants/materials, setting, methods: Study participants were the lead clinicians of the 155 reproductive medicine centres in the UK. The questionnaire explored the current practice of evaluation of ovarian reserve in each of the 155 UK reproductive medicine centres. The questionnaire and a stamped self-addressed return envelope were sent to each of the centres.

Main results and the role of chance: The response rate was 42%. The majority of respondents used AMH (92.3%) and AFC (78.5%) to assess ovarian reserve; no respondents used inhibin B. 86.2% considered AMH as a marker of ovarian responsiveness in women undergoing fertility treatment, 63.1% as a fertility marker in women concerned about fertility and 27.7% used AMH as a fertility marker in women trying to conceive naturally. 30.8% used AMH as part of the diagnosis of polycystic ovarian syndrome. The cost to patients of AMH testing was £50/test-£100/test in 53.8% of centres. 83.1% felt that AMH testing should be available to all infertile women, 3.1% felt both AMH and AFC should be available and 9.2% stated that neither AMH nor AFC should be available; no respondents supported inhibin B testing.

Limitations, reason for caution: The results of our study pertain to current practice in the UK and caution is needed in extrapolating these findings to practices in other countries.

Wider implications of the findings: Use of AMH as a fertility marker in women concerned about fertility (as opposed to women undergoing assisted conception treatment) is not evidence based and may have been extrapolated from studies on assisted conception cycles. This survey identifies that this role for AMH testing is widespread in UK practice and may point to an educational need among clinicians and patients. There is strong support for AMH testing for infertile couples on the National Health Service.

Study funding/competing interest(s): There are no competing interests.

Trial registration number: Not applicable.

P-472 The role of gonadotrophin releasing hormone agonist downregulation in artificially prepared frozen embryo transfer cycles

A. van de Vijver, C. Blockeel, M. Camus, N. Polyzos, L. Van Landuyt, and H. Tourmaye
 UZ Brussel, Centre of Reproductive Medicine, Brussels, Belgium

Study question: Among patients undergoing a frozen embryo transfer in an artificially prepared hormonal cycle, does the use of GnRH agonist downregulation result in a lower cancellation rate due to premature progesterone rise?

Summary answer: Cancellation rates due to premature progesterone rise in artificially prepared frozen cycles do not exceed 2%. Although the use of GnRH agonist eliminates premature progesterone rise, its low incidence does not justify routine use in everyday clinical practice.

What is known already: The association of a GnRH agonist in an artificially prepared endometrium for a frozen-thawed embryo transfer, results in a similar ongoing pregnancy rate, clinical pregnancy rate, miscarriage rate and cycle cancellation rate. Trials with small sample sizes report cancellation rates for premature progesterone rise up to 7%.

Study design, size, duration: In this retrospective cohort study in the Centre of Reproductive Medicine of the Brussels University Hospital, 1129 patients who underwent an oestrogen and progesterone supplemented cycle before the transfer of a homologous frozen-thawed embryo between 1 July 2009 and 1 June 2012 were included.

Participants/materials, setting, methods: Of all 1129 patients, 280 received hormonal supplementation, with GnRH agonist co-treatment (group A), whereas 849 patients only received hormonal supplementation (group B). Demographic characteristics, indication for fertility treatment and endometrial thickness at day of planning, did not differ significantly between the 2 groups.

Main results and the role of chance: Forty-one cycles did not result in an embryo transfer in group A (14.6%) and 116 in group B (13.7%) (p = 0.69). Premature

progesterone rise occurred in 1.9% in group B (16/849), versus 0% in group A (0/280), which is significantly different ($p = 0.02$).

Regarding secondary outcome parameters, 77/280 (27.5%) had a positive pregnancy test after transfer in group A, 237/849 (27.9%) in group B ($p = 0.94$). Mean number of embryos transferred (1.41 in group A (SD 0.49) versus 1.43 in group B (SD 0.50)) was comparable ($p = 0.81$).

Limitations, reason for caution: Given the retrospective design of this trial, results must be interpreted with caution. Future randomized trials are needed to confirm our findings.

Wider implications of the findings: Our results confirm the outcomes from previous smaller studies suggesting low cancellation rates and promising pregnancy rates in women undergoing artificially prepared frozen embryo transfer cycles with or without the use of a GnRH agonist. The low cancellation rate due to premature progesterone rise implies that the use of a GnRH agonist should not be routinely recommended, since it does not increase pregnancy rates whereas it increases patients' burden and costs related to medication.

Study funding/competing interest(s): This study was not funded and there is .

Trial registration number: NA

P-473 Combined oral contraceptive use increases serum prostate-specific antigen levels in patients with polycystic ovary syndrome

N. Öztürk Turhan¹, D. Hizli², Z. Kamalak³, A. Kösüs⁴, N. Kösüs², and H. Kafali²
¹Mugla Sıtkı Kocman University, *Obstetrics and Gynecology, Mugla, Turkey*,
²Fatih University, *Obstetrics and Gynecology, Ankara, Turkey*, ³Nene Hatun Hospital, *Obstetrics and Gynecology, Erzurum, Turkey*, ⁴Fatih University, *Obstetrics and Gynecology, Ankara, Turkey*

Study question: Does combined oral contraceptive (COC) use alter serum prostate-specific antigen (PSA) levels in patients with polycystic ovary syndrome (PCOS)?

Summary answer: Use of COC seems to significantly increase serum PSA levels in patients with PCOS. Therefore, PSA should not be used for the monitoring of PCOS patients on COC treatment.

What is known already: Universal pathology in PCOS is androgen excess that is responsible for signs and symptoms of hyperandrogenism. Several studies have demonstrated a significant reduction in ovarian androgen production during the use of COCs. PSA has been detected in female serum using ultrasensitive assays and has been proposed as a marker of androgen excess in hirsute women. However, the results are conflicting. In most of the study, significant decrease in serum PSA concentrations has been demonstrated in hirsute women on antiandrogen treatment. However, similar PSA decrease was not reported after COC treatment in several small studies. Moreover, in a study it was stated that COCs may increase serum PSA levels.

Study design, size, duration: Seventy women who had a new diagnosis of PCOS between January 2011 and April 2012 were included in this prospective study.

Participants/materials, setting, methods: PCOS was defined by the 2003 Rotterdam criteria. All patients with PCOS were treated with a COC containing 0.035 mg ethinylestradiol and 2 mg cyproterone acetate for 6 months. Serum PSA levels, ovarian volume, antral follicle count, serum testosterone level and HOMA index were measured before and after the treatment.

Main results and the role of chance: The median serum PSA levels were 0.040 (range 0.02-0.09) ng/ml and 0.060 (range 0.02-0.09) ng/ml before and after COC use, respectively. Serum PSA levels significantly increased after the treatment ($p < 0.001$). Serum PSA levels were not correlated to ultrasonographic, laboratory and demographic parameters.

Limitations, reason for caution: The main limitation of our study was the absence of control group. However, it might be the secondary outcome of the study, because our primary outcome was to evaluate the effect of COC use on serum PSA levels in patients with PCOS. The follow-up period might be longer than 6 months. However, in most of the studies clinical and laboratory changes were evaluated after 3 or 6 cycles of COC treatment.

Wider implications of the findings: The present study seems to be the largest prospective trial revealing the effect of COC use on serum PSA levels in women with PCOS. Our results suggest that use of COC may increase serum PSA level of PCOS women. Therefore, PSA should not be used as a monitoring marker of PCOS patients receiving COC.

Study funding/competing interest(s): This study was supported by the Scientific Research Fund of Fatih University. There is .

Trial registration number: P53011107_B 1533.

P-474 Probability of live birth in women with extremely low anti-Müllerian hormone levels

A. Lukaszuk¹, M. Kunicki², J. Liss³, A. Bednarowska², G. Jakiel⁴, K. Lukaszuk³, M. Lukaszuk³, and B. Olszak-Sokolowska³
¹Medical University Bydgoszcz, *Medical Division, Bydgoszcz, Poland*, ²Invicta, *Fertility Clinic, Warsaw, Poland*, ³Invicta, *Fertility Clinic, Gdansk, Poland*, ⁴The Medical Center of Postgraduate Education, *1st Department of Obstetrics and Gynecology, Warsaw, Poland*

Study question: What is the likelihood of clinical pregnancy and live birth rates in women with extremely low anti-Müllerian hormone (AMH) levels.

Summary answer: Our results confirmed that even without supplementation, the likelihood of live birth is still a reasonable reason to begin treatment in women with extremely low live birth rates.

What is known already: Anti-Müllerian hormone (AMH) is an established marker of ovarian reserve and predicts both high and low responses in controlled ovarian stimulation cycles. A current diagnostic issue for clinicians is the treatment of women with extremely low AMH levels. In that group of patients, we expect poor ovarian response, which can lead to cycle termination, thus lowering the probability of pregnancy.

Study design, size, duration: We retrospectively analyzed a computer database of women with extremely low AMH (< 0.4 ng/ml) levels treated with intracytoplasmic sperm injection (ICSI) in our IVF unit between May 2007 and January 2011.

Participants/materials, setting, methods: During the study period, 194 cycles of 106 women were investigated. The median age of all patients included in the study population was 37 years. We divided women into three age categories: < 35 , $35-39$, and > 39 . Clinical pregnancy rate and live birth rate was recorded.

Main results and the role of chance: The mean AMH levels in all women/cycles were 0.25 ± 0.12 ng/ml.

Fourteen clinical pregnancies were recorded (7.2% per cycle start and 13.2% cumulative) and 14 live births in 13 women (one pair of twins). Four live births occurred after the first cycle, seven live births occurred after the second cycle, two live births occurred after the third cycle, and one live birth occurred after the fourth cycle. Only one woman miscarried.

When evaluated according to age, we found significantly higher clinical pregnancy and live birth rates in women younger than 35 years [9 (23.7%)] compared to women between 35 and 39 years [3 (10.3%)] and older than 39 years [1 (2.6%)].

Limitations, reason for caution: Small group. Two center study.

Wider implications of the findings: It seems clear that clinicians should communicate the probability of live birth when the woman has extremely low AMH levels to allow both the couples and the doctors to either begin treatment (when a low probability of live birth is accepted) or present other possibilities to achieve pregnancy (e.g., oocyte donation program).

Study funding/competing interest(s): None

Trial registration number: No

P-475 Use of ovarian reserve parameters for predicting live births in women undergoing in vitro fertilization

K. Lukaszuk¹, M. Kunicki², J. Liss¹, G. Jakiel³, A. Bednarowska², T. Wasniewski⁴, M. Neuberg², and M. Lukaszuk¹
¹Invicta, *Fertility Clinic, Gdansk, Poland*, ²Invicta, *Fertility Clinic, Warsaw, Poland*, ³The Medical Center of Postgraduate Education, *1st Department of Obstetrics and Gynecology, Warszawa, Poland*, ⁴University of Warmia and Mazury, *Department of Obstetrics and Gynecology, Olsztyn, Poland*

Study question: Which of the common clinical determinants, including patient age; levels of anti-Müllerian hormone (AMH), inhibin B, and follicle-stimulating hormone (FSH); antral follicle count (AFC); and number of oocytes retrieved, is the best marker in predicting live births in women undergoing *in vitro* fertilization.

Summary answer: In this assessment of various indices (i.e., age; levels of AMH, inhibin B, and FSH; AFC; and quantity of oocytes retrieved) for predicting live births for IVF patients, AMH, AFC and the quantity of oocytes retrieved constituted the most reliable determinants.

What is known already: A number of clinical and laboratory parameters are currently under investigation as potential indices of successful pregnancy with assisted reproductive technology. Parameters presently viewed as reflecting ovarian stimulatory response, and thus aiding in clinical counseling of patients, are antral follicle count (AFC), FSH levels at day 3, and inhibin B levels. AMH is a relatively novel marker of ovarian reserve.

Study design, size, duration: Retrospective cohort study of women treated with intracytoplasmic sperm injection (ICSI) from May 2007 to January 2011.

Participants/materials, setting, methods: 2495 women undergoing ICSI for the first time were reviewed retrospectively, and serum levels of AMH, inhibin B, and FSH, as well as AFC (days 1 and 4 of pre-ICSI menstrual period) and patient age were analyzed as determinants of live birth rates.

Main results and the role of chance: Of the patients studied, 35.71% (891/2,495) became pregnant, with live births achieved in 32.20% (806/2,495) of cycles initiated and in 46.37% (806/1,738) of embryo transfers. Clinical pregnancy rate was 35.71% (891/2,495) for cycles initiated and 51.26% (891/2,318) for embryo transfers. Using multivariate logistic regression, only AMH (OR = 1.89; 95% CI, 1.00-3.60; $p < 0.05$) and AFC (OR = 1.86; 95% CI, 1.02-3.40; $p < 0.05$) showed statistically significant associations with live birth.

Area under the curve for ROC (ROC_{AUC}) indicated that AMH (AUC = 0.60) surpassed AFC (AUC = 0.59), number of oocytes retrieved (AUC = 0.59), inhibin B (AUC = 0.55), FSH (ROC_{AUC} = 0.54) and chronological age (ROC_{AUC} = 0.53) in predicting live birth.

Limitations, reason for caution: The limitation of our study is the lack of comparison data on BMI and smoking habits in our patient population. Additionally the lack of information regarding gonadotropin dosage could influence the results.

Wider implications of the findings: The novel concept of grouping subjects by both circulating AMH concentrations and age has great potential to facilitate decisions concerning ART. It is our opinion, however, that AMH assay alone has limited value from a live birth perspective. If one has not identified a nadir threshold for AMH, below which live birth is simply not feasible, then couples will still want to proceed with treatment.

Study funding/competing interest(s): None

Trial registration number: No

P-476 MTHFR gene polymorphisms and the correlation with estradiol serum levels and assisted reproduction outcomes

V. Cavalcanti, C. Peluso, B.L. Lechado, E.B. Cordts, D.M. Christofolini, C.P. Barbosa, and B. Bianco

Faculdade de Medicina do ABC, Human Reproduction and Genetics Center, Sao Paulo, Brazil

Study question: To correlate the C677T and A1298C polymorphisms of the *MTHFR* gene with estradiol serum level and assisted reproduction outcomes.

Summary answer: The present study found no statistically significant difference for polymorphisms correlated with serum levels of estradiol, ovarian stimulation and assisted reproduction outcomes.

What is known already: *MTHFR* gene has two polymorphisms that lead to a decreased activity of the *MTHFR* enzyme. Recent studies indicate that the synthesis of androgens and estrogens also suffer the influence of these polymorphisms in the *MTHFR* gene. Different concentrations of these hormones in the serum may have negative correlations with the assisted reproduction outcomes as number of oocytes retrieved, implantation rate and abortion.

Study design, size, duration: Cross sectional study that included 142 infertile women infertile women that underwent *in vitro* fertilization (n = 41 tuboperitoneal factor, n = 66 male infertility and n = 35 idiopathic infertility).

Participants/materials, setting, methods: All patients were ≤ 38 years old, had normal prolactin and TSH levels, both ovaries without morphological abnormalities, ovulatory cycle, BMI ≤ 30 , no previous history of poor ovulatory response, and no evidence of endocrine disorders and/or endometriosis. Genotyping were performed using *TaqMan* methodology. The measurement of estradiol was performed by ELISA.

Main results and the role of chance: Considering the controlled ovarian hyperstimulation response 66.9% (95/142) showed good response, 24.6% (35/142) poor response; 5.6% (8/142) hyper response, and 2.8% (4/142) developed ovarian hyperstimulation syndrome. The results of ovarian stimulation, serum

estradiol levels and assisted reproduction outcomes (such as number of retrieved oocytes, embryo transferred, good embryos quality and/or pregnancy rate) showed no statistically significant difference when correlated to the *MTHFR* polymorphisms.

Limitations, reason for caution: Number of patients is low.

Wider implications of the findings: Previous studies observed that women underwent assisted reproduction techniques carrying the T allele of the *MTHFR* C677T polymorphism showed reduced ovarian response to recombinant FSH (FSHr). Furthermore, these patients have significantly lower concentrations of estrogen and showed significantly less oocytes retrieved. However, the present study found no statistically significant difference for polymorphisms correlated with serum levels of estradiol, ovarian stimulation and assisted reproduction outcomes.

Study funding/competing interest(s): CNPq/PIBIC

Trial registration number: Not applicable

P-477 Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60,000 cycles

C.A. Venetis, E.M. Kolibianakis, J. Bosdou, and B.C. Tarlatzis
Aristotle University of Thessaloniki, Unit for Human Reproduction - 1st Dept. of Obstetrics and Gynaecology, Thessaloniki, Greece

Study question: Is progesterone elevation (PE) on the day of human chorionic gonadotropin (hCG) administration associated with the probability of pregnancy in in-vitro fertilization (IVF) cycles?

Summary answer: In women undergoing fresh embryo transfer (ET) after IVF, PE on the day of hCG is associated with a significantly decreased probability of pregnancy achievement. On the other hand, an adverse effect of PE does not seem to be present in frozen-thawed and donor/recipient cycles.

What is known already: Numerous studies on the association of PE with the probability of pregnancy have been published so far with discrepant and inconclusive results. This has led many clinicians to highly doubt the presence of an association of PE with the probability of pregnancy, and others to implement specific strategies to avoid or manage PE. Moreover, data concerning the effect of PE on the outcome of frozen-thawed or donor/recipient cycles have not yet been systematically appraised.

Study design, size, duration: A systematic review and meta-analysis was performed by conducting a literature search (until August 2012) in Medline, Scopus, Central and ISI Web of Science aiming to identify studies comparing the probability of pregnancy in patients with or without PE after ovarian stimulation with gonadotrophins and GnRH analogues.

Participants/materials, setting, methods: Eligible studies were those evaluating the effect of PE on the day of hCG on pregnancy rates in fresh, frozen-thawed and donor/recipient cycles. Studies reporting that the levels of P affected patient management or that significantly different numbers of embryos were transferred between the groups compared were excluded.

Main results and the role of chance: Sixty-three eligible studies were identified evaluating 55,199 fresh IVF cycles, nine studies evaluating 7,229 frozen-thawed cycles and eight studies evaluating 1,330 donor/recipient cycles. To assess the effect of PE on pregnancy rates, five different PE threshold groups were constructed, based on the PE thresholds used in the eligible studies (0.4-0.6 ng/mL, 0.8-1.1 ng/mL, 1.2-1.4 ng/mL, 1.5-1.75 ng/mL, 1.9-3.0 ng/mL). An adverse effect of PE on pregnancy achievement was detected in four out of five PE threshold groups evaluated: 0.4-0.6 ng/mL: OR = 0.39, (95% CI: 0.14-1.08); 0.8-1.1 ng/mL: OR = 0.79, (95% CI: 0.67-0.95); 1.2-1.4 ng/mL: OR = 0.67, (95% CI: 0.53-0.84); 1.5-1.75 ng/mL: OR = 0.64, (95% CI: 0.54-0.76); 1.9-3.0 ng/mL: OR = 0.68, (95% CI: 0.51-0.91). No adverse effect of PE was observed in the frozen-thawed and the donor/recipient cycles.

Limitations, reason for caution: The included studies are characterized by heterogeneity in terms of the population evaluated, as well as in terms of the clinical protocols used for ovarian stimulation and embryo transfer. The actual magnitude of the effect detected might vary in specific subpopulations or with the use of different clinical protocols.

Wider implications of the findings: The presence of a detrimental effect of PE on the day of hCG on pregnancy rates in fresh cycles and the absence of such an effect in donor/recipient and frozen-thawed cycles suggests that PE impairs endometrial receptivity and not embryo quality. Randomized controlled trials exploring the optimal way of managing PE are urgently warranted.

Study funding/competing interest(s): None

Trial registration number: N/A

P-478 Pregnancy outcomes according to endometrial thickness in ovulation induction cycles with clomiphene citrate

M. Onal, D.N. Gungor, M. Acet, and S. Kahraman

Istanbul Memorial Hospital, ART and Reproductive Genetics Unit, Istanbul, Turkey

Study question: Is there any difference in pregnancy outcomes between endometrial thicknesses above and below 7mm?

Summary answer: The objective of this study is to understand the pregnancy outcomes comparing thin endometrium (<7mm) with thick endometrium (≥7mm) in CC cycles.

What is known already: Thin endometrium has been observed in some women who received clomiphene citrate(CC) for ovulation induction and this is because of the antiestrogenic effect of the drug. Endometrial lining is often accepted as “thin” when it is under 7mm. Some patients has thin endometrium whether some of them has thick endometrium in CC cycles. It is not clearly known the meaning of thickness on pregnancy outcomes.

Study design, size, duration: This is a retrospective study. 414 CC COS cycles were included to study. Patients were divided into two groups: Group A with endometrial thickness less than 7mm, Group B with more and equal to 7mm. Patients who underwent controlled ovarian stimulation with CC from January 2001 to October 2012.

Participants/materials, setting, methods: All patients were under 40 years old. 100mg CC was given between days 3-7. hCG day endometrial thickness was noted. pregnancy/ongoing pregnancy and miscarriage rates were calculated. Mann Whitney U, Kruskal Wallis, Chi square test and Fisher tests were used for statistical analysis. $P < 0.05$ is accepted as statistically significant.

Main results and the role of chance: 414 CC COS cycles were included to study. 110 patients had endometrial thickness lower than 7mm(5.93 ± 0.72)(Group A). 304 patients had endometrial thickness 7mm and more (9.10 ± 1.63)(Group B). Baseline characteristics were similar in both groups. (Age, BMI, fertilisation rates, MII oocyte number, number of previous trials) There were no statistically difference in miscarriage/clinical pregnancy and ongoing pregnancy rates between group A and B.

Limitations, reason for caution: Pregnancy outcomes can not be depended only on endometrial thickness. Other factors should be taken to consideration.

Wider implications of the findings: Thin endometrium in CC cycles does not have any effect on pregnancy outcomes so there is no need to give medication or to step up gonadotropins to increase endometrial thickness.

Study funding/competing interest(s): No funding is used in this study

Trial registration number: this is not a RCT

	Group A <7mm	Group B ≥7mm	
Miscarriage rate %	10(11/110)	11.5(35/304)	$p > 0.05$
Clinical pregnancy rate %	26,3(29/110)	24,6(75/304)	$p > 0.05$
Ongoing pregnancy rate %	21,8(24/110)	18,7(57/304)	$p > 0.05$

P-479 Mid-pregnancy and perinatal reproductive endocrinology in singletons and twins

E. Kuijper¹, J. Twisk², M. Caanen³, T. Korsen³, P. Hompes³, M. Kushnir⁴, A. Rockwood⁴, W. Meikle⁴, and C.B. Lambalk³

¹Vrije Universiteit Medical Center, Obstetrics and Gynaecology, Amsterdam, The Netherlands, ²Vrije Universiteit Medical Center, Epidemiology, Amsterdam, The Netherlands, ³Vrije Universiteit Medical Center, Obstetrics and Gynaecology, Amsterdam, The Netherlands, ⁴ARUP Institute for Clinical & Experimental Pathology, Pathology, Salt Lake City, The Netherlands

Study question: Is there a difference in reproductive hormone status in maternal serum (mid-gestation and at delivery) and umbilical cord blood, between singletons and twins.

Summary answer: Estrogens are higher in maternal serum in twins during gestation but lower in umbilical cord blood compared to singletons. In boys from opposite-sex twins we found lower LH, testosterone and Inhibin B at time of birth compared to boys from DZ same-sex twins. Values in girls from opposite-sex twins did not differ from same-sex twins.

What is known already: until now it is believed that children born as part of a twin are exposed to higher estrogen levels during gestation and that in opposite-sex twins girls are influenced by androgens produced by their brothers and boys by estrogens produced by their sisters.

Study design, size, duration: Design: Prospective cohort study

Size: we have included 248 singleton mothers at mid-gestation, at delivery we have collected blood from 178 mothers and 178 children. For the twins we have included 209 mothers at 20 weeks, 174 at birth and blood from 155 twins.

Duration: January 2004-October 2009

Participants/materials, setting, methods: Maternal venous serum samples at mid-gestation and during labour were collected, at that time umbilical cord blood was sampled as well. Estrogens, androgens, gonadotropins, SHBG, AMH, inhibin and progesterone levels were compared using multilevel analyses and adjusted for age, BMI, smoking status, parity, mode of conception, ethnicity, birthweight and gestational age.

Main results and the role of chance: Mothers of twins have higher estrogen levels at 20 weeks and at time of delivery compared to mothers of singletons ($p = 0.000$), however cord blood estrogens are lower in twin compared to singleton neonates (E1 $p = 0.000$, E2 $p = 0.016$).

Girls of a girl/girl DZ twin show no difference compared to girls of an opposite-sex twin, however boys of an opposite-sex twin have lower LH, testosterone and inhibin B compared to DZ same-sex twins.

Limitations, reason for caution: Despite its prospective nature in a substantial number of cases umbilical cord blood was not sampled after delivery

Wider implications of the findings: We have demonstrated for the first time; 1) that in contrast to what is commonly believed twin children are not exposed to higher estrogens compared to singletons, estrogens are even lower in cord blood and 2) girls of an opposite-sex twin do not seem to show early signs of androgen effects caused by their male co-twin, but boys from an opposite-sex twin show lower LH, inhibin B and testosterone levels compared to boys from a DZ same-sex twin. These findings need to be taken into consideration when biomedical and psychological observations assume a relation with certain perinatal sex-hormone exposures.

Study funding/competing interest(s): Not applicable

Trial registration number: Not applicable

P-480 Does combined oral contraceptive use change serum 25-hydroxy vitamin D levels in patients with polycystic ovary syndrome?

D. Hizli¹, Z. Kamalak², A. Kosus¹, N. Kosus¹, N.O. Turhan³, and H. Kafali¹
¹Fatih University, Obstetrics and Gynecology, Ankara, Turkey, ²Nene Hatun Hospital, Obstetrics and Gynecology, Erzurum, Turkey, ³Mugla Sitki Kocman University, Obstetrics and Gynecology, Mugla, Turkey

Study question: Is there an effect of combined oral contraceptive (COC) use on serum 25-hydroxy vitamin D (25OHD) levels in patients with polycystic ovary syndrome (PCOS)?

Summary answer: Use of COC seems to have no significant effect on serum 25OHD levels in patients with PCOS.

What is known already: Vitamin D deficiency is common in women with PCOS, with the 67–85% of women with PCOS having serum concentrations of 25OHD <20 ng/ml. Accumulating evidence indicates that vitamin D deficiency may be a causal factor in the pathogenesis of insulin resistance and PCOS. For years, COCs

have been the first-line drugs for the treatment of PCOS. However, the impact of COC use on serum 25OHD levels has not been evaluated to date.

Study design, size, duration: From January 2011 to April 2012, seventy women who had newly diagnosis of PCOS were included in this prospective study.

Participants/materials, setting, methods: PCOS was defined by the 2003 Rotterdam criteria. All patients with PCOS were treated with a COC containing 0.035 mg ethinylestradiol and 2 mg cyproterone acetate for 6 months. Serum 25OHD levels, ovarian volume and antral follicle count were measured before and after the treatment.

Main results and the role of chance: The median 25OHD levels were 9.40 (range 4.40-24.50) $\mu\text{g/l}$ and 7.00 (5.00-13.50) $\mu\text{g/l}$ before and after COC use, respectively. Serum 25OHD levels decreased after the treatment, however the difference was not statistically significant ($p = 0.055$).

Limitations, reason for caution: The main limitation of our study was the absence of control group. However, it might be the secondary outcome of the study, because our primary outcome was to evaluate the effect of COC use on serum 25OHD levels in patients with PCOS. The follow-up period might be longer than 6 months. However, in most of the studies clinical and laboratory changes were evaluated after 3 or 6 cycles of COC treatment.

Wider implications of the findings: The present study seems to be the first prospective trial revealing the effect of COC use on serum 25OHD levels in women with PCOS. Although the decrease in serum 25OHD levels in patients with PCOS with of the use of COC alone, did not reach to statistically significance level after 6 months. Vitamin D supplementation might be recommended in patients who are receiving COC for PCOS until the results of larger prospective RCT's with longer duration are reported.

Study funding/competing interest(s): This study was supported by the Scientific Research Fund of Fatih University.

There is Trial registration number: P53011107_B 1533

P-481 Prenatal androgen excess programs metabolic derangements in pubertal female rats

X. Yan, X. Dai, J. Wang, N. Zhao, Y. Cui, and J. Liu

First Affiliated Hospital Nanjing Medical University, State Key Laboratory of Reproductive Medicine Clinical Center of Reproductive Medicine, Nanjing, China

Study question: The clinical manifestations of PCOS often emerge before or during puberty and peripubertal metabolic dysfunction is one of the first phenotypic traits observed in adolescent girls with PCOS. However, the molecular mechanisms involved in metabolic derangements in adolescents with PCOS are yet to be elucidated.

Summary answer: Our findings validate the contribution of prenatal androgen excess to metabolic derangements of PCOS in pubertal female rats, and the impaired insulin signaling through IRS and AKT may result in the peripheral insulin resistance during pubertal development.

What is known already: Clinical, experimental and genetic evidence supports an interaction between genetic susceptibility and the influence of maternal environment in the pathogenesis of PCOS. The emerging field of epigenetics has revealed that abnormal endocrine and metabolism in mothers perturb the *in utero* environment and alter the developmental trajectory of multiple organ systems in the fetus, thus greatly enhancing the likelihood of diseases in the offspring.

Study design, size, duration: To determine whether prenatal androgen excess induced PCOS-related metabolic derangements during pubertal development, we administered pregnant rats with 5α -dihydrotestosterone (DHT) and observed their female offspring from postnatal 4 to 8 weeks.

Participants/materials, setting, methods: The ovarian morphology, serum steroid hormone and metabolic parameters in the prenatally androgenized (PNA) rats were observed at the age of 4-8 weeks. We determined whether the PNA rats manifested impaired glucose tolerance and insulin resistance and whether the resulting metabolic derangements were mediated by peripheral insulin signaling during puberty.

Main results and the role of chance: The PNA rats exhibited more numerous total, cystic and atretic follicles than control. Fasting glucose, insulin and leptin levels were elevated in the PNA rats at the age of 5-8 weeks. Following glucose load, glucose and insulin levels did not differ between two groups; however, the PNA rats showed significantly higher 30- and 60-min glucose levels than control after insulin stimulation during 5-8 weeks. In addition,

prenatal DHT treatment significantly decreased insulin-stimulated phosphorylation of IRS1 and AKT in the skeletal muscles and liver of 6-week-old PNA rats. These findings validate the contribution of prenatal androgen excess to metabolic derangements in pubertal female rats, and the impaired insulin signaling through IRS and AKT may result in the peripheral insulin resistance during puberty.

Limitations, reason for caution: In our study, evidence of hyperandrogenism was not apparent in PNA rats during puberty, possibly because androgen levels were low during pubertal development and difficult to be evaluated. In addition, the use of RIAs for testosterone measurements in pubertal PNA rats may be a potential limitation of our study.

Wider implications of the findings: In this study, we found that prenatal androgen excess programmed both the reproductive and metabolic derangements of PCOS in pubertal female rats, similar to the development of PCOS in adolescents. These findings substantiate a direct relationship of prenatal androgen exposure with the metabolic features of PCOS in adolescents. Further studies are warranted to determine how an early perturbation from *in utero* androgen excess programs the target tissue of the fetus during prenatal developmental periods.

Study funding/competing interest(s): This work was supported by the 973 Program of China (grant numbers 2012CB944902, 2012CB944703) and the National Natural Science Foundation of China (grant number 81070465).

Trial registration number: This study was a basic trial.

P-482 Serum AMH levels in women with a history of early onset preeclampsia indicate a role for vascular factors in ovarian ageing

F. Yarde¹, A.H.E.M. Maas², A. Franx³, M.J.C. Eijkemans⁴, J.T. Drost⁵, B.B. van Rijn³, J. van Eyck⁶, Y.T. van der Schouw⁷, and F.J.M. Broekmans⁸

¹University Medical Center Utrecht, Reproductive Medicine, Utrecht, The Netherlands, ²Radboud University Nijmegen Medical Center, Cardiology, Nijmegen, The Netherlands, ³University Medical Center Utrecht, Obstetrics, Utrecht, The Netherlands, ⁴University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, The Netherlands, ⁵Isala klinieken Zwolle, Cardiology, Zwolle, The Netherlands, ⁶Isala klinieken, Gynaecology & Obstetrics, Zwolle, The Netherlands, ⁷University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, The Netherlands, ⁸University Medical Center Utrecht, Reproductive Medicine, Utrecht, The Netherlands

Study question: To assess whether women with a history of early onset preeclampsia (EOP) have signs of advanced ovarian ageing compared to women with normotensive pregnancies, using AMH as marker for ovarian reserve status. Moreover, to study the possible associations between vascular factors and AMH.

Summary answer: We demonstrate that women with a history of a vascular complicated pregnancy have significantly lower age-adjusted AMH levels compared to women with uncomplicated pregnancies. Also, an association between hypertension at follow up and serum AMH level was found.

What is known already: This is the first study to demonstrate that women with a history of EOP have signs of advanced ovarian ageing compared to women with normotensive pregnancies.

Study design, size, duration: The current study, focusing on women with a history of EOP in a follow up assessment on ovarian reserve status, was designed as a retrospective cohort study. The association between vascular factors (blood pressure, BMI, waist circumference, lipid profile, glucose metabolism) and AMH was assessed in a cross sectional design.

Participants/materials, setting, methods: Clinical data and blood samples of participants of the Preeclampsia-Risk-Evaluation-in-FEMales-study was used; $n = 338$ with a history of EOP and $n = 329$ after a normotensive pregnancy. Linear regression analysis, censored for undetectable AMH levels, was used to investigate the association between EOP and vascular factors and AMH, adjusted for age and smoking.

Main results and the role of chance: Mean age at index pregnancy was 29.8 ± 3.8 in the EOP group compared to 28.6 ± 4.1 years in the reference group and follow-up was 9.1 ± 3.6 versus 10.6 ± 3.0 years, respectively. Mean AMH level, measured at follow-up, was 2.00 ± 1.87 mcg/L in the EOP group, compared to 2.26 ± 2.56 mcg/L in the reference group. Women with a history of

EOP had significantly lower AMH levels than women with normotensive pregnancies. A relative reduction in AMH level by 4% at any age could be calculated (coefficient 0.96, 95%CI 0.93-0.99). Also, an association between hypertension at follow-up and serum AMH was found (coefficient 0.96, 95%CI 0.93-0.99). Other vascular factors were not associated with serum AMH.

Limitations, reason for caution: A small portion of women reported hypertension before the index pregnancy and not all women were primigravida.

Wider implications of the findings: Calculations based on a reference population indicate that a relative decrease in AMH level of 4% corresponds to an advancement in reproductive age of ~4 years. These results support the hypothesis that compromised vascular health could act as a causative mechanism in the ovarian ageing process.

Study funding/competing interest(s): Not applicable

Trial registration number: Not applicable

P-483 Should all young women have their AMH level checked and at what age?

F. Martyn, B. Anglim, and M. Wingfield

Merrion Fertility Clinic/National Maternity Hospital, Obstetrics and Gynaecology/Fertility Centre, Galway, Ireland

Study question: A significant number of young women referred to our fertility clinic had unexpected low AMH levels. This prompted 2 questions (1) should ovarian reserve screening be considered in a woman planning to defer pregnancy or if not in a position to conceive and (2) at what age should screening commence?

Summary answer: 40% of women (201/509) under 35 years referred to a fertility clinic already had AMH <10pmol/L and 19% (95/509) had AMH <5pmol/L. Corresponding figures for those under 30 years were 27% (30/112) and 13% (15/112). 67% of those with AMH <5pmol/L, had no identifiable risk factor for reduced ovarian reserve.

What is known already: Ovarian reserve declines as a woman ages and her reproductive capacity diminishes. In recent years AMH has emerged as a useful marker of ovarian reserve without the intra- and inter- cycle variability of other tests (FSH) and less operator dependence than antral follicle count (AFC). An AMH level of <5 pmol/L significantly impairs reproductive outcome in ART. It also indicates impending loss of ovarian reproductive capacity.

Study design, size, duration: Retrospective study involving 1016 women (18 to 49 years) attending two fertility clinics (Aug 2011 to Dec 2012). Serum AMH levels were measured as part of fertility work-up. Case histories of those <35 years (n = 509) were reviewed to determine risk factors for reduced ovarian reserve and to correlate AMH with FSH and AFC.

Participants/materials, setting, methods: Samples assayed using Beckman Coulter AMH Gen II assay. Women stratified according to age: 30, 31-35, 36-40, 40 and AMH levels as: <1, 1 – 4.9, 5 – 9.9, 10 pmol/L. Clinical history was reviewed. AFC and FSH were correlated with AMH. Statistical analysis: Chi square test and Spearman correlation.

Main results and the role of chance: Of women aged 35 years or less: 39.6% (201/509) had an AMH level of <10 pmol/L at referral; 18% (94/510) of these women had AMH <5 pmol/L and 4% (21/510) less than 1 pmol/L. Among very young women (<30 years), 27% (30/112) had an AMH <10, 13% (15/112) had an AMH <5 and 8% (9/112) had a very worrying AMH <1 pmol/L. Of these women under 35 years with an AMH of <5, no significant correlation was found between AMH levels and endometriosis (p = 0.2) or ovarian surgery (p = 0.2). There was a statistically significant relationship between an AMH <5 pmol/L and a family history of premature ovarian failure (p = 0.01). There was a significant correlation between AMH levels and AFC (significant at 0.01 level).

Limitations, reason for caution: Participants all had subfertility so might be expected to have poorer levels than the fertile population. However, only 33% of those under 35 with AMH <5pmol/L had a risk factor for reduced ovarian reserve which might have prompted earlier attendance. Future research will investigate AMH levels in fertile women attending our hospital.

Wider implications of the findings: Poor ovarian reserve is generally not expected in women <35 years without significant risk factors. Knowledge of poor ovarian reserve may influence life decisions regarding conception. Alternatively, young women may wish to consider oocyte cryopreservation. Our findings

suggest that (1) research should now focus on identifying at an early age those at risk of premature reduction in ovarian reserve and (2) routine AMH screening should be considered for all women in their late 20s who have not yet conceived.

Study funding/competing interest(s): none

Trial registration number: none

P-484 Dehydroepiandrosterone (DHEA) as a Holy Grail in the treatment of Premature Ovarian Failure (POF) patients; hype or hope?

T. Fang, G.J. Yan, H.X. Sun, and Y.L. Hu

The Affiliated Drumtower Hospital Reproductive Medicine Center, Department of Obstetrics and Gynecology, Nanjing Jiangsu, China

Study question: DHEA is being reported as a Holy Grail for the treatment of POF women, while the debate on its actual biological function is still ongoing. The aim of the present study was to evaluate its efficacy in Chinese Han women with POF.

Summary answer: Limited number of options for Ob/Gyners resort to in POF treatment. It is understandable that many of them resort to treatments that are not sufficient evidence based. Our results together with other reported datum indicate that DHEA is still not sufficient to prove their effectiveness in reversing POF outcome.

What is known already: Current best available evidence suggests that DHEA improves ovarian function, increases clinical pregnancy rates (CPR) and lowers miscarriage rates. DHEA also appears to improve ovarian reserve. Animal data support androgens in promoting preantral follicle growth and reduction in follicle atresia.

No significant difference between those treated with androgen supplementation compared with those without androgen supplementation (RR 1.29, 95% CI 0.71, 2.35) was showed in Meta-analysis of the non-randomized controlled studies for the outcome of CPR.

Study design, size, duration: 95 women diagnosed as POF returned monthly for follow-up after DHEA treatment. The mean age at follow-up was 31 ± 4.09 years (range 19-38 years), and the mean follow-up time was 38 months (range 24-50months).

DHEA: at 75 mg a day for 1 months was given each follow-up.

Participants/materials, setting, methods: 95 China Han women with POF exclude of autoimmune diseases, who wanted to become pregnant, were enrolled. This is an open uncontrolled study conducted in outpatient department.

Assessments of ovarian function classified by the Hoogland and Skouby score, thickness of endometrium, and sex hormone levels, as well as overall feeling.

Main results and the role of chance: Among the 95 patients undergoing treatment, 4 lost follow-up. 6 women had one follicle of 10 mm or greater (4 of them had thawed embryo transfers but all failed). 3 clinical pregnancy was achieved, among which only 2 indicate deliveries.

Our data suggest that treatment with DHEA improves the patients overall feeling (55/91). While no significant change in serum FSH levels had been detected throughout the study. Still, AFC and ovarian volume were no significantly higher than pre-treatment levels. (Mean values for FSH, AFC, and ovarian volume were 112 ± 35.2 IU/L, 4 ± 1.6 , and 1030 ± 40.3 mm³ respectively.)

In addition, no difference in ovarian function score was present.

Pregnancies were achieved only in patients whose FSH levels were once lower than 20 mIU/mL.

Limitations, reason for caution: Robust data from RCTs showing an improvement in CPR following DHEA supplementation in women with POF are lacking.

As dietary supplement, the purity and potency of commercially available formulations of DHEA are questionable. Analysis showed that DHEA content ranged from 0 to 150% of the labeled amount.

Wider implications of the findings: Autoimmune etiology and young age are good prognostic factors for future fertility despite POF.

Large-scale, well-designed confirmatory studies are necessary to prove the efficacy of DHEA before it can be recommended for routine use. Our findings raise some immediate practical concerns regarding the use of DHEA.

Since FDA has no requirements for the composition of DHEA, it is also important to choose a standard label to fully analyze its clinical function.

Study funding/competing interest(s): State Key Development Program of Basic Research of China Grant

Competing interest: None stated.

Trial registration number: 973 Project No. 2010CB945104

P-485 The prevalence of insLQ, Asn291Ser and Ser312Asn polymorphisms of luteinizing hormone receptor gene in Czech population and patients with ovarian hyperstimulation syndrome

J. Chrudimská, P. Krenková, M. Macek jr., and M. Macek sr.
University Hospital Motol, Department of Biology and Medical Genetics, Prague 5, Czech Republic

Study question: The aim of this study is to ascertain the prevalence of polymorphisms **Asn291Ser** (rs1470652), **Ser312Asn** (rs2293275) and **insLQ** (insertion of leucine and glutamine, rs58356637) in the luteinizing hormone chorionic gonadotropin receptor (LHCGR) gene in Czech fertile men and women and in patients with ovarian hyperstimulation syndrome (OHSS).

Summary answer: No significant difference was found between Czech control males and females, between Czech, Swedish and Dutch females, between Czech and German males in the genotypes/alleles prevalence of studied polymorphisms. No difference was disclosed between Czech control females and OHSS patients.

What is known already: Polymorphisms Asn291Ser, Ser312Asn and insLQ have been associated with increased LHCGR sensitivity (Altmäe et al., 2011). So far, only the relation of the insLQ polymorphism to the OHSS development was studied by Kerkelä et al (2007) on rather small number of patients without significant association to this life-threatening complication of the controlled ovarian hyperstimulation (COH).

Study design, size, duration: Total of 102 fertile men-controls, 149 fertile female-controls (with documented delivery of at least two children) and 59 patients affected by OHSS type III-V were analyzed. Our OHSS patients were examined within years 2008 – 2012.

Participants/materials, setting, methods: Detection of the Asn291Ser and Ser312Asn polymorphisms was performed using TaqMan SNP Genotyping Assays. The insLQ variation was detected by the capillary electrophoresis with fluorescence-labeled primers. Association studies were analyzed by Pearson chi-squared (χ^2) test.

Main results and the role of chance: The genotype frequencies of Asn291Ser, Ser312Asn and insLQ polymorphisms don't differ between Czech men and women ($P = 0,6320; 0,7922; 0,8329$ respectively), the same was true for allelic frequencies ($P = 0,6168; 0,5528; 0,705$ respectively). No significant differences were disclosed between genotype frequencies of Asn291Ser, Ser312Asn and insLQ in Czech female controls and data from our OHSS patients ($P = 0,6697; 0,4446; 0,3471$ respectively) and the same was true for allelic frequencies ($P = 0,5418; 0,2533; 0,9734$ respectively).

The genotypes/allelic frequencies of studied polymorphisms of Czech women controls did not differ from so far studied women from The Netherland (Valkenburg et al, 2009) and Sweden (Kerkelä et al, 2007). Similarly, no difference was found between Czech and German males (Simoni et al, 2008).

Limitations, reason for caution: The OHSS pathogenesis is very complex. It is necessary to integrate association with mutations/polymorphisms of other biomarkers as luteinizing hormone, follicle stimulating hormone, hormone receptors and others (Altmäe et al, 2011). Our study will be extended to increase number of controls and severe OHSS patients to confirm these results.

Wider implications of the findings: Detected prevalence of genotypes/alleles of insLQ, Asn291Ser and Ser312Asn polymorphisms in Czech fertile males and females will be used for further association studies in dysfertility, hormone-dependent diseases and cancers and for the individualization of the hormonal treatment for COH to increase its efficacy and decrease its risks.

Study funding/competing interest(s): Supported by grants IGA NT13770-4/2012 (Ministry of Health, CZ), project for conceptual development of research organization 00064203 (University Hospital Motol, CZ) and OPK CZ.2.16/3.1.00/24022 (EU).

Trial registration number: No RCT project

P-486 Low-dose human chorionic gonadotropin at oocyte retrieval after gonadotropin-releasing hormone agonist triggering: retrospective study

J. Teixeira da Silva¹, M. Cunha², J. Silva², P. Viana², A. Gonçalves³, N. Barros², C. Oliveira¹, M. Sousa⁴, and A. Barros⁵

¹Centre for Reproductive Genetics Prof. Alberto Barros, IVF-Clinics, Porto, Portugal, ²Centre for Reproductive Genetics Prof. Alberto Barros, IVF-Lab, Porto, Portugal, ³Centre for Reproductive Genetics Prof. Alberto Barros, Andrology-Lab, Porto, Portugal, ⁴Institute of Biomedical Sciences Abel Salazar UMIB University of Porto, Dept. Microscopy Lab. Cell Biology, Porto, Portugal, ⁵Centre for Reproductive Genetics Prof. Alberto Barros Dept. Genetics FMUP, IVF-Director, Porto, Portugal

Study question: The aim of the study was to compare the pregnancy rates and risk of ovarian hyperstimulation syndrome (OHSS) after GnRH-agonist trigger and low-dose hCG for luteal support versus hCG/rhCG trigger (conventional protocol).

Summary answer: A clinically significant OHSS was avoided using a tailored GnRH-antagonist controlled ovarian stimulation (COS)/GnRH-agonist trigger protocol and 1500 UI hCG at the day of oocyte retrieval for luteal support, without compromising the pregnancy rates.

What is known already: OHSS is the most frequent complication of COS during ART, being hCG to induce oocyte maturation the main cause for the development of this syndrome. The alternative, using a GnRH-agonist in a GnRH-antagonist COS protocol, virtually eliminates OHSS but can lead to a significant decrease in pregnancy rates, even with intensive luteal-phase steroid supplementation. Using this approach, two options are in debate: cryopreservation of all embryos or a change of the luteal support.

Study design, size, duration: From November-2011-December-2012, 86-IVF/ICSI antagonist COS protocol cycles were divided in two groups: 39-cycles used Triptorelin 0,2mg s.c (study-group) and 47-cycles used hCG/rhCG (control-group), to trigger final oocyte maturation. In the study-group, besides intravaginal progesterone (600mg/day), 1500 UI hCG at oocyte retrieval and oral oestradiol (4mg/day) was added for luteal support.

Participants/materials, setting, methods: The study included high-risk OHSS patients < 38 years of age and with at least one of the following conditions: ≥ 20 follicles, estradiol ≥ 3000 pg/mL on the day of oocyte trigger, or ≥ 13 oocytes. The choice of either GnRH-agonist or hCG/rhCG trigger was based on physician option.

Main results and the role of chance: Data showed no significant differences between the study and control groups for female age, time of infertility, polycystic ovarian syndrome patients, and estradiol on trigger day. In the study-group we observed a lower body mass index ($p = 0,044$), a higher number of follicles ($p < 0,001$), although the total dose of FSH/hMG ($p = 0,004$) was lower. Regarding laboratory data, there were no significant differences in the number of oocytes obtained, oocyte maturation and fertilization rates. The mean number of embryos transferred was significantly higher in the control-group ($p < 0,01$). There were no differences in the implantation (55.1% vs 47.9%), clinical pregnancy (63.2% vs 59.1%), ongoing pregnancy (42.1% vs 52.3%), twins (12.5% vs 34.6%), miscarriage (33.3% vs 11.5%) and clinically significant OHSS (0.0% vs 8.5%) rates between the two groups.

Limitations, reason for caution: Although not significant, the miscarriage rate found in the study-group indicates the need for adjustments in timing/dosages of hCG. In a subgroup of patients with estradiol ≥ 4000 pg/mL on GnRH-agonist trigger-day we did not observe any miscarriage, so we might consider a second hCG dose in lower OHSS risk patients.

Wider implications of the findings: Clinically significant OHSS was avoided using a tailored GnRH-antagonist COS/GnRH-agonist trigger protocol and 1500 UI hCG at oocyte retrieval-day for luteal support. Although not statistically significant, we had 4-OHSS cases in the control-group (2 cycles cancelation on embryo transfer day and 2 late-onset OHSS hospitalizations). In our opinion this is a promising protocol in which a similar ongoing pregnancy rate is possible in parallel with an important reduction in the risk of clinically significant OHSS.

Study funding/competing interest(s): None.

Trial registration number: None.

P-487 Prediction of live birth from IVF: enhanced discrimination and calibration with inclusion of AMH in the IVFpredict model

S.M. Nelson¹, S.M. Lloyd¹, A. McConnachie¹, A. Khader¹, R. Fleming¹, and D.A. Lawlor²

¹University of Glasgow, Centre for Population and Health Sciences Reproductive & Maternal Medicine, Glasgow, United Kingdom, ²University of Bristol, Department of Social Medicine, Bristol, United Kingdom

Study question: To assess whether a nationally derived, externally validated, model of live birth prediction for IVF (IVFpredict) works at an individual clinic level and if inclusion of AMH improves model performance.

Summary answer: After recalibration for the higher live birth success rate in this clinic compared to that predicted by IVFpredict, the IVFpredict model was accurate in a population from a regional single clinic. Inclusion of log(AMH) produced a slight improvement in model performance.

What is known already: Prediction models like IVFpredict allow pretreatment stratification of the likelihood of live birth after IVF. AMH is a marker of ovarian reserve and through its relationship with functional ovarian reserve has been associated independent of age with live birth after IVF.

Study design, size, duration: Prospective cohort study comprising 656 patients undertaking IVF from 1 January 2009 to 1 December 2009 in a single private IVF centre.

Participants/materials, setting, methods: All patients undertaking an IVF cycle had an AMH measured using the DSL assay and their baseline phenotypic data collected in accordance with the Human Fertilisation and Embryology Authority Act 1990 and UK regulatory legislation. Miscalibration of the IVFpredict model was assessed using graphical methods and logistic regression. The association between AMH and live birth rates (adjusting for IVFpredict model predictions) was assessed using logistic regression. The potential of AMH to improve model performance was assessed using the receiver operator characteristic area under curve (AUC) and net reclassification index (NRI).

Main results and the role of chance: The IVFpredict model underestimated the likelihood of live birth, which for this centre was higher than the national average. Adjusting for this miscalibration, resulted in accurate prediction. Addition of log(AMH) to the IVFpredict model prediction was associated with a small improvement in model performance: difference in AUC 0.0217 (95% CI 0.0040, 0.0393); NRI +7.67% (95% CI +0.9%, +14.0%; $p = 0.04$). Recalibration reduced the observed improvement in NRI attributable to AMH though this still remained positive; NRI 3.9% (-0.2%, 8.0%), $p = 0.06$

Limitations, reason for caution: Risk within quintiles was imprecisely estimated (i.e. wide confidence intervals) because of the relatively small sample size. The miscalibration of IVFpredict observed in this study may not be representative of other clinic populations.

Wider implications of the findings: Adjusting IVFpredict to take into account an individual clinic's performance relative to the national benchmark allows accurate prediction of live birth at an individual clinic level. With recalibration inclusion of AMH in the model is associated with a small improvement in the overall IVFpredict performance, with 3.9% more couples being correctly classified, due largely to improved identification of couples who are not successful with a live birth.

Study funding/competing interest(s): The UK Medical Research Council (G0600705) and the University of Bristol provide core funding for the MRC Centre of Causal Analyses in Translational Epidemiology.

Trial registration number: Not applicable

P-488 Intrafollicular endocrine milieu after addition of different doses of hCG to rFSH throughout controlled ovarian stimulation for IVF

L. Thuesen¹, A. Nyboe Andersen¹, A. Loft¹, and J. Smitz²
¹Copenhagen University Hospital, The Fertility Clinic Section 4071, Copenhagen, Denmark, ²Vrije Universiteit Brussel UZ Brussel, Follicle Biology Laboratory, Brussels, Belgium

Study question: Does hCG supplementation to rFSH during controlled ovarian stimulation (COS) affect the intrafollicular hormone concentrations in relation to hCG dose, follicular sizes, and embryo quality?

Summary answer: Increasing hCG dose induced a marked stimulation on the intrafollicular concentration of estradiol and androgens; with a shift towards an androgenic milieu. In large follicles with oocytes giving rise to good quality embryos, the fluids were significantly more estrogenic than in small follicles with oocytes developing into poor quality embryos.

What is known already: Results from large clinical trials have indicated that highly purified human Menopausal Gonadotrophin (HP-hMG), containing LH/hCG activity, induced significant differences in hormone profiles in serum and follicular fluids (FF) accompanied by improvement of embryo quality. Our published data showed that supplementation of hCG throughout COS increased the number

of top-quality embryos and influenced the level of androstenedione, progesterone, and estradiol significantly (Thuesen et al., 2012).

Study design, size, duration: In a prospective randomised dose-response study at a University Hospital, 62 normo-ovulatory IVF patients were treated using a GnRH agonist protocol with rFSH 150 IU/day and randomised to addition of hCG from stimulation Day 1: D0: 0 IU/day, D50: 50 IU/day, D100: 100 IU/day, and D150: 150 IU/day.

Participants/materials, setting, methods: From separately collected follicles ($n = 816$), the oocytes were followed individually, and 334 FF were selected for analyses according to predefined criteria. In small (<2.1 mL) and large follicles (≥ 2.1 mL), the relationship between intrafollicular endocrine milieu and development capacity of oocytes was assessed in relation to hCG dose.

Main results and the role of chance: In large follicles, hCG-dosing induced a significant nearly 3-fold increase of estradiol (D0: 1496 nmol/l; D100: 4338 nmol/l ($P < 0.001$)) and androstenedione (D0: 63 nmol/l; D100: 208 nmol/l ($P < 0.001$)), and a 5-fold increase of testosterone (D0: 15 nmol/l; D100: 72 nmol/l ($P < 0.001$)). The estradiol/testosterone ratio decreased significantly with the lowest ratio in D100 and the highest in D0. Good quality embryos originating from large follicles and poor quality embryos from small follicles were differentiated by significantly higher estradiol and progesterone levels, estradiol/testosterone, estradiol/androstenedione, and progesterone/estradiol ratios.

Limitations, reason for caution: The embryo quality is not only associated with the hormone levels present in FF, but is a combination of various factors including physiology and accompanying diseases. However, we tried with strict patient inclusion criteria to minimize these potential biases.

Wider implications of the findings: Based on our clinical results, we are able to propose for normo-ovulatory patients that an optimal dose of hCG addition to rFSH throughout COS for IVF is in a range up to 100 IU/day. In clinical routine, hCG is used in doses around 20–30 IU per day when using HP-hMG. A trial with a larger sample size is evidently needed to substantiate this proposal.

Study funding/competing interest(s): Ferring Pharmaceuticals, Research and Development, provided funds for the hormone analyses.

Trial registration number: ClinicalTrials.gov (NCT00844311).

P-489 Accuracy of glycated hemoglobin (HbA1c) in detecting abnormal glucose metabolism in women with PCOS

M. Abdel-Rahman¹, S. Ismail¹, J. Silk², M. Abdellah³, and A.H. Abdellah⁴
¹Sohag University Hospital, OB/GYN, Sohag, Egypt, ²Case Western Reserve University, OB/GYN, Cleveland, U.S.A., ³Sohag School of Medicine, OB/GYN, Sohag, Egypt, ⁴Qena School of Medicine, OB/GYN, Qena, Egypt

Study question: Does glycated hemoglobin improve detection of abnormal glucose metabolism in women with PCOS?

Summary answer: Glycated hemoglobin could be used as a marker of abnormal glucose metabolism in women with PCOS. Cut-off point of 5.75 may be used for detecting diabetes and 5.45 for pre-diabetes.

What is known already: A change of the diagnostic tool for type-2-diabetes from an oral glucose tolerance test (OGTT) to hemoglobin A1c (HbA1c) has been suggested in general population. The aim of this study was to assess the accuracy of HbA1c in detecting abnormal glucose metabolism (diabetes and pre-diabetes) in women with PCOS.

Study design, size, duration: Women with PCOS from North East Ohio, USA ($N = 172$) were recruited during July 2010 and January 2012. PCOS was diagnosed according to Rotterdam criteria (2003) and abnormal glucose metabolism was diagnosed using the American Diabetes Association guidelines (2010).

Participants/materials, setting, methods: All participants received 75-g OGTT as a gold standard. HbA1c was analyzed using ion exchange chromatography (Bio-Rad Laboratories). Receiver operating characteristic (ROC) curve was developed to assess the accuracy of HbA1c compared to OGTT as a gold standard.

Main results and the role of chance: Using the ADA standards; the sensitivity, specificity, positive likelihood ratio (LR) and negative LR of HbA1c in diagnosing DM II was 100% (CI: 93-100), 31% (CI: 26-36), 1.4 (CI: 1.3-1.5) and 0 respectively. Nevertheless; the sensitivity, specificity, positive LR, negative LR of HbA1c in diagnosing pre-DM was 50% (CI: 34-66), 70% (CI: 62-77), 1.7 (CI: 1.1-2.5) and 0.7 (CI: 0.5-1.0) respectively. The best cut-off point for diagnosing DM II in the study group was 5.75 according to the ROC curve with an area under the curve (AUC) of 0.91. The area under the curve was lower (0.65) when diagnosing

pre-DM using the HbA1c compared to OGTT as a gold standard. A cut-point of 5.45 resulted in 75% sensitivity and 50% specificity.

Limitations, reason for caution: The sample size of the study is small to reach a firm conclusion. More patients will be recruited to get a proper sample size.

Wider implications of the findings: Glycated hemoglobin is a simple and accurate test for abnormal glucose metabolism screening in women with PCOS

Study funding/competing interest(s): None

Trial registration number: N/A

P-490 Endocrine profile during controlled ovarian stimulation with recombinant versus urinary gonadotropins (FSH + LH)

F. Ruiz, M. Cruz, M. Piro, D. Collado, J.A. Garcia-Velasco, and A. Requena
IVI Madrid, Gynaecology, Madrid, Spain

Study question: This study aims to compare the endocrine profile in serum and follicular fluid derived from a controlled ovarian stimulation with either recombinant (rFSH + rLH) gonadotropins or urinary (uFSH + uLH) gonadotropins.

Summary answer: Data showed similar hormonal profiles regardless of the presence of urinary hCG in urinary preparations. Although progesterone levels are slightly but not significantly higher with recombinant preparations, testosterone and estradiol levels are indicative of a good conversion from progesterone to estrogens, suggesting that recombinant LH is as effective as uHMG.

What is known already: It is well-known that the LH activity in the hMG preparations is derived from the hCG rather than the LH content. Exposure to hCG from the start of stimulation leads to a specific endocrine profile characterized by low levels of progesterone and higher serum androgen and estradiol concentrations. The question is if the introduction of recombinant LH from the beginning of the protocol leads to a similar endocrine profile than those obtained with exogenous hCG.

Study design, size, duration: A prospective, observational study was performed with 50 oocyte donors undergoing ovarian stimulation along 2012. Two groups were established: a) Pergoveris®: combination of recombinant gonadotropins (rFSH + rLH) or b) Menopur®: mixture of urinary gonadotropins (uFSH + uLH) plus Bravelle® (uFSH). Blood samples were obtained on day 5 and the day of hCG administration.

Participants/materials, setting, methods: Subjects were assigned to receive either an ovarian stimulation protocol with Pergoveris® (150 IU rFSH + 75 IU rLH) (n = 25) or Menopur® (75 uFSH + 75 IU uLH) plus Bravelle® (75 IU uFSH) (n = 25). Patients were tested for serum and follicular (>17 mm) levels of estradiol, progesterone and testosterone during the stimulation protocol.

Main results and the role of chance: Estradiol levels were slightly higher although the difference was not statistically significant on day 5 and the day of triggering in the recombinant group compared to the urinary group (436.4 ± 181.9 vs 323.4 ± 80.6 pg/ml, $p = 0.241$; 3377.7 ± 703 vs 2547 ± 527.9 pg/ml, $p = 0.053$). Progesterone levels were 0.39 ± 0.07 vs 0.34 ± 0.07 ng/ml, $p = 0.347$ (day 5) and 0.89 ± 0.17 vs 0.72 ± 0.20 ng/ml, $p = 0.156$ (hCG day) for the recombinant and urinary groups respectively; testosterone levels were 0.57 ± 0.19 vs 0.53 ± 0.11 ng/ml, $p = 0.683$ (day 5) and 0.79 ± 0.13 vs 0.68 ± 0.09 ng/ml, $p = 0.165$ (hCG day) also for recombinant and urinary preparations respectively. No statistically significant differences were found between the groups.

In follicular fluid no statistically significant differences were found between both groups.

Ongoing pregnancy rates were similar (46.1% vs 46.1%, $p = 1.000$) for recombinant and urinary gonadotropins respectively.

Limitations, reason for caution: The small sample size could act as a limitation to explain the results.

Wider implications of the findings: Hormonal profile associated with exogenous LH/hCG supplementation during ovarian stimulation may differ because there are different types of exogenous compounds with LH activity, i.e. rLH or hCG.

Our data suggests that is possible to use either recombinant LH or hCG in ovarian stimulation in order to provide a good hormonal milieu for oocyte maturation and, in consequence, good pregnancy rates.

Study funding/competing interest(s): The study was privately funded. No competing interests to declare.

Trial registration number: There is no trial registration number.

P-491 FSH-responsiveness as well as Anti-Mullerian hormone secretion is higher in granulosa cells in vitro from naturally matured follicles than from gonadotropin stimulated follicles

Z. Kollmann¹, N.A. Bersinger¹, B. McKinnon², S. Schneider¹, M.D. Mueller¹, and M. von Wolff¹

¹University of Berne, Obstetrics and Gynaecology, Berne, Switzerland,

²University of Berne, Clinical Research, Berne, Switzerland

Study question: Are granulosa cells (GCs) from naturally matured follicles – as in Natural Cycle IVF (NC-IVF) – responding better to FSH secretion than GCs from conventional gonadotropin stimulated IVF (cIVF) and is the response associated with increased production of Anti-Mullerian hormone (AMH) and AMH-mRNA?

Summary answer: FSH stimulation increased *in vitro* progesterone concentration 4-fold in cultures of GCs obtained from a NC-IVF whereas GCs from cIVF-cells did not respond. This increase was in line with elevated AMH production in cultures of GCs from NC-IVF when compared with AMH release in cIVF-cells.

What is known already: Culture of GCs is a suitable system to study the regulation of AMH by FSH *in vitro*. Granulosa cells express FSH receptors by the secondary stage of follicle maturation at the time of rising AMH production. AMH levels in follicles of IVF patients correlate with follicle sensitivity to FSH. Moreover, AMH inhibits follicle recruitment and selection. Follicular fluid in naturally matured follicles – as in NC-IVF – has been found to contain higher concentrations of AMH.

Study design, size, duration: Lutein GCs from the dominant follicle were isolated and cultured in absence or presence of recombinant FSH. We included 29 NC-IVF and 24 cIVF cycles performed in 2011 and 2012. Mean age was 35.1 ± 4.8 (SD) and 37.6 ± 3.4 years in the NC-IVF and cIVF groups.

Participants/materials, setting, methods: cIVF was performed following antagonist protocol. GCs were collected from the leading follicle, plated at $10,000/\text{cm}^2$ in complete Iscove's medium and cultured for 6 days +/- FSH. Progesterone and AMH were determined in the supernatant by RIA and ELISA. AMH, FSH-R and CYP 19 gene expression was assessed by QPCR.

Main results and the role of chance: Progesterone concentrations in FSH treated cells after 6 d. were 212.9 ± 84.2 and 90.6 ± 22.2 pmol/10,000 cells for NC-IVF and cIVF derived cycles, respectively (mean \pm SEM). This difference was significant ($P < 0.0001$). In the control cultures, these values were 63.3 ± 11.4 and 93.0 ± 15.0 pmol/10,000 cells. After 48 h and 6 days, AMH levels were higher in luteinized GCs obtained from NC-IVF than from cIVF (17.3 ± 4.3 vs. 8.7 ± 1.8) and (28.1 ± 7.3 vs. 20.1 ± 2.9). AMH concentration was significantly correlated with progesterone level ($P < 0.0001$, $r = 0.77$). Estradiol (E2) could not be detected. However, CYP 19 mRNA expression was higher in GCs from NC than from cIVF (6.9 ± 4.7 vs. 0.17 ± 0.03 , delta Ct). AMH mRNA expression was 2.4 ± 0.9 vs. 1.00 ± 0.56 and in positive correlation with FSH receptor mRNA

Limitations, reason for caution: The absence of detectable E2 in cultured GCs indicates a loss or strong reduction in aromatase activity *in vitro*. Hence, *in vitro* studies don't ideally reflect the *in vivo* situation during follicular maturation. However progesterone production is an accepted marker for GCs responsiveness.

Wider implications of the findings: The experiment shows that GCs from NC-IVF, in contrast to those derived from cIVF, resume their responsiveness to FSH stimulation *in vitro* after short time culture. The reduced AMH production of GCs obtained from cIVF follicles is in line with reduced AMH concentrations in follicular fluid in cIVF follicles, and indicates that treatment with exogenous gonadotropins might deblock the follicle recruitment by reducing intrafollicular AMH and forcing the production of oocytes even from non-competent follicles.

Study funding/competing interest(s): Public university (salaries) and private industry (consumables).

Trial registration number: Not applicable.

P-492 Gonadotropin stimulation in In vitro fertilization (IVF) significantly alters the hormone concentrations in follicular fluid ñ a comparative study between Natural Cycle and conventional IVF

A. Vaucher¹, Z. Kollmann¹, N.A. Bersinger¹, B. Weiss¹, P. Stute¹, U. Marti², and M. von Wolff¹

¹University Womens Hospital, Gynecological Endocrinology and Reproductive Medicine, Bern, Switzerland, ²MCL, Medical Laboratories, Niederwangen, Switzerland

Study question: Is the steroid hormone profile in the follicular fluid (FF) at the time of ovum pick up different in naturally matured follicles as in Natural Cycle IVF (NC-IVF) in comparison to conventional, gonadotropin stimulated IVF (cIVF)?

Summary answer: Testosterone (T) and estradiol (E2) concentrations are around 3-fold and androstendione (A2) around 1.5-fold higher in NC-IVF than in cIVF follicles. The high concentrations are positively correlated with Anti Mullerian hormone (AMH), which concentration is also 3-fold higher in NC-IVF follicles. **What is known already:** In cIVF the implantation rate of unselected embryos appears to be lower than in NC-IVF, which is possibly due to negative effects of high dosages of gonadotropins on the follicular metabolism. AMH, which seems to be regulated by intrafollicular testosterone, appears to be a marker of oocyte quality as found in cIVF. We have previously shown, that AMH is concentrated around 3 fold higher ($p < 0.0001$) in NC-IVF- follicles in comparison with cIVF-follicles.

Study design, size, duration: Cross-sectional study involving 37 NV-IVF and 39 cIVF cycles performed in 2011 and 2012. Thirteen women within this population underwent one NC-IVF and one cIVF cycle each. cIVF was performed by controlled ovarian stimulation with HMG following a GnRH antagonist protocol.

Participants/materials, setting, methods: Mean age was 35.3 ± 4.6 (SD) and 34.2 ± 3.7 years in the NC and cIVF groups (ns). Follicular fluid was collected from the leading follicles. T, A2, Dehydroepiandrosterone (DHEA) and E2 were determined by immunoassays. For statistical analysis the non-parametric Mann-Whitney U or Wilcoxon tests were used.

Main results and the role of chance: There was no significant difference in the mean age and follicular size between both groups. Hormone analysis in serum excluded relevant impact of serum concentration on FF hormone concentrations. In FF, median levels of T in NC-IVF and cIVF were 32.8 and 11.1 pmol/L ($p < 0.0001$), of A2 290 and 201 nmol/L ($p = 0.003$), of DHEA 6.7 and 5.6 pg/ml (n.s.) and of E2 3290 and 1199 nmol/L ($p < 0.0001$), respectively. Median AMH levels were 32.8 in NC-IVF and 11.1 pmol/L in cIVF ($p < 0.0001$). T and E2 correlated positively (T: $r = 0.36$, $p = 0.002$; E2: $r = 0.37$, $p = 0.001$) with FF-AMH concentration. Significant differences in concentrations for E2 and AMH were also found in the 13 patients who performed both NC-IVF and cIVF when analysed separately in pairs.

Limitations, reason for caution: The correlation analysis suggests but does not prove a direct metabolic link between the different hormones. A correlation between the hormone concentrations and the implantation potential of the oocytes could not be investigated as the oocytes in cIVF were not treated separately.

Wider implications of the findings: The alteration of the endocrine milieu in follicles in gonadotropin stimulated IVF (cIVF) could be a cause for the lower oocyte quality as it was described in cIVF. The reasons for the altered hormone concentrations might be non-physiological LH activity due to the use of GnRH antagonists plus HMG or changes in follicular aromatase activity. In addition, we are hereby presenting "normal" values of the follicular steroid milieu which can be used for further optimisation of stimulation protocols.

Study funding/competing interest(s): Public university (salaries) and private industry (consumables). No competing interests

Trial registration number: Not applicable

P-493 The live-birth rates of women with thyroid autoimmunity and/or subclinical hypothyroidism following IVF

J. Chai, W.Y.T. Yeung, C.Y.V. Lee, W.H.R. Li, P.C. Ho, and H.Y.E. Ng
The University of Hong Kong, Obstetrics and Gynaecology, Hong Kong Island, Hong Kong

Study question: Whether the live-birth rate of IVF is affected in infertile patients with thyroid autoimmunity and/or subclinical hypothyroidism.

Summary answer: The live birth rate and miscarriage rate of women with thyroid autoimmunity and/or subclinical hypothyroidism following IVF were not impaired.

What is known already: Both thyroid autoimmunity and subclinical hypothyroidism have independently been associated with adverse pregnancy outcomes.

Study design, size, duration: Retrospective study of a total of 627 women undergoing their first IVF cycle.

Participants/materials, setting, methods: Women without past or current history of thyroid disorder undergoing their first IVF cycle. Their archived

serum samples were assayed for thyroid function tests and thyroid autoimmunity. The live birth rate of those with and without thyroid autoimmunity +/- subclinical hypothyroidism were compared.

Main results and the role of chance: The clinical pregnancy rate, live birth rate and miscarriage rate were similar among women with or without thyroid autoimmunity and/or subclinical hypothyroidism using a TSH threshold of up to 4.5 mIU/L. Thyroid autoantibodies level did not affect these IVF outcomes.

Limitations, reason for caution: Limited by its retrospective design and lack of data regarding antenatal complications or neonatal outcomes.

Wider implications of the findings: Screening for thyroid disorder before IVF may not be cost-effective. Also the cut-off value for subclinical hypothyroidism is uncertain.

Study funding/competing interest(s): Nothing to declare.

Trial registration number: HKCTR-1573

P-494 Increased femoral bone mineral density according to increase of skeletal muscle mass in 547 Korean women; a retrospective cohort study over 2.7-year period

S.M. Kim¹, S.H. Kim², B.C. Jee³, S. Ku², C.S. Suh³, Y.M. Choi², J.G. Kim², and S.Y. Moon²

¹*Seoul National University Hospital Health Care System Kangnam Center, obstetrics and gynecology, GangNam-Gu Seoul, Korea South,* ²*Seoul National University Hospital, obstetrics and gynecology, Seoul, Korea South,* ³*Seoul National University Bundang Hospital, obstetrics and gynecology, Seongnam, Korea South*

Study question: What is the impact of change of abdominal fat amount which is directly measured with computed tomography (CT), body composition, and metabolic risk factors on the amount of change of bone mineral density (BMD) in Korean women.

Summary answer: The increase of skeletal muscle mass (SMM) had positive correlation with the increase of femoral BMD.

What is known already: Although increased body mass index (BMI) has been reported to have a protective effect on the bone mineral density (BMD), the main impact is estimated to be caused by straining force exerted by skeletal muscle mass on the bone. Central obesity, which is considered to be the essential component of the metabolic syndrome, has been suggested to have inverse relationship between bone mineral density, though the results are inconsistent according to study population and measurement protocols.

Study design, size, duration: Retrospective cohort study involving 547 Korean women aged 29 ~ 78, during Jan 2004 to Dec 2010 (average 2.7 years)

Participants/materials, setting, methods: Women who had visited Seoul National University Gangnam Center more than twice for routine health check-up and who had undergone both dual energy X-ray absorptiometry (DEXA) and abdominal adipose tissue analysis with CT scans more than twice during study period.

Main results and the role of chance: The mean age of the participants were 52.7 and the mean follow up interval was 2.7 yrs. The mean values (g/cm^2) of BMD at L1-4, femoral neck and total femur were 1.1, 0.88 and 0.93 at initial visit. The change of BMD over time was -0.003 (-0.27%) at L1-4, -0.02 (-2.3%) at femoral neck and -0.016 (-1.7%) at total femur. There were significant positive correlation between increase of skeletal muscle mass and increase of femoral BMD. Otherwise there was no significant relationship found among abdominal fat, metabolic syndrome (MetS) risk factors or other body composition parameters with BMD at each visit and throughout the study period after adjustment for age (time interval).

Limitations, reason for caution: Since this is a retrospective cohort study conducted in a single health check-up center located in urban area of metropolitan city of Seoul, selection bias might have worked. We could not evaluate the quantitative effect of exercise on SMM and BMD.

Wider implications of the findings: Generally there have been significant positive correlations found among BMDs with body mass index (BMI). BMDs at lumbar and femur decreases and abdominal fat amount increases with aging. If one can increase SMM (probably by regular exercise), regardless of increasing abdominal fat amount, she/he could keep the BMD, especially at femur.

Study funding/competing interest(s): This study was supported by Seoul National University Hospital Research Grant no. 0420100350

Trial registration number: n.a.

P-495 The effect of additional low dose hCG with vaginal progesterone gel in luteal phase of IVF cycles

J.H. Lee¹, S.G. Kim¹, Y.Y. Kim¹, H.J. Kim¹, K.H. Lee², I.H. Park², and H.G. Sun²
¹Mamapapa&baby Obstetrics Gynecology Clinic, Infertility lab, Ulsan city, Korea South, ²Mamapapa&baby Obstetrics Gynecology Clinic, OB&GY, Ulsan city, Korea South

Study question: Does additional low dose hCG supplementation with vaginal progesterone gel is equally effective with intramuscular progesterone as luteal phase support of controlled ovarian hyperstimulation (COH) in vitro fertilization (IVF) cycles?

Summary answer: In COH IVF cycles, additional low dose hCG supplementation with vaginal progesterone gel makes similar pregnancy rate with intramuscular progesterone as luteal phase support.

What is known already: In a past meta-analyses, clinical pregnancy and delivery rates were significantly improved with intramuscular (IM) progesterone as compared with vaginal progesterone. On the other hand, in some recent RCTs and meta-analyses provided some evidence that no difference existed regarding the clinical pregnancy rate between vaginal progesterone and IM progesterone for LPS. However, many clinicians still prefer IM progesterone to vaginal progesterone due to concern of decreased pregnancy rate with vaginal progesterone.

Study design, size, duration: This retrospective cohort study included 543 women undergoing IVF between January 2011 and December 2012.

Participants/materials, setting, methods: We retrospectively reviewed 543 patients undergoing IVF using GnRH agonist and antagonist protocols. After COH and oocyte aspiration, women received IM progesterone only (Group A) or vaginal progesterone with additional low dose hCG (1000 IU) three times (Group B) (OPU day, ET day, ET + 3 day).

Main results and the role of chance: 349 patient received IM progesterone only as LPS and vaginal progesterone with low dose hCG was given to 194 women. In each group, age (35.9 ± 4.28 vs. 35.06 ± 4.37), endometrial thickness (10.39 ± 2.11 vs. 10.33 ± 2.13), number of transferred embryos (2.0 vs. 2.0) and percentage of top quality embryo (60.7% vs. 59.3%) did not show significant difference. There were no significant differences between two groups in the pregnancy rate [52.7%(184/349) vs. 49.0%(95/194), $P = 0.2$], spontaneous abortion rate [9.8%(18/184) vs. 8.4%(8/95), $P = 0.3$], ongoing pregnancy rate [47.6%(166/349) vs. 44.8%(87/194), $P = 0.14$].

Limitations, reason for caution: This is a retrospective study. A prospective randomized study would have minimized potential limitations. And we don't have comparison group using vaginal progesterone only as LPS due to previous experience of decreased pregnancy rate with vaginal progesterone only [PR 35.7%(5/14)].

Wider implications of the findings: The intravaginal route of progesterone supplementation in IVF has gained wide application as a first choice luteal support regimen, mainly due to comfort and effectiveness of patients. In spite of some RCTs and meta-analyses proving efficacy of vaginal progesterone, many clinicians have met episode of vaginal bleeding before pregnancy test and some have experienced decreased pregnancy rate. With additional low dose hCG, vaginal progesterone could be more secure LPS and really equivalent to IM progesterone.

Study funding/competing interest(s): None

Trial registration number: None

P-496 Is fertility sustained until the late reproductive age in the IVF-ET cycles of PCOS patients?

Y.I. Hwang, N.Y. Sung, M.H. Choi, S.H. Cha, C.W. Park, J.Y. Kim, K.M. Yang, I.O. Song, M.K. Koong, I.S. Kang, and H.O. Kim
 Cheil General Hospital and Women's Health Care Center, Dept. of OB/GYN Division of Reproductive Endocrinology and Infertility, Seoul, Korea South

Study question: The aim of this study was to evaluate the characteristics and IVF outcomes in PCOS patients according to age and major purpose was to compare the clinical pregnancy rates in the late reproductive age of PCOS patients.

Summary answer: The clinical pregnancy rate in PCOS patients may be sustained until the age of 38 but in the late reproductive age over 38, clinical pregnancy rate drops sharply even in PCOS patients.

What is known already: As a woman is getting older, the ovaries commence a poorer response to gonadotropin. As a result, less and less oocytes are retrieved after ovarian stimulation according to age. But despite ageing, the number of retrieved oocyte seems somewhat maintained in PCOS patients. It has been suggested that women with PCOS display sustained fertility until the age of 41 with advancing age as compared with infertile eumenorrhic women.

Study design, size, duration: 307 PCOS women and 364 non-PCOS infertile women with tubal factor who received IVF program from January 2003 to August 2012 were analyzed retrospectively. Four age groups were made in the PCOS patients and controls. (group A: 30-32 y-o, group B: 33-35 y-o, group C: 36-38 y-o, group D: 38-41 y-o)

Participants/materials, setting, methods: Characteristics of two groups (age, BMI, cycle number, Day 3 E2, FSH, LH, AMH, stimulation duration, total gonadotropin unit, E2 level in hCG day, endometrial thickness on hCG day, number of oocyte retrieved, mature oocyte, inseminated oocyte, fertilized embryo, transferred embryo, embryo score) were compared.

Main results and the role of chance: Serum AMH level was 4 fold higher in PCOS group. hCG positive rate and clinical pregnancy rate were about 10% higher in PCOS group (50.8% vs. 62.9%, $p = 0.002$ / 42.9% vs. 52.4%, $p = 0.013$). Interestingly, there was no significant difference in the number of retrieved oocytes among four age groups in PCOS unlike control group. Clinical pregnancy rate in PCOS women was sustained until the age of 38 (group A, 57.9%; group B, 53.3%; group C, 47.2%). But hCG positive rate and clinical pregnancy rate showed significantly steeper decrease between the age group C and D in the PCOS patients (62.3% vs. 31.3%, $p = 0.03$; 47.2% vs. 18.8%, $p = 0.044$). Besides, there was no difference in the embryo score and oocyte maturation rate among the age groups.

Limitations, reason for caution: Limitation of this study is that the oocyte quality and embryo score were demonstrated but the routine morphological scoring system cannot reflect age related biological changes.

Wider implications of the findings: The result of this study will be helpful when counseling the infertile patients with PCOS who is planning to receive IVF program.

Study funding/competing interest(s): none

Trial registration number: none

P-497 Hormone replacement therapy versus combined oral contraceptives for prevention of osteoporosis in young hypoestrogenic women: a prospective randomized trial

C. Haines, W.Y. Wong, W.S. Kong, L.P. Cheung, T.K. Choy, and P.C. Leung
 The Chinese University of Hong Kong, Dept of Obstetrics and Gynaecology, Hong Kong, Hong Kong

Study question: Is hormone replacement therapy better than oral contraception for preventing osteoporosis in young hypoestrogenic women?

Summary answer: Hormone replacement therapy is significantly more effective than oral contraception in preventing bone loss in this group of women

What is known already: There are no data on the effect of hormone replacement therapy on bone mineral density in young hypoestrogenic women. There are contradictory data on the effect of oral contraceptives in these women

Study design, size, duration: Prospective randomized trial comprising 100 subjects treated for 12 months

Participants/materials, setting, methods: This was a prospective, randomized trial comparing the effect of ethinylestradiol 0.03mg, levonorgestrel 0.15 mg daily for 21 days with 7 days lactose tablet (Microgynon 30 ED OC, Bayer) with oestradiol 2mg daily for 14 days, oestradiol 2 mg daily plus dydrogesterone 10mg daily for 14 days (Femoston, Solvay Pharmaceuticals). Subjects aged 20-45 years with either 12 months of amenorrhoea or a menstrual cycle of average length 35 days or longer in the previous 12 months were recruited from the gynaecological endocrine clinic of a large teaching hospital.

Main results and the role of chance: Over 12 months of treatment, increases in BMD were greater in all areas of the lumbar spine and the hip in the HRT group compared with the OC group. The differences were statistically significant at L2 ($P < 0.01$), L3 ($p < 0.001$), and for the total spine ($p < 0.01$). Whilst the trend

was the same for all areas of the hip, the changes there were only statistically significant for Total BMD of the hip $p < 0.01$.

Limitations, reason for caution: The finding of statistically significant changes in BMD over such a short period of time were unexpected. We plan to lengthen the duration of the study to determine whether the statistical significance remains.

Wider implications of the findings: These data strongly suggest that young hypoestrogenic women respond better in terms of BMD change to treatment with HRT than with OC. At this preliminary stage we don't wish to speculate on the reasons for the differences, but both the type of exogenous steroids used and the dosing schedule may prove to be important. These results have the potential to change common prescribing practice for young hypoestrogenic women whatever the underlying pathology

Study funding/competing interest(s): This study was funded by a Hong Kong RGC Direct Grant

Trial registration number: ISRCTN55207514

P-498 Expression and effects of amphiregulin on the maturation of human cumulus cell-oocyte complexes recovered from antral follicles in IVM cycles

R. Fadini¹, G. Coticchio¹, M. Mignini Renzini¹, M.C. Guglielmo¹, F. Brambillasca¹, A. Hourvitz², D.F. Albertini³, P. Novara¹, M. Merola¹, and M. Dal Canto¹

¹Biogenesi Reproductive Medicine Centre, Istituti Clinici Zucchi, Monza, Italy; ²IVF Unit and Fertility Research Laboratory, Sheba Medical Center, Tel Hashomer, Israel; ³Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, U.S.A

Study question: Animal studies suggest that amphiregulin (AREG), a member of the EGF-like proteins family, mediates mid-cycle LH-induced oocyte meiotic resumption and maturation. Therefore, AREG is a possible candidate to improve the yield and quality of human oocytes matured in vitro.

Summary answer: AREG may improve maturation rate of GV-stage cumulus cell-oocyte complexes (COCs) recovered from antral follicles in IVM cycles. Expression of AREG and its receptor EGFR is higher in oocytes competent to mature in vitro. Importantly, oocytes matured in the presence of AREG display normal meiotic spindle and cytoskeletal characteristics.

What is known already: In women undergoing ART treatment, intrafollicular AREG levels considerably increase following hCG administration. AREG and EGFR transcripts appear to be expressed in human cumulus cells (CCs) obtained from antral follicles. In vitro, AREG, in combination with epiregulin but not alone, has been tested in a largely imperfect model, i.e. leftover germinal vesicle (GV) oocytes from conventional ovarian stimulation cycles.

Study design, size, duration: The expression of AREG, EGFR and genes involved in oocyte maturation were evaluated in CCs of COCs matured in vitro (IVM) or in vivo (IVO) in stimulated cycles. Yield and quality of metaphase II (MII) oocytes matured in vitro were evaluated following IVM in the presence of AREG.

Participants/materials, setting, methods: Supernumerary COCs were donated by consenting normo-ovulatory women undergoing IVM or controlled ovarian stimulation treatment. Immature COCs derived from IVM cycles were matured in vitro with FSH/hCG, AREG, or FSH/hCG/AREG. mRNA detection was achieved in duplicate samples by quantitative real time-PCR. Oocyte cytoskeleton was evaluated by confocal microscopy.

Main results and the role of chance: In COCs matured in vitro, ERBB1 expression was higher in CCs associated to MII oocytes compared to CC enclosing either GV or MI oocytes. AREG expression increased following maturation with FSH/hCG, being higher in COCs containing MII oocytes. In comparison to IVO COCs, HAS2 and PTGS2 expression was reduced in IVM COCs treated with or without AREG. Maturation rates in the presence of 25 or 50 ng/ml AREG alone were low (24% and 29%, respectively). However, maturation rate tended to increase with FSH/hCG/AREG supplementation in comparison to FSH/hCG controls (58% vs. 48%). Confocal analysis of 10 oocytes matured with AREG revealed normal cortical actin. Six oocytes showed MII spindles with canonical bi-polar organization and equatorial chromosome alignment, while 4 oocytes were classified as telophase I.

Limitations, reason for caution: Data are numerically limited and require further confirmation. Oocyte quality should be investigated also by criteria different from

cytoskeletal integrity. AREG appears to have little influence on the expression of genes involved in the maturation of the COC, such as HAS2 and PTGS2.

Wider implications of the findings: This study is the first in which AREG was tested in human immature COCs derived from IVM cycles, i.e. the material authentically used in IVM treatments, rather than leftovers from stimulated cycles. The trend towards an increased maturation rate and unaltered cytoskeletal attributes of IVM oocytes support the hypothesis that the clinical efficiency of IVM can be ultimately improved by the use of AREG and possibly other paracrine factors mediating oocyte maturation in vivo.

Study funding/competing interest(s): This study was partly funded by the Italian Ministry of Health. The author declare to have no interests conflicting with the study.

Trial registration number: Not applicable.

P-499 AMH vs FSH blood determination: which one is the most decisive and comfortable? It is time to be pragmatic

J.A. Agirreigoikoa Iza, J.L. DePablo, C. Anarte, A. Domingo, E. Abanto, and G. Barrenetxea

Quirón Bilbao, Assisted Reproduction Center, Bilbao, Spain

Study question: To know if antimüllerian hormone (AMH) blood determination is as efficient as FSH determination to predict the ovarian response in our patients.

Summary answer: AMH blood measurement is more effective than FSH to determine ovarian response in IVF cycles. Although both hormones are good indicators of ovarian response in younger women, AMH determination is a better predictive test in older women.

What is known already: The clinical applications of AMH have increased due to results shown in different studies. Circulating AMH is correlated strongly with oocyte yield and has shown to be proportional to the number of small antral follicles.

In addition, AMH can be measured regardless of the day of the cycle whereas FSH can only be measured on days 3 to 5. Therefore AMH is more comfortable both for patients as well as clinicians.

Study design, size, duration: IVF patients who were treated in our clinic in 2012 were analysed in this retrospective study to determine their AMH and FSH blood levels before undergoing ovarian stimulation. AMH determination was performed on 270 patients and FSH on 356.

Participants/materials, setting, methods: Ovarian stimulation was started using 200-300 IU rFSH following an antagonist protocol depending on the AMH and FSH results, age and AFC.

Four different groups were studied depending on the age of the patients (<35, 35-37, 38-40 and >40) and the amount of retrieved oocytes was analysed in each group.

Main results and the role of chance: Bilateral correlation of AMH and FSH blood determination is statistically significant [$p = (0,009)$].

Both (AMH and FSH) are correlated to age and to the amount of retrieved oocytes.

However, when patients were divided into groups depending on their age. AMH determination was more accurate than FSH measurement in older women. The results of AMH and the amount of retrieved oocyte correlation was statistically significant in patients who were 38-40 years old ($p = 0,014$), but FSH determination was not ($p = 0,419$). Similar results were observed in patients older than 40 ($p = 0,006$ vs. $p = 0,072$).

Limitations, reason for caution: Although a lot of tests have been used to analyse and ovarian response in IVF treatments, the only way to know it is to stimulate ovaries and look at how they react. We should analyze these results as another factor (considering age, AFC . . .) to determine our stimulation protocol.

Wider implications of the findings: Historically ovarian reserve has been analysed by using different and complicated tests.

AMH levels do not change thorough the ovarian cycle, so it can be done as soon as possible and regardless of the menstruation, which makes it the first choice for hormone determination.

Making the sterile couple diagnosis more simple and comfortable for the patients should be our objective and priority.

Study funding/competing interest(s): Financial support provided by Quiron Bilbao

Trial registration number: No registration number needed

P-500 Patient- and cycle-related prognostic factors affecting the outcome of mild IVF: a multivariate analysis of 2876 treatment cycles from a single centre

R. Kato, S. Kawachiya, D. Bodri, M. Kondo, and T. Matsumoto
Kobe Motomachi Yume Clinic, Assisted Reproduction Unit, Kobe, Japan

Study question: Which patient- and cycle-related prognostic factors affect the probability of live birth following natural cycle IVF and clomiphene-citrate based minimal ovarian stimulation?

Summary answer: Our findings suggest that mild IVF approaches (especially clomiphene-based minimal ovarian stimulation) might be most effective in younger women (<38 years) starting their IVF treatment and in those who have a previous offspring.

What is known already: A recent systematic review (van Loendersloot et al, 2010) analyzed the effect of several putative factors (female age, parity, basal FSH, subfertility duration and indication, number of oocytes retrieved, fertilization method, number of embryos transferred, embryo quality) that could predict IVF outcome. However it only included studies where conventional ovarian stimulation was used. So far no large scale analysis was made in the setting of mild IVF.

Study design, size, duration: A single-centre cohort of 727 consecutive infertile patients (mean age 38.4 ± 4.5 , range: 26-52 years) who underwent a total of 2876 treatment cycles (55% natural cycle IVF and 38% minimal ovarian stimulation) coupled with a universal single embryo transfer policy between November 2008 and December 2011 was retrospectively analyzed.

Participants/materials, setting, methods: For programme inclusion no selection was made by female age, basal FSH or previous ART history. The effect of 9 variables (female and male age, BMI, basal FSH, female infertility type, parity, previous IVF treatment, current cycle rank and stimulation type) on live birth was evaluated by a multivariate analysis.

Main results and the role of chance: A total of 2876 treatment cycles resulted in 966 (34%) embryo transfers and 274 (9.5%) live births. Although a univariate analysis showed that all the above-mentioned 9 prognostic factors had a statistically significant impact, in a multivariate logistic regression analysis only female age, parity, previous IVF treatment and stimulation type were associated with live birth. Female age (26-34 as reference, 35-37, 38-40, 41-44 years) had the strongest effect on success rates (adjusted OR: 0.80, 0.39, 0.08) with no live births obtained in ≥ 45 years patients. Previous parity was a positive predictive factor (aOR: 1.59) whereas any previous IVF treatment had a negative effect (aOR: 0.67). Minimal ovarian stimulation with clomiphene-citrate was more effective than natural cycle IVF treatment (aOR:3.95).

Limitations, reason for caution: Only those patient- and cycle-related prognostic factors were evaluated which were available before treatment start whereas other embryological laboratory variables (number of retrieved oocytes, fertilization method and embryo quality) were not taken into account.

Wider implications of the findings: Although mild IVF approaches seem to be the most effective in good-prognosis patients they could also be of interest in patients (elevated basal FSH values did not impact outcome significantly) who otherwise are discouraged to do treatment with their own eggs. Natural cycle IVF treatment probably should be only reserved to young good-prognosis patients.

Study funding/competing interest(s): none

Trial registration number: n/a

P-501 Cost-effectiveness comparison between pituitary downregulation with a GnRH agonist short regimen in alternate days and an antagonist protocol for assisted fertilization treatments

L.G.L. Maldonado¹, A.S. Setti², D.P.A.F. Braga², A. Iaconelli Jr.³, and E. Borges Jr.³

¹Fertility - Centro de Fertilização Assistida, Clinical department, Sao Paulo SP, Brazil, ²Fertility - Centro de Fertilização Assistida and Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Scientific department, Sao Paulo SP, Brazil, ³Fertility - Centro de Fertilização Assistida and Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Clinical and scientific department, Sao Paulo SP, Brazil

Study question: Is there a difference in the cost-effectiveness between pituitary downregulation with a GnRH agonist (GnRHa) short regimen in an alternate

day schedule and a GnRH antagonist (GnRHant) multi-dose protocol on ICSI outcome?

Summary answer: We aimed at developing a protocol in which fewer GnRH injections and gonadotrophin amounts are required, reducing the IVF cost. A significant reduction in the pituitary suppression cost per cycle was observed. The experimental group showed significantly lower pregnancy and higher miscarriage rates, resulting in a higher cost per pregnancy.

What is known already: The achievement of a simple, safe and cost-effective treatment protocol is of pivotal importance to improve the quality of care in assisted reproduction. An interesting approach would be to unite the reduced costs of pituitary suppression with GnRH GnRHa in alternate days and the administration of recombinant hCG (rhCG) microdoses.

Study design, size, duration: Randomised clinical trial performed in a private IVF centre. Sample size calculation was based on the assumption that a 10% difference in mean cost would mean a clinically significant difference. Patients were randomized in two groups: GnRHa (n = 48) and GnRHant groups (n = 48).

Participants/materials, setting, methods: In the agonist group, a dose of triptorelin was administered in alternate days and ovarian stimulation was achieved with recombinant FSH (rFSH) and rhCG microdoses. In the antagonist group, ovarian stimulation was achieved with rFSH, rhCG microdoses and cetrorelix acetate daily.

Main results and the role of chance: A significant lower number of patients underwent embryo transfer in the GnRHa group (87.5 vs. 100%, $p = 0.026$). A trend towards a reduced Implantation rate was observed on the GnRH agonist group (15.9 vs. 28.1%, $p = 0.061$). Clinical pregnancy rate was significantly lower (31.0 vs. 52.1%, $p = 0.042$) and miscarriage rate was significantly higher (38.4 vs. 8.0%, $p = 0.031$) in the GnRHa group. It was observed a significant lower cost per cycle in the GnRHa group as compared to the GnRHant group ($\$5327.8 \pm 387.3$ vs. $\$5900.4 \pm 472.5$, $pp < 0.001$).

Limitations, reason for caution: In our experimental protocol no cycle was cancelled before oocyte retrieval because of premature LH surge, therefore all patients had oocyte retrieval. However, three patients developed OHSS and had all the embryos frozen. Two of these patients became pregnant in a subsequent embryo thawing cycle.

Wider implications of the findings: Despite more practical than a long agonist schedule, less costly and as effective as the antagonist protocol in terms of pituitary suppression, the short agonist protocol administered in alternate days increases the risk for OHSS, therefore, it is not a safe protocol to be included in routine. It is important to identify which patients would benefit from this protocol, if any, in order to obtain a less costly and friendlier COS without compromising the treatment outcomes.

Study funding/competing interest(s): No funding. s.

Trial registration number: ClinicalTrials.gov: NCT01468441

P-502 Supplementation with hCG microdoses brings costs and effectiveness together resulting in higher oocyte quality and blastocyst formation in young women undergoing controlled ovarian stimulation

C. Iaconelli¹, A.S. Setti², D.P.A.F. Braga², R.C.S. Figueira³, A. Iaconelli Jr.⁴, and E. Borges Jr.⁴

¹Fertility - Centro de Fertilização Assistida, Clinical department, Sao Paulo SP, Brazil, ²Fertility - Centro de Fertilização Assistida and Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Scientific Department, Sao Paulo SP, Brazil, ³Fertility - Centro de Fertilização Assistida, IVF lab, Sao Paulo SP, Brazil, ⁴Fertility - Centro de Fertilização Assistida and Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Clinical and scientific department, Sao Paulo SP, Brazil

Study question: Does the supplementation with recombinant hCG (rhCG) in the late stages of controlled ovarian stimulation (COS) affect oocyte and embryo quality, blastocyst formation, ICSI outcomes and treatment costs in young patients undergoing ICSI?

Summary answer: The rhCG supplementation can be used to reduce the FSH amounts required in COS protocols, resulting in a significantly costless treatment, improved oocyte quality, fertilization rate, embryo quality and blastocyst formation. Its effect on implantation and pregnancy remains controversial.

What is known already: GnRH antagonists are associated with a shorter treatment length and friendlier treatment schedule. There is evidence that LH activity

may be useful, particularly when antagonists are used, to improve cycle outcome by providing improved embryo development. However, it is not clear whether or not the supplementation with rhCG microdose is beneficial for oocyte and embryo quality.

Study design, size, duration: This retrospective cohort study, performed in a private assisted fertilization center (January to December 2011), enrolled 330 female patients (age ≤ 35 years) undergoing ICSI. The couples were assigned to two groups according to the COS protocol used: control group ($n = 178$) and microdose group ($n = 152$).

Participants/materials, setting, methods: COS was achieved by the administration of rFSH in a step-down manner and cetorelix acetate. In the control group, the rFSH and GnRH antagonist were administered until the day of ovulation trigger; and in the microdose group, the rhCG and GnRH antagonist were administered until the day of ovulation trigger.

Main results and the role of chance: A lower mean total dose of FSH administered was observed in the microdose group (1434 ± 277 vs. 2051 ± 472 IU, $p < 0.001$) as well as a higher mean estradiol level on the day of ovulation trigger (2767 ± 1830 vs. 1310 ± 1017 pg/ml, $p < 0.001$). A significant lower rate of oocytes presenting intracytoplasmic dysmorphisms was observed in the microdose group (0.9 vs. 5.3%, $p < 0.001$, OR: 0.17, CI: 0.07–0.43). Significant higher mature oocyte (78.9% vs. 72.8%, $p = 0.004$), fertilization (79.1% vs. 70.9%, $p < 0.001$), high-quality embryos (78.8% vs. 58.2%, $p = 0.007$) and blastocyst formation rates (32.7% vs. 23.6%, $p < 0.001$, OR: 1.57, CI: 1.23–2.01). A significant lower COS cost per pregnancy was observed in the microdose group ($\$6,429.5 \pm 893.5$ vs. $\$8,923.0 \pm 1623.8$, $p < 0.001$).

Limitations, reason for caution: This is a retrospective study and therefore subjected to bias.

Wider implications of the findings: Any simplification in pharmacological treatment is a welcome development for infertile couples undergoing ICSI. The results of this study suggest that the use of rhCG microdoses in GnRH antagonist COS protocols is recommended for young patients undergoing ICSI. In addition, this protocol may be an alternative for developing countries, countries where the ICSI treatment does not qualify for reimbursement and for couples with limited financial resources.

Study funding/competing interest(s): No funding.

Trial registration number: Not applicable, due to the retrospective design of the study.

P-503 Low follicular fluid tyrosine concentration in infertile patients with ovarian hyperstimulation syndrome

K. Kitaya, S. Taguchi, M. Funabiki, Y. Tada, T. Hayashi, and Y. Nakamura
Oak Clinic Inc., IVF Center, Osaka, Japan

Study question: Are the follicular fluid tyrosine concentration altered in the infertile patients who developed ovarian hyperstimulation syndrome (OHSS) in controlled ovarian stimulation (COS) and IVF cycles?

Summary answer: Follicular fluid branched-chain amino acid concentration was similar between the moderate-to-severe OHSS group and normoresponder group, whereas follicular fluid tyrosine concentration was significantly lower in the OHSS group than in the normoresponder group.

What is known already: Tyrosine is a precursor amino acid of dopamine. Dopamine receptor agonists, such as cabergoline, are the potential preventive drugs for OHSS.

Study design, size, duration: Retrospective cross-sectional study. From July 2012 to Sep 2012, follicular fluid was retrieved during oocyte pickup from 40 infertile patients undergoing COS-IVF [20 moderate-to-severe OHSS patients (mean \pm SD age; 34.0 ± 3.3 , mean \pm SD BMI; 22.1 ± 2.3) and 20 normoresponders (mean \pm SD age; 33.5 ± 3.7 , mean \pm SD BMI; 22.4 ± 2.8)] under informed consent. All the participants underwent COS with FSH-HMG-GnRH agonist short protocol or FSH-HMG-GnRH antagonist protocol.

Participants/materials, setting, methods: Follicular fluid branched-chain amino acid and tyrosine concentration were measured using an enzymatic assay.

Main results and the role of chance: The number of oocytes retrieved was significantly higher in the moderate-to-severe OHSS group than in the normoresponder group. Follicular fluid branched-chain amino acid concentration was similar between the two groups ($p = 0.55$), whereas follicular fluid tyrosine

concentration was significantly lower in the moderate-to-severe OHSS group than in the normoresponder group ($p = 0.027$).

Limitations, reason for caution: This retrospective study may have some biases in participant selection.

Wider implications of the findings: Low follicular fluid tyrosine concentration may be associated with development of OHSS. The findings support the idea that tyrosine plays a role in ovarian integrity in COS-IVF cycles and dopamine receptor agonists are the potential preventive drugs for OHSS.

Study funding/competing interest(s): None.

Trial registration number: None.

P-504 Sexual development in girls with central precocious puberty during 5 years after completion of long-term treatment with GnRH agonist

M. Snajderova¹, D. Zemkova¹, V. Lanska², and L. Teslik³

¹2nd Faculty of Medicine Charles University and University Hospital Motol, Department of Paediatrics - Endocrine Division, Prague 5, Czech Republic,

²Institute of Clinical and Experimental Medicine, Department of Statistics,

Prague, Czech Republic, ³2nd Faculty of Medicine and University Hospital Motol, Department of Gynecology and Obstetrics, Prague 5, Czech Republic

Study question: To evaluate development of the reproductive axis and sexual development in girls with central precocious puberty during 5 years after long-term treatment with GnRH agonist

Summary answer: Suppression of pituitary-gonadal axis after long-term depot GnRH agonist therapy in girls with CPP was fully reversible. Volume of uterus and ovaries corresponds with the rapid increase of gonadotropin levels within first year after therapy withdrawal. Incidence of irregular menses is significantly more frequent in girls with organic central precocious puberty.

What is known already: Gonadotropin-releasing hormone agonists (GnRH agonists) are very effective in suppression of the pituitary-gonadal axis in girls with central precocious puberty. Only a few data on the long-term follow-up of sexual development in girls with CPP after completion of long-term therapy are available.

Study design, size, duration: Observational study, follow-up during 5 years after the end of GnRH agonist therapy

Participants/materials, setting, methods: 34 girls with central precocious puberty ($n = 21$ idiopathic; $n = 13$ organic), were treated 1.0–7.2 years by D-Trp6-GnRH agonist (Diphereline S. R. 3 mg; Ipsen). Standard GnRH test (LHmax/FSHmax), volume of uterus, ovaries and menstrual cycle were assessed at the end of therapy, 3, 12, 24, 36, 48 and 60 months after.

Main results and the role of chance: Similar trends were found in both girls with idiopathic (ICPP) and organic (OCP) central precocious puberty. LHmax/FSHmax (end of therapy 0.3 ± 0.3 (mean \pm SD), 3 months after therapy 1.2 ± 0.7 ($p < 0.001$), 3.0 ± 3.2 ($p < 0.01$) at 12 months. Ovarian volume 2.2 ± 0.9 ccm increased at 3 months to 3.3 ± 2.0 ccm ($p < 0.01$), 12 months 4.4 ± 1.7 ccm ($p < 0.05$), 48 months 6.8 ± 3.0 ccm (ns). Uterus volume 4.2 ± 1.7 ccm increased within 3 months to 9.8 ± 5.0 ccm ($p < 0.05$), at 12 months 21.7 ± 7.8 ccm ($p < 0.05$), 24 months 37.0 ± 17.6 ccm ($p = 0.05$), with no change later. Menarche was reached at age 12.7 ± 1.2 years (bone age 13.8 ± 1.0 years); 1.6 ± 1.0 years after the end of therapy, earlier in ICPP group. Irregular and regulated menstrual cycle 5 years after GnRH agonist therapy was found in 10% ICPP and 54% OCP girls.

Limitations, reason for caution: none

Wider implications of the findings: further knowledge on the sexual development after treatment

Study funding/competing interest(s): no

Trial registration number: no

P-505 Luteal hormonal profile of the same oocyte donors stimulated with either GnRH antagonist or agonist compared with natural cycles

R. Nunez - Calonge¹, L. Ortega², A. Garcia¹, S. Cortes¹, A. Guijarro³, and P. Caballero Peregrin³

¹Clinica Tambre, Embryology, Madrid, Spain, ²Clinica Tambre, Andrology, Madrid, Spain, ³Clinica Tambre, Gynecology, Madrid, Spain

Study question: The aim of the present study was to characterize hormonal profile during the early and the mid-secretory phase of unsupplemented in vitro fertilization cycles (IVF) in the same egg donors, stimulated in association with rFSH and either GnRH antagonist or GnRH agonist, compared with control, natural cycles.

Summary answer: In the present study, luteal LH serum concentrations were extremely low in the early luteal phase of the stimulated cycles compared with natural cycles. On the contrary, oestradiol and progesterone concentrations were significantly higher in the stimulated cycles, especially in the agonist cycles when peak values were attained.

What is known already: Due to inherent differences between GnRH antagonists and agonists, their effect on ovarian steroidal production during the luteal phase of IVF cycles may differ. However, despite the wide introduction of GnRH antagonists and GnRH agonists for controlled ovarian stimulation (COS), studies analyzing the luteal phase of unsupplemented GnRH antagonist or agonists cycles are lacking, mainly, because of the difficulty of conducting such a trial.

Study design, size, duration: A prospective clinical study was conducted at Clinica Tambre, including 15 oocyte donors who underwent each one: a COS with rFSH and GnRH antagonist, a COS with rFSH and GnRH agonist and a natural cycle during the period of July 2011 to December 2012.

Participants/materials, setting, methods: Blood samples were collected during the early (LH +2) and mid-luteal phase (LH + 7) of a natural cycle and, subsequently, on hCG +2 and hCG +7 of a stimulated cycle with GnRH antagonist and finally, a GnRH agonist treatment. Oestradiol, LH, FSH, Progesterone, Prolactin and Testosterone were evaluated.

Main results and the role of chance: During the early luteal phase (D + 2), oestradiol levels were significantly lower in natural cycles ($178,08 \pm 140,15$ ng/l) ($p < 0,001$) compared with agonist ($1574,1 \pm 658,7$ ng/l) and antagonist cycles ($870,45 \pm 352,8$ ng/l); likewise, progesterone concentration ($3,41 \pm 3,71$ nmol/l in natural cycle, $52,65 \pm 9,8$ nmol/l with agonist and $34,54 \pm 26,1$ nmol/l with antagonist cycles) ($p < 0,001$). Oestradiol level were significantly higher in agonist cycle compared with natural and antagonist cycle on the day +2 as well on day +7 ($p < 0,01$). Similarly, serum progesterone and testosterone concentrations were significantly higher in agonists cycles compared with antagonist and natural cycles ($p < 0,01$) on the day +2, but not in day +7.

FSH and LH serum concentrations were significantly lower in the stimulated cycles than the corresponding day in natural cycles ($p < 0,01$).

Limitations, reason for caution: The serum oestradiol and progesterone levels were significantly higher in the stimulated cycles, but whether such a difference confers a more favourable luteal environment for implantation is unclear and needs additional study.

Wider implications of the findings: This is the first study analyzing by paired samples, the hormonal profiles from the same woman during luteal phase transition both in a natural and in a subsequent stimulated cycle, which seems an essential condition to minimize the impact of inter-patient variability.

Studies on the luteal phase are mandatory as it is during that period of time that embryonic implantation takes place and low pregnancy rates have been associated with an abnormal luteal phase profile.

Study funding/competing interest(s): No one

Trial registration number: FT/108

P-506 Impact of ovarian hyperstimulation syndrome on thyroid function after assisted reproductive technologies

M. Bellavia¹, M.H. Pesant¹, D. Wirthner², L. Portman³, D. de Ziegler⁴, and D. Wunder¹

¹Unité de Médecine de la Réproduction CHUV, Gynécologie et Obstétrique, Lausanne, Switzerland, ²Centre de Procréation médicale assistée, Cpma, Lausanne, Switzerland, ³CHUV, Service d'endocrinologie diabétologie et métabolisme, Lausanne, Switzerland, ⁴Cochin, Unité de Médecine de la Réproduction, Paris, France

Study question: To compare the impact of ovarian hyperstimulation syndrome (OHSS) on thyroid function after assisted reproductive technologies (ART).

Summary answer: The results of the current study show that subclinical hypothyroidism is in the current study a consequence of OHSS in 92% of basically euthyroid patients.

What is known already: The impact of maternal thyroid hormones on pregnancy and foetal development are unequivocal. Moreover, hypothyroidism during the

first half of pregnancy is associated with an increased risk of adverse neurodevelopmental outcomes in the child.

Study design, size, duration: This case-control study included 34 patients, a cohort recruited between 2007 and 2010.

Participants/materials, setting, methods: Thyroid stimulating hormone (TSH) and free thyroxine (fT₄) levels were evaluated

in the University hospital before starting ART in all patients and during OHSS in the OHSS group (12 cases) respectively 14 days after embryo transfer (ET) in the control group (22 cases).

Main results and the role of chance: All patients showed baseline TSH values within the normal range. After ET, mean TSH increased above the recommended TSH values of <2.5 mU/L in all patients, but statistically significant differences were reached only in the OHSS group ($P = 0.03$). fT₄ levels remained normal for all patients.

Limitations, reason for caution: Limitations of our study include the statistical power due to the small sample size.

Wider implications of the findings: Our results show that subclinical hypothyroidism is extremely frequent in patients with OHSS after ART. This is an important finding with potential clinical consequences for the embryo/foetus. All of the patients with clinical hypothyroidism in this study have been substituted by levothyroxine and the outcome of all pregnancies has been excellent, i.e. no early miscarriage. It has to be further investigated if levothyroxine substitution during the first trimester plays a role concerning pregnancy outcome.

Study funding/competing interest(s): None.

Trial registration number: Institutional review board approved this study with trial registration number 472/11.

P-507 The reproductive outcome in women with premature ovarian failure

X. Chen, S.H.L. Chen, Y.D. Liu, T. Tao, L.J. Xu, X.L. Tian, D.S.H. Ye, and Y.X. He Nanfang Hospital Southern Medical University, Department of Obstetrics and Gynecology, GuangzhouGuangdong, China

Study question: How do women, who were found growing follicles after diagnosis of premature ovarian failure (POF), approach reproductive outcomes and what is the efficacy of follicles reoccurred?

Summary answer: The results indicated that the resumption of ovarian activity had a high prevalence of empty follicle syndrome and successful births were achieved from competent oocytes with assisted reproductive technology (ART).

What is known already: Several studies have investigated the intermittent ovarian activity in women identified as POF is not a rare phenomenon, but there is a lack of data concerning the oocyte quality and reproductive outcome for these phenomena.

Study design, size, duration: This was a prospective cohort study. Forty-seven infertility women with diagnosis of POF with normal chromosomal karyotype attending our centre for fertility treatment were prospectively recruited and followed up with estrogen replacement therapy (ERT) from March 2009 to March 2011. No women were lost to follow-up.

Participants/materials, setting, methods: Overall 47 patients with POF and normal chromosomal karyotype were included. The patients received 4 mg/day of estradiol valerate for 4 weeks in ERT. Transvaginal ultrasounds were performed once a week. If no growing follicle was detected for three weeks, they were supplemented with oral dydrogesterone 10 mg daily for one week. If the follicle reached 14 mm, oocyte retrieval was scheduled.

Main results and the role of chance: There were 23 patients having growing follicle among 47 patients (23/47; 48.9%) during estrogen replacement therapy. Three patients restored ovarian reserve partly, 7 patients attempted natural pregnancy, and another 13 patients performed 27 follicular punctures. The empty follicle rate per oocyte retrieval was 70.4% (19/27); 8 oocytes were recovered: 1 (12.5%) was in GV, 2 (25.0%) were in MI, 1 (12.5%) was in MII, and 4 (50%) were atresia. Five deliveries were achieved resulted from 1 natural pregnancy and 4 ART pregnancies.

Limitations, reason for caution: These are preliminary data with a limited sample size.

Wider implications of the findings: These findings have implications in patient management and family counseling. Estrogen replacement therapy combined

with regularly monitoring by transvaginal ultrasound may give women on this condition the best chance to have a family of their own.

Study funding/competing interest(s): This work was supported by National Natural Science Foundation of China (81170574), Comprehensive Strategic Sciences Cooperation Projects of Guangdong Province and Chinese Academy (04020416), Guangzhou Science and Technology Program Key Projects (11C22120737) and Scientific Research Foundation of Southern Medical University.

Trial registration number: None.

P-508 Antagonist cycles using GnRH trigger without hCG luteal phase support achieve similar clinical pregnancy rates to standard hCG trigger protocols in PCO patients undergoing IVF/ICSI

A. Carby, E. Barsoum, S. El-Shawarby, G. Trew, and S. Lavery
IVF Hammersmith, Hammersmith Hospital, London, United Kingdom

Study question: Are similar clinical pregnancy rates achieved with GnRH agonist trigger without using hCG as luteal phase support in PCO patients undergoing IVF/ICSI with antagonist protocol

Summary answer: Comparable clinical pregnancy rates are achieved using GnRH trigger and without hCG luteal phase support in PCO patients undergoing IVF/ICSI with antagonist protocol.

What is known already: The risk of OHSS (ovarian hyperstimulation syndrome) is reduced with the use of GnRH (gonadotrophin releasing hormone) agonist trigger in antagonist cycles in hyper-responders, however previous studies have shown clinical pregnancy rates to be lower with routine luteal phase support using estrogen and progesterone supplementation. To counteract this, low dose hCG (human chorionic gonadotrophin) given in the luteal phase has been used to increase clinical pregnancy rates, however this carries an inherent risk of hyperstimulation

Study design, size, duration: PCO patients using antagonist protocol receiving GnRH trigger (2mg busserelin) as part of an OHSS minimization programme commencing on 1/1/2012 (study group) were matched with PCO patients receiving hCG trigger (0.25mg Ovitrelle™) in the preceding 12 months (control). Patients were matched according to age, starting dose FSH and BMI.

Participants/materials, setting, methods: The setting was a UK tertiary referral Unit. Study group included 95 patients receiving GnRH trigger (luteal phase support with 2mg oral estrogen bd, 100mg IM progesterone or 8% vaginal progesterone gel). The control group received hCG trigger and luteal phase progesterone suppository 400mg bd or 100mg im progesterone.

Main results and the role of chance: The mean number of eggs collected and embryos created were comparable in the study and control groups, and similar numbers of embryos were transferred (1.4 and 1.6 respectively). 12 patients in the study and 4 patients in the control group failed to reach embryo transfer. Implantation rates were 60.4 and 67.9% for study and control groups respectively ($p > 0.05$). A higher biochemical pregnancy rate was seen in the study versus control group (58.9 v 45.3%; $p = 0.0049$). Clinical pregnancy rate per cycle started for GnRH trigger and hCG trigger groups were 38.9% and 42.1% respectively ($p > 0.05$; odds ratio 1.13; 95% CI 0.64-1.99). Onset of early OHSS was 10.5% in the control group with no cases of OHSS in the study group ($p < 0.0001$).

Limitations, reason for caution: Forms of progesterone supplementation in study and control groups were not standardized, and were continued until at least 4 weeks of pregnancy in the study group. GnRH antagonist commenced on day 5, or when the lead follicle measured 14mm. Live birth data are still awaited for the study group.

Wider implications of the findings: Our large series indicates GnRH trigger without hCG supplementation provides effective, safe oocyte maturation with no hyperstimulation risk or necessity for embryo cryopreservation which may be financially and emotionally challenging. Previous trials show variable clinical pregnancy rates in GnRH triggered cycles without hCG support although doses, routes and formulations of GnRH agonist vary. Given the OHSS rate in the control group a randomized controlled trial to gain more robust evidence may be ethically questionable.

Study funding/competing interest(s): There are no competing interests declared by the authors.

Trial registration number: Not applicable

P-509 Three day pre-treatment with GnRH antagonists prior to beginning of controlled ovarian stimulation in IVF/ICSI: efficacy from a clinical and embryological perspective

N. Mishieva¹, N. Barkalina², I. Korneeva¹, T. Ivanets¹, and A. Abubakirov¹

¹*Research Centre of Obstetrics Gynaecology and Perinatology named after academician V.I. Kulakov, reproductive department, Moscow, Russia C.I.S.*

²*Research Centre of Obstetrics Gynaecology and Perinatology named after academician V.I. Kulakov currently: Nuffield Department of Obstetrics and Gynaecology The University of Oxford UK, reproductive department, Moscow, Russia C.I.S.*

Study question: Does three-day administration of GnRH antagonists (GnRHant) in early follicular phase prior to the start of controlled ovarian stimulation (COS) with rFSH in IVF/ICSI cycles improve embryo quality and clinical pregnancy rate in young patients with good ovarian reserve, compared to standard GnRHant protocol?

Summary answer: Three-day pre-treatment with GnRHant prior to the COS in IVF/ICSI cycles in young non-PCOS patients with good ovarian reserve is associated with a trend towards increase in clinical pregnancy rate, and similar profiles of early embryo development, compared to standard fixed GnRHant protocol.

What is known already: GnRHant are increasingly applied in IVF/ICSI owing to their potential to decrease OHSS risks and treatment costs, improve patient satisfaction and deliver good success rates. Recently, a modified IVF/ICSI protocol with three-day GnRHant pretreatment before COS has been suggested as a cycle programming option. There is evidence that such approach increases the number of retrieved oocytes, compared to standard fixed GnRHant protocol, however no significant effect upon clinical pregnancy rate has been observed so far.

Study design, size, duration: Open controlled parallel study including 256 women, carried out from January 2011 till January 2013.

Participants/materials, setting, methods: Inclusion criteria: age <40yrs, regular cycle, basal FSH <12mIU/ml, normal BMI, history of ≤ 2 unsuccessful IVF attempts. Exclusion criteria: PCOS, severe male infertility cases. In the study group ($n = 126$), GnRHant were administered daily starting from cycle day 2 for 3 consecutive days (0.25mg/d), and then COS with rFSH was initiated. In the control group ($n = 130$), COS was started immediately on cycle day 2. Clinical pregnancy rate per cycle was used a primary outcome.

Main results and the role of chance: Age of the patients, causes and duration of infertility, ovarian reserve parameters, mean duration of COS, and total/starting dose of rFSH did not differ significantly between the groups. GnRHant pre-treatment protocol was associated with a significantly larger number of COCs retrieved (10.12 (95%CI 9.21-11.04) vs 8.53 (95%CI 7.73-9.34); $p < .05$). No differences were observed with regards to the proportion of MII oocytes, fertilization rate, cleavage rate, and blastocyst formation rate. Three-day pretreatment with GnRHant was associated with a trend towards increase in clinical pregnancy rate, however the difference has not reached statistical significance (54.7% vs 37.8%, $p = .062$, NS).

Limitations, reason for caution: The protocol of this study excluded women with PCOS, and therefore, validation of efficacy of three-day GnRHant pre-treatment strategy in PCOS patients is required before recommending clinical application in this sub-group of infertile women.

Wider implications of the findings: A three-day GnRHant pre-treatment protocol represents a promising alternative to conventional GnRHant protocol, with an additional benefit of cycle programming option, which does not compromise treatment success.

Study funding/competing interest(s): Since October 2012, Dr Natalia Barkalina is affiliated with the Nuffield Department of Obstetrics and Gynaecology, University of Oxford (UK) as a PhD student on an independently funded non-related project.

Trial registration number: N/A (not attributable)

P-510 Regulation of hyaluronan (HA) synthesis and signalling systems in ovine granulosa cells

R. Chavoshinejad¹, G.m. Hartshorne¹, W. Marei², and A.a. Fouladi-nashta²

¹*Warwick Medical School, Clinical Sciences Research Laboratories, Coventry, United Kingdom,* ²*Royal Veterinary College, CBS, London, United Kingdom*

Study question: Are there differential or synergic effects of endocrine hormones on expression of HA synthases (HAS2-3) and HA cell membrane receptor (CD44) in granulosa cells?

Summary answer: Follicle stimulating hormone (FSH) has synergistic effects with oestradiol (E₂) or insulin to up-regulate HAS2 mRNA expression. E₂, FSH and insulin have additive effects on HAS3 mRNA expression. E₂ and insulin have positive effects on CD44. These results provide evidence for endocrine regulation of HA system in the sheep ovary.

What is known already: Hyaluronan, produced by HAS, is a glycosaminoglycan component of extracellular matrix in ovaries. It functions as a structural component of tissues and also a signalling molecule after binding to CD44. Hyaluronan and CD44 are present in the follicular fluid and the role of HA in cumulus expansion is well characterised. We have previously shown that HAS2-3 proteins in ovarian follicular cells have a differential distribution in different classes of follicles.

Study design, size, duration: Sheep ovaries were collected from abattoir, granulosa cells (GC) were isolated from follicles by aspiration of follicular fluid. The experiments were repeated on at least three separate occasions with at least ___ replicates. The data (mean ± SEM) were analysed using mixed linear model and the differences (P < 0.05) were determined.

Participants/materials, setting, methods: Isolated GCs were cultured in serum-free conditions supplemented with different combinations of 1 ng/ml FSH, 10 ng/ml Insulin, 10 ng/ml IGF1, 40 ng/ml E₂ and 25 ng/ml LH. After 48h of culture, RNA was extracted and reverse transcribed to cDNA then used for quantification of mRNAs expression.

Main results and the role of chance: Supplementation with E₂, FSH, LH or IGF1 alone had no effect on HAS2 or HAS3 mRNA expression. Combined treatment with E₂ and FSH in the presence or absence of IGF1 increased HAS2 expression. This effect was not observed for E₂ + LH. Combined treatment of E₂, FSH and insulin in the absence of IGF1 up-regulated HAS3 expression. Combined treatments (FSH + insulin or IGF1) or (E₂ + FSH, LH or insulin) did not affect HAS3 expression. Treatment with LH or E₂ or insulin up-regulated CD44 mRNA expression, whereas FSH, IGF1 had no effect. Combination of E₂ with FSH or LH or insulin up-regulated CD44 expression. Combination of FSH and IGF1 increased CD44 expression. This effect was not observed in the presence of insulin + E₂.

Limitations, reason for caution: These studies were carried out in vitro with fixed physiological concentrations of hormones. Testing of the findings in an in vivo situation is needed to assess the clinical robustness of the in vitro data presented here.

Wider implications of the findings: It has been postulated that hyaluronan in the extracellular compartment of the ovarian follicle relates to cell metastasis and granulosa tumour. Therefore, improved knowledge of the basic function and components of the ovarian HA system and its regulatory mechanisms is important for understanding the pathophysiology of the ovaries in mammals.

Study funding/competing interest(s): These studies were funded in part by a BBSRC research grant to Ali Fouladi-Nashta. Ramyar Chavoshinejad is a self-funded PhD student.

Trial registration number: Not applicable for in vitro studies.

P-511 Randomize study: the prevalence of the metabolic syndrome in Greek women with polycystic ovary syndrome

G. Kyrkou¹, E. Trakakis², C.H. Chrelias², E. Alexiou², K. Lykeridou³, and G. Mastorakos⁴

¹University hospital of Athens 'Attikon', 3rd Pediatric Department N.I.C.U., Athens, Greece, ²University hospital of Athens 'Attikon', 3rd Obstetric & Gynecological Department, Athens, Greece, ³Technological Education Institute of Athens, Midwifery Department, Athens, Greece, ⁴University Hospital Of Athens "Areteion", 2nd Obstetric & Gynecological Department, Athens, Greece

Study question: The objective of the study was to investigate the prevalence of metabolic syndrome (MS) in Greek women with polycystic ovary syndrome (PCOS) because women with PCOS have an increased risk of cardiovascular disorders and incidence of diabetes.

Summary answer: The prevalence of MS in Greek women with PCOS was 10%, also we found that it increases gradually with age. So to prevent future disorders we recommend systematic follow up patient education.

What is known already: Polycystic ovary syndrome is among the most common endocrine disorders of women in the reproductive age group. PCOS has been strongly associated with MS and insulin resistance, so there is an increased prevalence of MS in women with PCOS. The PCOS and the MS appear to be interrelated and the PCOS-MS interrelationship is not restricted to Caucasian women with PCOS. In women with PCOS indicated differences in the prevalence of MS among people.

Study design, size, duration: In this study participated only Greek women, during their reproductive age; they were from different residence areas, different origin and independent socio-economic class and were tested during the period 2004 to 2009. They underwent laboratory and clinical evaluation for the diagnosis of PCOS and MS.

Participants/materials, setting, methods: 230 Greek women, which have been diagnosed with PCOS according to the criteria of the Rotterdam ESHRE, aged from 12 to 44 years, correlation for the diagnosis of MS the abdominal circumference, the fasting glucose, the TG, the HDL-C, the systolic and the diastolic pressure, using criteria of IDF. This is the first study in Greece that correlates PCOS with MS.

Main results and the role of chance: The prevalence of MS of Greek women with PCOS was 10% (IDF criteria). Pathological variables in the MS to the general population with PCOS: abdominal circumference (>80cm) rate of 63%, HDL-C (<50mg/dl) 26, 5%, TG (>150mg/dl) 10,4%, fasting glucose (>100mg/dl) 7,4%, diastolic blood pressure (>85mm/Hg) 6,1%, systolic blood pressure (130mm/HG) 4,3%. The prevalence of MS according to age: minimum age to 19 years 0%, 20 to 24 years 6,76%, 25 to 29 years 9,62%, 30 to 34 years 17,14% and 35 to maximum age 55,56%. The pathological variables of MS in all age groups have the primary finding of increased abdominal circumference and secondary finding the decreased levels of HDL-C, also a gradual increased observed in diastolic blood pressure and fasting glucose.

Limitations, reason for caution: This study was limited by the fact that the data was collected only from the Gynecological Endocrinology outpatient clinic, of the 3rd Obstetrics and Gynecology Clinical of the University General Hospital 'Attikon'.

Wider implications of the findings: Our findings agree with the most researches of the literature and prove that the prevalence of the MS varies depending on each country's habits and life style and also is not limited to Caucasian race, -for example the prevalence of MS in USA is 43% (criteria ATP-III), in Australia 40% (criteria IDF), in India 46,2% (criteria IDF), in South Italy 8,2% (criteria ATP-III) and in Germany 33,8%(criteria IDF).

Study funding/competing interest(s): This study was not funded by any source.

Trial registration number: Validation date 26/7/2010

Number/protocol 10865/31/8/2010

P-512 Exogenous gonadotropins have little impact on follicular but considerable effect on serum cytokine concentrations - a comparison between natural cycle and stimulated IVF

N. Bersinger, Z. Kollmann, M.D. Mueller, A. Vaucher, and M. von Wolff
University of Berne, Obstetrics and Gynaecology, Berne, Switzerland

Study question: Is the intrafollicular production of inflammatory cytokines and growth factors such as vascular endothelial growth factor (VEGF) and their release stimulated and negatively influenced by the use of controlled ovarian stimulation with gonadotropins?

Summary answer: Serum levels of the studied cytokines at the moment of oocyte retrieval were generally higher in the stimulated than in the natural cycles. However, this was not the case in the follicular fluid. We attribute the difference to the increased number of active follicles present after controlled ovarian stimulation.

What is known already: Throughout follicular growth and subsequent corpus luteum formation the leukocyte number increases and follicular vascularisation changes. These processes are enhanced under cIVF with gonadotropins. Cytokines released by leukocytes contribute to further recruitment and vascularisation of the follicle, and they play an important role in regulating ovarian steroidogenesis by influencing theca and granulosa-lutein cell function. Changes in cytokine and VEGF concentrations in the ovary as a consequence of gonadotropin stimulation may negatively influence oocyte quality.

Study design, size, duration: Cross-sectional study involving 37 natural (NC) and 39 gonadotropin-stimulated (cIVF) cycles performed in 2011 and 2012. Thirteen women within this population underwent one NC and one cIVF cycle each. Collection of serum on the day of oocyte retrieval and of the follicular fluid (FF).

Participants/materials, setting, methods: Mean age was 35.3 ± 4.6 (SD) and 34.2 ± 3.7 years in the NC and cIVF groups (ns). A total of 14 cytokines from Bio-Plex panels I and II were determined in matched serum and FF samples using Luminex xMAP technology on the Bio-Plex® platform, using the serum protocol.

Main results and the role of chance: Tumour necrosis factor- α , RANTES, eotaxin and interferon- γ -induced protein-10 levels were lower in FF than in serum, and thus not further investigated. Interleukin (IL)-6, -8, -10, -15, -18, monocyte chemoattractant protein-1 (MCP-1), VEGF and leukaemia inhibitory factor (LIF) showed significantly higher median concentrations in FF than in serum, indicating possible ovarian production and thus being interesting targets for further analysis. Moreover, all of these (except IL-15 and LIF) showed significantly higher levels in the cIVF than in the NC cycles in the serum (by Mann-Whitney U test). For follicular fluid this was not the case; IL-8 was reduced in cIVF but correlated strongly and positively with IL-10, IL-15, MCP-1, VEGF and LIF. In the serum, MCP-1 correlated strongly with all interleukins above, and so did VEGF.

Limitations, reason for caution: The selection of the cytokines depended on their availability in the Bioplex panels. The serum results for some of them (IL-15 and LIF) could not be analysed due to insufficient assay sensitivity.

Wider implications of the findings: Follicular concentrations of the studied interleukins, MCP-1, VEGF and LIF did not differ between cIVF and NC groups, but increased serum levels were observed after stimulation. This is probably due to the high number of follicles present after stimulation and releasing hormones, cytokines and growth factors into the circulation. These results thus suggest an application potential of multiplexed analyses for the monitoring of the stimulation phase in the serum before and up to oocyte retrieval.

Study funding/competing interest(s): Public university (salaries) and private industry (consumables).

Trial registration number: Not applicable.

P-513 Dopamine receptor D2 agonist (D2-ag) post-transcriptionally inhibits VEGF production and secretion by lutein granulosa cells through non classical D2 transduction pathways

H. Ferrero¹, R. Gómez¹, C.M. García-Pascual¹, C. Simón¹, and A. Pellicer²
¹Fundación IVI/INCLIVA, Research, Paterna (Valencia), Spain, ²Instituto Valenciano de Infertilidad (IVI), Reproductive Medicine, Valencia, Spain

Study question: The goal of this study was to elucidate whether the inhibition of VEGF secretion by lutein granulosa cells in response to D2-ag is exerted at the production or secretion levels and to explore which of the classical D2 transduction pathways is/are involved in D2-ag induced VEGF inhibition in LGC.

Summary answer: The D2-ag (Cb2) inhibits VEGF secretion not only by interfering with the translation of the peptide but also through interference with the release of the protein once it is produced. None of the classical D2 transduction pathways is responsible for the effects observed.

What is known already: By using *in vitro* and *in vivo* models we showed that LGCs express D2 and that D2-ag prevents increased ovarian vascular permeability (VP) in OHSS by inhibiting VEGF secretion in those cells with D2-antagonist reverses the effect. As Protein but not VEGF mRNA are affected by D2-ag the regulation is exerted at the post-transcriptional level. In D2 expressing cells other than LGCs D2-ag exerts its different actions through the G₀/G₁, G_z and β -arrestin transduction pathways.

Study design, size, duration: Follicular fluid-derived LGC were isolated and cultured with hCG in presence/absence of the D2-ag cabergoline (Cb2) and treated with: Study 1: Brefeldin-A (protein secretion inhibitor) for 8h. Study 2: Pertussis toxin, Wortmannin, Phorbol 12-myristate 13-acetate for 48h, to inhibit each one of the different classical D2 transduction pathways.

Participants/materials, setting, methods: LGCs from egg donors (~25years, oocytes < 20, E2 < 2000pg/ml, BMI < 30) were cultured as described above. Conditioned medium was collected at the end point to measure the amount of (secreted) VEGF by ELISA. LGCs were fixed in paraformaldehyde or lysed for

quantification of intracellular (produced) VEGF through image analysis or ELISA respectively.

Main results and the role of chance: In order to determine how the D2-ag affected VEGF protein production and/or secretion, we measured intracellular and extracellular VEGF in LGCs treated with/without D2-ag in the presence/absence of Brefeldin-A. VEGF accumulation was higher in D2-ag treated cells (154 ± 15.26 pg/ml) vs control cells (29.14 ± 12.5 pg/ml) in the absence of Brefeldin-A, implying that D2 activation interferes with mechanisms of VEGF secretion. Intracellular VEGF was lower in D2-ag treated cells (235.9 ± 30.4 pg/ml) vs control cells (405.9 ± 18.56 pg/ml) when incubated with Brefeldin-A, uncovering an inhibitory effect of D2-ag on VEGF protein production. Incubation of LGCs with different D2-downstream pathways inhibitors (PTX, WT and PMA) to block G₀/G₁, G_z and β -arrestin D2 signalling transduction pathways respectively failed to counteract the effects of D2-ag on LGCs VEGF secretion.

Limitations, reason for caution: Despite decreases in VEGF by D2-ag are D2 mediated (D2 antagonists reverses the effects), D2 D2-ag effects might be being exerted through non-yet described D2 pathways or through alternative mechanisms not yet discovered. Indeed transduction pathways have not been fully characterized and of course are poorly described in LGC.

Wider implications of the findings: D2-ag are not only used for OHSS but also for ovarian cancer treatment. It is likely both therapies benefit from D2-ag compounds through a similar mechanism of action: Inhibition of VEGF production by ovarian tissue. If this is a universal mechanism of action, deciphering the by now paradoxical/alternative molecular mechanisms through which this effect occurs is a scientific challenge expected to render more specific therapies and/or implement new ones.

Study funding/competing interest(s): Participation of H.Ferrero was supported by 'Instituto de Salud Carlos III, FI09/00549'. This project has been financed by grant SAF2008-03546 from Spanish government and by European Regional Development Fund.

Trial registration number: This is a Basic science work. A trial registration number is only required for clinical trials.

P-514 Effect of follicle-stimulating hormone receptor N680S polymorphism in the efficacy of follicle stimulating hormone stimulation on donor ovarian response

A. Turienzo¹, B. Lledo¹, J. Guerrero², J.A. Ortiz¹, R. Morales¹, J. Ten², J. Llacer³, and R. Bernabeu³

¹Instituto Bernabeu Biotech, Molecular Biology, Alicante, Spain, ²Instituto Bernabeu, Reproductive Biology, Alicante, Spain, ³Instituto Bernabeu, Reproductive Medicine, Alicante, Spain

Study question: Investigate whether Asn680Ser (N680S) FSHR polymorphism has a predictive value for ovarian response to stimulation with gonadotropins and cycle outcome in our egg donor program.

Summary answer: In a population of fertile egg donors FSHR gene polymorphism at position 680 is associated with different ovarian response to COH (Controlled Ovarian Hyperstimulation). FSHR gene genotype is an important factor for determining the prognosis of COH cycles on normo ovulatory fertile women.

What is known already: An individual variability in response to drugs exists. Genetic factors could explain these differences. FSH and its receptor (FSHR) play a major role in follicular development and regulation in the ovary. Ovarian response to FSH, however, varies widely among women undergoing ovarian stimulation. Clinical studies have demonstrated that N680S polymorphism determines ovarian response to FSH stimulation in patients undergoing IVF. Patients with the S680 allele need more FSH during the stimulation phase.

Study design, size, duration: FSHR polymorphism N680S has been studied in 145 oocyte donors. Patients underwent ovarian stimulation (n = 355) using urinary FSH, in a GnRH-antagonist protocol and triggering the LH surge with a bolus of GnRH-agonist. The main outcome measures were oocyte yield, stimulation days, gonadotropin dosages, biochemical pregnancy, ongoing pregnancy and miscarriage rates.

Participants/materials, setting, methods: Oocyte donors were selected according to Instituto Bernabeu egg donation program requirements and ASRM and ESHRE guidelines for oocyte donation. This research was set at Instituto Bernabeu Fertility center.

Main results and the role of chance: Significant differences were reported in antral follicles count (16.5 ± 5.0 for NN, 14.5 ± 4.7 for NS and 14.1 ± 3.8 for SS), number of eggs retrieved (21.5 ± 9.2 for NN, 18.5 ± 8.2 for NS and 19.8 ± 8.9 for SS) and gonadotropin doses (2098.5 ± 639.4 IU for NN, 2023 ± 490.1 IU for NS and 2149.5 ± 552.3 IU for SS) between genotypes. Differences in terms of biochemical pregnancies, miscarriage and ongoing pregnancy rates were not affected by the N680S polymorphism on FSHR gene in egg donors.

Limitations, reason for caution: Application of pharmacogenetics to measure ovarian reserve and predicting ovarian response is true. However an individual is embedded with the context of that individual's entire genome and environment. In fact, some others genes related to follicular growth could also play an important role in determining the response to OH.

Wider implications of the findings: In spite of the final clinical outcome is not different, this investigation reveals that in a population of fertile egg donors, FSHR gene polymorphism at position 680 is associated with different ovarian response to COH. Genotyping FSHR N680S together with some additional markers may therefore provide a means of identifying a group of poor responders before infertility treatment is initiated.

Study funding/competing interest(s): Conflicts of interest and source of funding none declared.

Trial registration number: This study is not a trial

P-515 Oxidative stress status in follicular fluid and oocyte quality in women with polycystic ovary syndrome treated with inositol

V. De Leo¹, R. Focarelli², A. Capaldo², A. Stendardi¹, L. Gambera¹, A. La Marca³, and P. Piomboni¹

¹University of Siena, Molecular medicine and Development Department, Siena, Italy, ²University of Siena, Life Sciences Department, Siena, Italy, ³University of Modena and Reggio Emilia, Mother-Infant Department, Modena, Italy

Study question: The oxidative stress (OS) status in the follicular fluid (FF) of women with polycystic ovary syndrome (PCOS) undergoing in vitro fertilization (IVF) could be decreased after a three months therapy with D-chiro-inositol (DCI). **Summary answer:** A reduced protein oxidation level in FF, demonstrated by a proteomic analysis of the free -SH group, and a better oocyte quality were detected in PCOS women undergoing to ovarian hyperstimulation protocol when pre-treated with a three-months therapy with DCI in comparison to untreated patients.

What is known already: Reactive oxygen species are involved in the modulation of several reproductive functions. PCOS has been associated with oxidative imbalance, linked to insulin resistance typical feature of the syndrome. Inositol is an intracellular mediator of insulin and its main action takes place via insulin sensitization. Little is known about the effects on follicular microenvironment. Through a proteomic approach, the oxidative damage on FF proteins can be evaluated by the quantitative analysis of free-SH groups.

Study design, size, duration: FF were collected in a period of one year and analysed by a proteomic approach to evaluate OS level and the results statistically compared between two groups: 22 samples collected from PCOS patients treated with 500 mg DCI for 3-months before ovarian stimulation and 12 FF samples collected from PCOS women not subjected to DCI treatment. Mean age, BMI and smoking status were similar in the two groups.

Participants/materials, setting, methods: A total of 34 FF samples were collected and analysed. FF proteins were labeled with 3-(N-Maleimidopropionyl)-biocytin, separated in SDS-PAGE and transferred to nitrocellulose in order to detect the protein with free-SH residues for OS level quantification. This analysis was performed by specific software and the results were statistically evaluated using the one-way variance test.

Main results and the role of chance: An inverse relationship was found between protein labeling intensity and oxidative stress. The results revealed a reduced presence of free-SH groups in the follicular fluid proteins and low quality oocytes in untreated women with PCOS when compared to DCI treated women. These results suggest that in PCOS there is an increase of the oxidation of -SH groups in FF proteins, correlated to a deteriorated follicular microenvironment. The administration of D-chiro-inositol seems able to decrease the OS level as demonstrated by the appearance in FF of new proteins with free thiol groups. The improved environment could be related to a greater oocyte quality.

Limitations, reason for caution: The direct evaluation of the OS level in cell-free biological fluids is very difficult since they lack the ROS producing cells. However

by our proteomic approach it's possible evaluate the oxidative damage of proteins and therefore indirectly determine the effect of OS. Previous studies have shown that oral administration of DCI has not side effects or contraindications

Wider implications of the findings: The present study demonstrated at molecular level the antioxidant action of D-chiro-inositol on follicular fluid protein of women with PCOS and therefore the improvement of follicular microenvironment that reflect a greater oocyte quality. This finding may be important for the overall outcome of the IVF treatment in these patients. Starting from a good oocyte, it's possible increase the chance to obtain a top quality embryo.

Study funding/competing interest(s): No competing interest was present. PAR funding from Siena University and Health Ministry funding 2010 from Modena University.

Trial registration number: Samples collection from patients undergoing IVF and molecular analysis were approved by the local Ethic Committee at Siena University Hospital.

P-516 Carotid intima-media thickness in women with polycystic ovary syndrome and matched controls

J.J. Kim¹, Y.M. Choi¹, J.H. Kang², K.R. Hwang³, S.J. Chae⁴, S.M. Kim¹, S.H. Yoon⁵, S.Y. Ku¹, S.H. Kim¹, J.G. Kim¹, and S.Y. Moon¹

¹Seoul National University Hospital, Obstetrics & Gynecology, Seoul, Korea South, ²Seoul National University Hospital, Radiology, Seoul, Korea South, ³Seoul National University Hospital, ObstRadiology, Seoul, Korea South, ⁴Maria Fertility Hospital, Obstetrics and Gynecology, Seoul, Korea South, ⁵Dongguk University, Obstetrics and Gynecology, Seoul, Korea South

Study question: Does carotid intima-media thickness (CIMT) increase in young untreated Korean women with polycystic ovary syndrome (PCOS)?

Summary answer: Despite the significant differences in some vascular risk factors, PCOS patients did not have a significantly higher CIMT compared with age-matched controls.

What is known already: Metabolic disturbances are well-recognized clinical features of PCOS. CIMT has been widely used as a surrogate marker of atherosclerosis and cardiovascular disease (CVD). The association between PCOS and CIMT has been investigated in many studies, but has not yet been in the Korean population. The aim of the present study was to compare the presence of subclinical atherosclerosis in young untreated Korean women with PCOS and age-matched controls, specifically by measuring their CIMT.

Study design, size, duration: Case-control study. CIMT profiles were evaluated in 56 PCOS patients and 56 age matched controls.

Participants/materials, setting, methods: Fifty-six women with PCOS (18-40 years) were recruited using the Rotterdam criteria. CIMT was measured by the one radiologist in 56 PCOS patients and 56 controls. To compare the CIMT according to PCOS phenotypes, women with PCOS were divided into two subgroups according to the presence of hyperandrogenism. Continuous parameters were compared using Student's t- or Mann-Whitney U test. Univariate regression analyses were conducted with CIMT as a dependent variable and traditional CVD risk factors and serum androgens as independent variables. All data analyses were performed using the Statistical Package for the Social Sciences software (version 19.0, IBM SPSS, NY, USA), and statistical significance was set at two-sided P values $<.05$. Power calculations were performed using the G-power v.3.1.5 software (<http://www.psych.uni-duesseldorf.de/abteilungen/aap/gpower3>). Given the specified sample size (56 PCOS patients and 56 controls), the power to detect a mean CIMT difference 0.5mm (an α value of 0.05) was 0.75.

Main results and the role of chance: Women with PCOS and controls were same in age (30.9 vs. 30.8 ; $p = .904$), but those with PCOS had a higher BMI than controls (21.2 vs. 19.8 ; $p = .004$). The CIMT ranged from 0.30 to 0.70 mm in women with PCOS and from 0.24 to 0.82 mm in controls. Despite the significant differences in some vascular risk factors between women with PCOS and controls, the mean CIMT was not different between the two groups (0.49 ± 0.09 mm in PCOS patients vs. 0.50 ± 0.11 mm in controls, respectively, $p = .562$). When the CIMT in the control group was compared with hyperandrogenic and non-hyperandrogenic PCOS groups, there were also no significant differences between the groups. CIMT was not correlated with any of the atherogenic or androgenic parameters among all subjects as well as the subset of PCOS patients.

Limitations, reason for caution: Given the specified sample size, this study has limited power for the small CIMT difference.

Wider implications of the findings: PCOS patients did not have a significantly higher CIMT (even in the hyperandrogenic subgroups). Although our study did not show the increased risk of subclinical atherosclerosis in PCOS patients, the role of CIMT continues to be investigated considering the importance of screening and monitoring CVD risk factors in women with PCOS.

Study funding/competing interest(s): exists. This study was supported by Supported by grant no 04-2008-0900 from the SNUH Research Fund.

Trial registration number: N/A

P-517 Can anti-Müllerian hormone (AMH) predict the diagnosis of polycystic ovary syndrome (PCOS) - a systematic review and meta-analysis

S. Iliodromiti¹, T.W. Kelsey², R.A. Anderson³, and S.M. Nelson¹

¹University of Glasgow, Reproductive and Maternal Medicine, Glasgow, United Kingdom, ²University of St. Andrews, School of Computer Science, St. Andrews, United Kingdom, ³Queens Medical Research Institute University of Edinburgh, MRC Centre for Reproductive Health, Edinburgh, United Kingdom

Study question: A systematic literature review was performed to assess the true accuracy of AMH in the prediction of PCOS and to perform an aggregation data analysis to determine the optimal diagnostic threshold.

Summary answer: Summary receiver operator curves and patient data meta-analyses suggest that PCOS can be diagnosed with appropriate sensitivity and specificity based on a single AMH value.

What is known already: Existing diagnostic biochemical tests for PCOS have poor sensitivity and specificity. Many women with PCOS have high AMH concentrations, thus this may be a useful addition to the diagnostic criteria.

Study design, size, duration: Systematic review and meta-analysis of the literature.

Participants/materials, setting, methods: Nine studies (n = 593 women with PCOS) reporting AMH values in the diagnosis of PCOS were included in the meta-analysis and the construction of the summary ROC. Individual data with AMH serum levels for 146 females with PCOS (according to the Rotterdam criteria) and 136 women with no PCOS were extracted from four studies.

Main results and the role of chance: Meta-analysis of the extracted data demonstrated specificity and sensitivity in diagnosing PCOS in symptomatic women of 79.4% and 82.8% respectively for a cut off value of AMH of 4.7 ng/ml. The AUC was 0.87 (95%CI 0.83, 0.92) similar to the summary ROC curve of 0.89.

Limitations, reason for caution: Heterogeneity was caused by study quality and characteristics, slight differences in study populations and the necessity to adjust for AMH assay used.

Wider implications of the findings: AMH may be a useful initial diagnostic test for PCOS particularly for those healthcare providers without ready access to transvaginal sonography, or where that investigation is inappropriate.

Study funding/competing interest(s): No specific funding was received for this study. RAA and SMN have undertaken consultancy work for Beckman Coulter and Roche Diagnostics.

Trial registration number: N/A

P-518 Is androgen production in association with immune system activation potential evidence for the existence of a functional adrenal/ovarian auto-immune system in women?

H.J. Lee³, A. Weghofer², V.A. Kushnir³, A. Shohat-Tal³, E. Lazzaroni³, H.J. Lee³, D.H. Barad¹, and N.N. Gleicher¹

¹The Center for Human Reproduction & Foundation for Reproductive Medicine, N/A, New York NY, U.S.A., ²Medical University Vienna & The Center for Human Reproduction, Department of Gynecologic Endocrinology and Reproductive Medicine, Vienna, Austria, ³The Center for Human Reproduction, N/A, New York NY, U.S.A

Study question: We investigated whether evidence of immune system activation is associated with androgen levels in women with diminished functional ovarian reserve (DFOR).

Summary answer: Women with evidence of immune system activation demonstrated, overall, statistically higher testosterone levels than women without evidence of immune system activation.

What is known already: Autoimmunity is a well-recognized etiology for DFOR, whether in association with premature ovarian failure (POF)/primary ovarian insufficiency (POI) or its precursor stage premature ovarian aging (POA)/occult primary ovarian insufficiency (OPOI). DFOR in association with POA/OPOI and in association with physiologic ovarian aging has recently also been associated with low testosterone levels, raising the potential of an association between androgen levels and autoimmunity.

Study design, size, duration: In a prospective cohort study we investigated 322 consecutive infertility patients at initial presentation, 135 with POA/OPOI (DFOR < age 38, Group 1), 155 women with physiologic DFOR (> 40 years, classical DOR, Group 2), and 32 controls (< 38 with normal age-specific FOR).

Participants/materials, setting, methods: We in all women assessed androgen levels and a broadly based immune profile, in previous studies proven effective in differentiating patients with and without immune activation.

Main results and the role of chance: Overall, women with immune abnormalities demonstrated higher total testosterone (TT, P = 0.004) and free testosterone (FT, P < 0.001) levels than women without. Groups 1, 2 and controls and the two immunologically defined groups (negative and positive) demonstrated significant statistical interaction in mean TT (P = 0.008): Mean TT and FT were significantly higher in women with positive immune findings in controls than in POA/OPOI (Group 1) and physiologic DOR (Group 2) patients (all 4 differences P < 0.001). Women without immune abnormalities, however, did not demonstrate such differences.

Limitations, reason for caution: Though results are statistically robust, caution is in order since objective definition of immune system activation is difficult, and utilized definition favors sensitivity over specificity. Moreover, associating immune activation with higher (i.e. normal) androgen levels, on first impression, appears counterintuitive.

Wider implications of the findings: The study, however, offers objective evidence for an association between androgen levels and immune system activation. Seeking a possible explanation, these findings raise the hypothesis of an immune system derived androgen-production factor (APF), which maintains normal androgen levels and is lacking in women with DFOR and/or absence of immune system activation. Since pregnancy represents a semi-allograft, induction of tolerance requires a degree of immune system activation.

Study funding/competing interest(s): Supported by Foundation for Reproductive Medicine and Center for Human Reproduction. N.G., A.W. and D.H.B. received research support, lecture fees and travel support, none, however, related to this project.

Trial registration number: N/A

P-519 A comparison of in-vitro maturation versus antagonist protocols for in-vitro fertilization in women with polycystic ovarian syndrome

T. Shavit, E. Shalom-Paz, O. Fainaru, M. Michaeli, E. Kartchovsky, and A. Ellenbogen

Hillel Yaffe Medical Center, IVF unit Department of Obstetrics and Gynecology, Hadera, Israel

Study question: Is there any differences in the outcomes of In Vitro Maturation (IVM) versus Antagonist

protocols for In-Vitro Fertilization (IVF) in women with Polycystic Ovarian Syndrome (PCOS) undergoing Assisted Reproductive Technology (ART) ?

Summary answer: IVM protocol may be an alternative for infertile women with PCOS who desire to prevent the potential adverse effects of gonadotropins treatment but with lower pregnancy rates and live birth rates

What is known already: Only several studies compared IVM to IVF in patients with PCOS, most of them did not found significant differences in pregnancies rate and live birth rates even though there was a trend toward IVF. We could not found any study comparing IVM to IVF antagonist protocol in patients with PCOS.

Study design, size, duration: A retrospective cohort study. Records of all treatment cycles of patients with PCOS that underwent IVM or IVF with antagonist protocols in our unit between 01.01.2010 to 31.12.2011 were reviewed. There were 62 IVM cycles (16 with gonadotropins priming and 46 with estradiol priming) and 50 GnRH antagonist IVF cycles.

Participants/materials, setting, methods: The two treatment protocols were compared regarding the number of oocytes retrieved, maturation rate (IVM protocol) or mature oocytes (antagonist protocol), fertilization and cleavage rates, quality of embryos and pregnancy and delivery rates. The amount of gonadotropins administered was calculated.

Main results and the role of chance: Comparing IVF antagonist protocol to IVM, no significant differences in the number of mature oocytes achieved (9.12 ± 6.56 vs. 8.14 ± 5.05 $p = 0.38$), fertilization rate (59% vs. 57%, $p = 0.66$) and quality of embryos was observed. The average dose of gonadotropins in the IVF-antagonist protocol was $2051 \text{ IU} \pm 838 \text{ IU}$ per cycle, compared to $116 \pm 198 \text{ IU}$ in the IVM group (P value < 0.001). Pregnancy rate in the IVM cycles and IVF cycles was 18% and 40% respectively ($p = 0.008$) and live birth rates 26% and 10% respectively ($P = 0.04$).

Limitations, reason for caution: 1. This is a retrospective cohort study

2. Two different protocols of IVM were used: one with limited gonadotropins stimulation for three days in the early follicular phase, and the second without priming but with the use of estrogen supplements during the follicular phase.

Wider implications of the findings: We compared two protocols applied for infertile patients with PCOS, being at increased risk for Ovarian Hyperstimulation Syndrome (OHSS). Both protocols may reduce the incidence of OHSS in this population. A prospective randomized study comparing IVM to antagonist protocol with GnRH agonist triggering of ovulation is suggested.

Study funding/competing interest(s): None

Trial registration number: Not relevant

P-520 Self-operated endo-vaginal tele-monitoring (SOET) as an alternative for traditional monitoring of the stimulation phase of IVF/ICSI cycles Preliminary results of a randomized comparison

J. Gerris¹, F. Vandekerckhove¹, A. Delvigne², N. Dhont³, B. Madoc⁴, J. Neyskens⁴, M. Buyle⁴, E. Vansteenkiste⁵, E. De Schepper⁶, L. Pil⁷, N. Van Keirsbilck⁸, W. Verpoest⁹, D. Debacquer¹⁰, L. Annemans¹¹, and P. De Sutter¹²

¹University Hospital Ghent, Centre for Reproductive Medicine - Dept. of OB/GYN, Ghent, Belgium, ²Hôpital St Vincent Rocourt, Centre for Reproductive Medicine - Dept. of OB/GYN, Liège, Belgium, ³Ziekenhuis Oost-Limburg, Centre for Reproductive Medicine - Dept. of OB/GYN, Genk, Belgium, ⁴University Hospital, Centre for Reproductive Medicine - Dept. of OB/GYN, Ghent, Belgium, ⁵Ghent University, Faculty of Engineering, Ghent, Belgium, ⁶Ghent University, Centre for bio-statistics, Ghent, Belgium, ⁷Ghent University, Health-economics, Ghent, Belgium, ⁸Ghent University Hospital, Centre for Reproductive Medicine, Ghent, Belgium, ⁹Brussels University Hospital, Centre for Reproductive Medicine, Brussels, Belgium, ¹⁰Ghent University Hospital, Public Health, Ghent, Belgium, ¹¹Ghent University Hospital, Health-economics, Ghent, Belgium, ¹²Ghent University Hospital, Centre for Reproductive Medicine, Ghent, Belgium

Study question: Non-inferiority study comparing SOET of the stimulation phase in IVF/ICSI with traditional monitoring.

Summary answer: SOET (S) is welcomed by $\approx 80\%$ of patients, is technically feasible and shows similar clinical, laboratory, patient reported and health-economic results as non-SOET (NS).

What is known already: Monitoring the follicular phase is needed to adapt gonadotrophin dose, avoid OHSS and plan HCG administration. Traditionally, patients visit care providers, entailing transportation costs and productivity loss. It stresses patients, partners, care providers and the environment. Patients who live at great distance from IVF centres have more difficult access to treatment.

Study design, size, duration: Non-inferiority RCT between S and NS. Sample size calculations based on the number of metaphase-II oocytes at retrieval showed we need 100 patients in each study arm. This also allows comparing patients reported outcomes (PRO) and health-economic data (HE). Interim report after 10 months; time horizon ≈ 2 years.

Participants/materials, setting, methods: Inclusion criteria: < 41 years, ICSI, no poor response, two ovaries. We used a PC with vaginal probe and a specific web application. Study participants were given sonographic training sessions at the centre. 58 randomized patients completed their study cycle with SOET ($n = 29$; 1 drop-out) or non-SOET ($n = 29$); 19/78 (20%) eligible patients declined participation for different reasons.

Main results and the role of chance: Patient characteristics (age, partner age, BMI, smoking, treatment rank, AMH) in SOET- and non-SOET are comparable.

Clinical results: similar conception rates (S: $10/28 = 36\%$; NS: $14/29 = 48\%$) ($p = 0.42$)

Laboratory results: similar follicles $> 15 \text{ mm}$ at OPU (S: 8.2 ± 3.1 ; NS: 8.7 ± 7.7), n ova at OPU (S: 11.8 ± 6.3 ; NS: 10.5 ± 7.9), n metaphase-II oocytes (S: 8.9 ± 4.9 ; NS: 8.4 ± 6.9), \log_2 n metaphase-II oocytes (S: 3.01 ± 0.887 ; NS: 2.72 ± 1.263), n transferable embryos available at ET (S: 4.3 ± 3.7 ; NS: 4.5 ± 3.4), n excellent embryos (S: 1.4 ± 0.6 ; NS: 1.1 ± 0.7) and n embryos frozen (S: 0.8 ± 1.4 ; NS: 1.5 ± 2.2).

PRO: S ($n = 22$) showed a significantly higher feeling of empowerment ($p = 0.02$) and more partner participation ($p = 0.04$) than NS ($n = 25$); comparing S patients with their own historic controls as NS ($n = 15$) showed higher empowerment ($p < 0.001$), partner participation ($p = 0.004$), feeling of discretion ($p = 0.002$), less stress ($p = 0.008$) and a trend towards more contentedness ($p = 0.09$).

HE analysis: SOET cycles have 8x less productivity loss ($74 \pm 163 \pm \text{€}$ vs. $361 \pm 440 \text{€}$; $p < 0.01$), 4-5x lower transportation cost ($49 \text{€} \pm 73 \text{€}$ vs. $224 \pm 345 \text{€}$; $p < 0.01$), > 10 x direct lower sonograms and consultation costs ($23 \pm 28 \text{€}$ vs. $248 \pm 149 \text{€}$; $p < 0.01$), but higher personnel cost ($100 \pm 42 \text{€}$ vs. $181 \pm 33 \text{€}$; $p < 0.01$).

Limitations, reason for caution: Preliminary results are in line with non-inferiority hypothesis. Mean average difference for primary outcome variable (n metaphase-II oocytes) = -0.29 (95% CI: -0.87 ; 0.28). Non-inferiority can be concluded with all these values < 0.32 .

Wider implications of the findings: SOET works. Innovative approach offering advantages: empowering patients in urban western settings, increasing and facilitating access to treatment in large countries, bringing care towards patients in poor resource settings by using the concept of "flying care providers", allowing a disconnection between monitoring and laboratory facilities. Trial continues to reach full power. Hard- and software to be further developed. RCT preliminary data highly suggestive.

Study funding/competing interest(s): Supported by an IOF (industrial research fund) of Ghent University and by Flanders Care (demonstration project).

Trial registration number: EC/2011/669 (Ethical Committee Ghent University Hospital) and B670201112232 (Belgian registration).

P-521 AMH, a putative follicular fluid marker of oocyte quality, is concentrated around 3-fold higher in follicular fluid of Natural Cycle-IVF than in gonadotropin stimulated IVF

M. Von Wolff, Z. Kollmann, A. Vaucher, B. Weiss, and N.a. Bersinger
University of Bern - Inselspital, Department of Endocrinology & Reproductive Medicine, Bern, Switzerland

Study question: Are Anti-Mullerian hormone (AMH) concentrations in follicular fluid at the time of ovum pick up higher in Natural Cycle IVF (NC-IVF) than in conventional gonadotropin stimulated In vitro fertilization (cIVF)?

Summary answer: An inter-individual as well as in an intra-individual comparison revealed that AMH levels are around 3-fold ($p < 0.0001$) higher in NC-IVF follicles than in cIVF-follicles (Antagonist protocols with HMG-stimulation) at the time of ovum pick up.

What is known already: In NC-IVF the implantation rate in women < 40 y has been reported to be around 25% in comparison to 15% in cIVF without embryo selection. The difference has been suggested to be due to negative effects of high dosages of exogenously administered gonadotropin on the follicular metabolism and thereby on the oocyte quality. The intrafollicular concentration of AMH has been shown in several studies to be negatively correlated with the oocyte fertilisation and implantation rates.

Study design, size, duration: Cross-sectional study involving 37 NV-IVF and 39 cIVF cycles performed in 2011 and 2012. Thirteen women within this population underwent one NC-IVF and one cIVF cycle each. Collection of serum on the day of oocyte retrieval and of the follicular fluid (FF).

Participants/materials, setting, methods: Mean age was 35.3 ± 4.6 (SD) and 34.2 ± 3.7 years in the NC and cIVF groups (ns).

Follicular fluid was collected from the leading follicles using a Gauge 17 needle, clarified by centrifugation and the supernatant stored at -70°C . AMH was determined by microplate ELISA (Hybritech/Beckman) in batch. For statistical analysis the non-parametric Mann-Whitney U or Wilcoxon tests were used for cross-sectional or paired comparisons, respectively.

Main results and the role of chance: There was no significant difference in the mean age and follicular size between both groups. Hormone analysis in serum excluded relevant impact of serum concentration on follicular fluid hormone concentrations.

Median follicular fluid AMH concentration was 32.8 (p25 = 18.1, p75 = 43.0) pmol/L in NC-IVF follicles and 11.1 (p25 = 7.8 p75 = 16.8) pmol/L in cIVF follicles ($p < 0.0001$). The differences of median AMH concentration was confirmed when the analysis was limited to the 13 patients receiving both kind of therapies and so excluding inter-individual variation: Median follicular fluid AMH concentration was 35.0 and 10.4 pmol/L in NC-IVF and cIVF follicles, respectively ($p = 0.01$).

Limitations, reason for caution: A comparative analysis of the implantation potential of the oocytes of both treatment groups could not be performed as the oocytes in cIVF were not treated separately.

Wider implications of the findings: Previous cIVF studies negatively correlated AMH concentration in follicular fluid with oocyte maturity and fertilization rate. In contradiction, follicles in NC-IVF, which are expected to be a model for the "ideal" follicle with highest oocyte quality, revealed much higher AMH-concentrations than in cIVF. This suggests first that the artificial exposure to high concentrations of gonadotropins in cIVF might have a negative effect on the follicle metabolism and second questioning a direct link of AMH-concentration and oocyte quality in cIVF.

Study funding/competing interest(s): No competing interests

Trial registration number: Not applicable

P-522 Pregnancy outcomes in poor responders after ICSI cycles

F.F. Verit¹, S. Keskin¹, A.K. Sargin², S. Karahuseyinoglu², O. Yucel¹, and S. Yalcinkaya¹

¹Suleymaniye Maternity Research and Training Hospital, Department of Obstetrics and Gynecology Infertility Research & Treatment Center, Istanbul, Turkey, ²Suleymaniye Maternity Research and Training Hospital, Department of Embryology Infertility Research & Treatment Center, Istanbul, Turkey

Study question: Is there any increased risk of adverse pregnancy and neonatal outcomes in poor responders after ICSI cycles?

Summary answer: Poor responders are associated with adverse pregnancy and neonatal outcomes

What is known already: Poor ovarian response is a common clinical problem which affects up to 26% of ART cycles. Pregnancy rates were decreased in poor responders and it has been also reported that trisomic pregnancies and miscarriage rates were increased in that group.

Study design, size, duration: One hundred thirty one poor responders and 130 age and body mass index matched normoresponders who attended to infertility clinic of Suleymaniye Maternity, Research & Training Hospital, Department of Obstetrics and Gynecology, between January 2009 and March 2012 that become pregnant after ICSI therapy, were enrolled in the study. Poor responder was defined as patients less or equal to 4 oocytes were retrieved. Patients older than 37 years were excluded from the study.

Participants/materials, setting, methods: Data from the all of the participants were analyzed in terms of pregnancy outcomes such as biochemical pregnancies, miscarriages, live births, multiple pregnancies, prematurity, small for gestational age, intrauterine growth restriction, preeclampsia, placenta previa, abruptio placenta, third trimester bleeding, stillbirth, macrosomia and neonatal complications such as perinatal asphyxia, hydronephrosis, necrotizing enterocolitis, congenital heart disease and chromosomal abnormalities.

Main results and the role of chance: Among all the study participants with positive pregnancy tests; miscarriage rates were higher in poor responders compared with normoresponders (21.4% versus 10.8%; $p < 0.0001$, respectively) and live birth rates were lower in the same group (48.1% versus 76.2%; $p < 0.0001$, respectively). The incidence of multiple pregnancy rates was decreased in poor responders compared with normoresponders (1.6% versus 12.1%; $p = 0.008$, respectively) and low birth weight (less than 2500g) and preterm delivery (less than 37 weeks) were increased in that group compared with controls (34.9% versus 16.2%; $p = 0.006$, respectively and 27.0% versus 10.1%; $p = 0.006$, respectively). Other adverse pregnancy and neonatal outcomes were also elevated in poor responders ($p < 0.0001$; $p = 0.001$, respectively). Moreover, the relative risk of obstetric and neonatal outcomes were (3.7, 95% confidence interval [CI] 2.01-6.70 and 2.7, 95% CI 1.51-4.80, respectively).

Limitations, reason for caution: One of the limitations of the study is its retrospective design. In addition it is a preliminary study with relatively low study subjects. Some additional studies are also needed to investigate long term outcome of these children.

Wider implications of the findings: It has been hypothesized that there is a close relationship between poor ovarian reserve and oocyte quality. The present study has shown that pregnancy and neonatal complications were increased in poor responders compared with normoresponders. We also confirmed that poor responders are less prone to multiple pregnancies. Poor responders undergoing IVF treatment should not only be informed about their low pregnancy rates, but also about their increased pregnancy and neonatal complications.

Study funding/competing interest(s): Any financial support has been taken for this study.

Trial registration number: EPC4637

P-523 Chronic kisspeptin administration advances the menstrual cycle in healthy women

A.N. Comninos¹, C.N. Jayasena¹, G.M.K. Nijher¹, A. Abbara¹, A. De Silva¹, J.D. Veldhuis², R. Ratnasabapathy¹, C. Izzi-Engbeaya¹, A. Lim³, D.A. Patel³, M.A. Ghatei¹, S.R. Bloom¹, and W.S. Dhillo¹

¹Imperial College London, Investigative Medicine, London, United Kingdom,

²Mayo Clinic, Centre for Translational Science Activities, Rochester, U.S.A,

³Imperial College Healthcare NHS Trust, Imaging Department, London, United Kingdom

Study question: What are the effects on the menstrual cycle of acute and chronic kisspeptin-54 administration in healthy women?

Summary answer: We demonstrate for the first time that exogenous administration of kisspeptin-54 (a product of the KISS1 gene) advances the menstrual cycle in healthy women.

What is known already: The KISS1 gene is a critical regulator of reproductive function. In humans, inactivating mutations of the KISS1 gene result in a failure of puberty while activating mutations result in precocious puberty. Administration of kisspeptin induces ovulation in rodents and sheep. In women with hypothalamic amenorrhoea, acute administration of kisspeptin leads to potent stimulation of gonadotrophins but chronic administration leads to profound tachyphylaxis. The effects of chronic kisspeptin administration in healthy women are not known.

Study design, size, duration: We performed a prospective, single-blinded, placebo-controlled, one-way crossover study.

Participants/materials, setting, methods: Five healthy women received twice-daily subcutaneous injections of kisspeptin-54 or saline for 7 consecutive days during days 7-14 of their menstrual cycle. Volunteers underwent serial assessment of reproductive hormones, luteinising hormone (LH) pulsatility, ultrasound parameters (ovarian activity), as well as acute sensitivity to gonadotrophin-releasing hormone (GnRH) and kisspeptin-54 injection.

Main results and the role of chance: Main results and the role of chance Kisspeptin treatment shortened the overall menstrual cycle (mean length (days): saline 28.6 ± 1.4 vs. kisspeptin 26.8 ± 3.1 , $P < 0.01$), advanced the onset of highest recorded serum LH (mean menstrual day of highest LH: saline 15.2 ± 1.3 vs. kisspeptin 13.0 ± 1.9 , $P < 0.05$), and advanced the onset of the luteal phase of menstrual cycle (mean day of progesterone increase: saline 18.0 ± 2.1 vs. kisspeptin 15.8 ± 0.9 , $P < 0.05$). On menstrual day 15, the largest ovarian follicle had a significantly larger diameter following 7 days of kisspeptin-54 administration when compared with saline (mean diameter of largest follicle (mm): saline 10.0 ± 2.2 vs. kisspeptin 15.5 ± 1.2 , $P < 0.05$). Furthermore, contrary to the effects previously seen in women with hypothalamic amenorrhoea, chronic kisspeptin-54 administration at the same dose did not abolish acute stimulation of LH following injection of GnRH or kisspeptin-54.

Limitations, reason for caution: In this study assessments were made on 10 separate visits during the menstrual cycle rather than daily. This was carefully planned to achieve a balance between accuracy and volunteer compliance as well as to minimise stress to the volunteer.

Wider implications of the findings: It had been previously assumed that chronic kisspeptin administration to healthy women would result in tachyphylaxis and hence limit its therapeutic potential. This study demonstrates for the first time that tachyphylaxis is not observed and furthermore the menstrual cycle timings

are in fact advanced. This suggests that kisspeptin can be used to modulate the menstrual cycle and hence can potentially be used in the treatment of women with reproductive disorders.

Study funding/competing interest(s): Wellcome Trust, National Institute for Health Research.

Trial registration number: Not applicable

P-524 Triggering of final oocyte maturation with triptorelin 0.3 mg increases pregnancy rates in oocyte donation cycles

M. Colodron¹, J.J. Guillén¹, D. García², O. Coll¹, R. Vassena¹, and V. Vernaev¹
¹Clinica EUGIN, Assisted Reproduction, Barcelona, Spain, ²Fundació EUGIN, Research, Barcelona, Spain

Study question: Final oocyte maturation is usually triggered with triptorelin 0.2 mg in controlled ovarian stimulation (COS) protocols with a Gonadotropin-Releasing Hormone (GnRH) antagonist, in oocyte donation cycles. Could we improve the efficiency of the cycle using a triptorelin triggering dose of 0.3 mg? **Summary answer:** Cycles triggered with 0.3mg of triptorelin lead to higher biochemical and ongoing pregnancy rates compared to 0.2mg. However, comparable numbers of cumulus-oocyte complex (COC) and mature oocytes (MII) are retrieved from the two treatments. Our results suggest that triptorelin 0.3mg promotes better cytoplasmic maturation, producing oocytes with higher developmental competence.

What is known already: GnRH agonist triptorelin is a triggering drug widely used in many donation programs. Different triggering doses (0.1, 0.2, 0.3, and 0.5mg) have been reported in the literature as safe and effective. Despite the extensive use of 0.2 mg, there is no available information over its ability to elicit a complete maturation of all suitable oocytes. We tested for the first time the hypothesis that a slightly higher dose could improve the efficiency of the cycle.

Study design, size, duration: This monocentric, retrospective, cohort study was carried out in a large private fertility clinic during a period of 3 months. A total of 399 subjects (236 triptorelin 0.3 mg / 163 triptorelin 0.2 mg) were included in the study.

Participants/materials, setting, methods: Donors received one of the two triptorelin doses on the triggering day (0.3mg or 0.2mg). Triggering criterion: ≥ 3 follicles ≥ 18 mm at last ultrasound. Average number of retrieved COC and MII, and pregnancy rates in recipients were analyzed using the Student's t-test and Chi-square test.

Main results and the role of chance: The average number of follicles of diameter ≥ 14 mm measured at last ultrasound before oocyte retrieval (as a predictor of the number of COC that will be obtained) was similar among treatments (13.1 ± 6.0 vs. 12.5 ± 5.2 , $p = 0.39$). Accordingly, both the average number of COC (17.3 ± 9.17 vs. 15.1 ± 6.98 , $p = 0.054$) and of MII retrieved (14.1 ± 7.37 vs. 12.3 ± 5.72 , $p = 0.06$) was similar among treatments. Interestingly however, biochemical (42.3% vs. 54.1% , $p = 0.0014$) and ongoing (29.1% vs. 40.3% , $p = 0.001$) pregnancy rates in recipients were significantly higher for triptorelin 0.3. Triggering with 0.3mg of triptorelin seems to increase the quality, rather than the quantity, of MII collected from COS.

Limitations, reason for caution: A randomized controlled clinical trial should be performed to confirm the results of this study. Because of the study design (not randomized), study groups could be unbalanced; however age, BMI, and treatment (dose and duration) were comparable in both groups. Caution should be exerted in generalizing our results.

Wider implications of the findings: We presume that triptorelin 0.3mg will allow for a surge of a sufficiently high LH peak and/or a more physiologically normal surge of progesterone to stimulate complete cytoplasmic maturation of a higher number of oocytes, which will lead to a higher quality of the MII retrieved. By increasing the performance of each donation cycle, we expect to reduce the number of cycles necessary for obtaining oocytes of higher quality, and increase each cycle risk-benefit ratio.

Study funding/competing interest(s): No competing interests are declared.

Trial registration number: NA

P-525 Comparing the effects of aerobic exercise and fennel (foeniculum vulgare) on pre-menstrual syndrome(PMS) in high school young girls

H. Pazoki¹, G. Bolouri², F. Farokhi¹, and M.A. Azarbayjani²

¹faculty of science Urmia University, Department of Biology, Urmia, Iran,

²Islamic Aazad University, Department of exercise Physiology, Tehran, Iran

Study question: Premenstrual syndrome (PMS) is a condition characterized by a number of behavioral, psychological and physical symptoms recurring cyclically during the luteal phase of the menstrual cycle. The present study was carried out to compare the effects of regular exercise and fennel extracts on the PMS on high school girls.

Summary answer: The exercise and fennel extracts could reduce the severity of PMS. The effects of fennel were greater than the exercise. Administration of the extracts of this herb is suggested for relieving the signs and symptoms of PMS.

What is known already: In recent years, use of herbs and aerobic exercise has been considered in the treatment of premenstrual syndrome. Foeniculum vulgare is herb that is traditionally used for menstrual disorders. Torke Zahrani, et al reported that fennel extract reduced the severity of dysmenorrhea, Fennel essence can reduce the frequency and intensity of contractions in rat uterus. Gannon in 1988 proposed the potential role of exercise on the alleviation of menstrual disorders and menopausal symptoms including PMS.

Study design, size, duration: In this Randomized clinical trial 36 students aged 16-18 were selected by filling the questionnaire form, participant divided into three equal groups; control, aerobic(eight weeks exercise), fennel(fennel extracts was 30 drops, every 8 hours, from 3 days before until 3 days after the onset of menstrual bleeding), in three months.

Participants/materials, setting, methods: 36 students were divided into three equal groups and received exercise, fennel extracts. The severity of PMS was measured by questionnaire at the end of the first and second menstrual cycles before the intervention and the results were compared with them after the intervention in first and second cycle.

Main results and the role of chance: There were not any significant differences in the means of premenstrual syndrome scores before the intervention among the three groups (34.31 ± 16 in exercise group, 33.27 ± 16 in fennel group and 31.36 ± 17 in control group, $p > 0.05$), but the differences were significant after the intervention (36.69 ± 16 in exercise group, 42.49 ± 16 in fennel group and 34.90 ± 17 in control group, respectively, ($p < 0.009$) in this study no significant differences were observed for age, body mass index, age at menarche, age at dysmenorrhea onset and duration of menstruation between the three groups.

Limitations, reason for caution: In addition, menstrual cycle intervals and family histories of PMS were similar among the groups and all students didn't have any family illness, and $p < 0.05$ was considered statistically significant. Limitations, reason for caution Further investigations with more participants and longer duration of therapy should be carried out to clarify the detailed benefits of fennel extract and aerobic exercise in women with PMS.

Wider implications of the findings: Wider implications of the findings Electrolyte symptoms in PMS are suggested to be related increase serum level of aldosterone and prostaglandin E2 and deficiencies of vitamin B6 and magnesium. The reasons for increased serum aldosterone level in late luteal phase are increased activity of rennin-angiotensin and decrease levels of progesterone and estrogen. Aerobic exercise and fennel is shown to decrease rennin and increase estrogen and progesterone level leading to decrease serum level of aldosterone and improvement of electrolyte symptoms.

Study funding/competing interest(s): Fennels extract and aerobic exercise together or each reduced the severity of premenstrual syndrome. The fennel extracts and exercise both were effective on symptoms of anxiety and depression.

Trial registration number: 12613000088741 from (ANZCTR)

P-526 Should serum anti-Müllerian hormone (AMH) or antral follicle count (AFC) be used for prediction of oocyte yield in patients with AMH-AFC discordances

M.S. Alebic¹ and N. Stojanovic²

¹Merkur Teaching Hospital, Human Reproduction, Zagreb, Croatia, ²Merkur Teaching Hospital, Medical Biochemistry and Laboratory Medicine, Zagreb, Croatia

Study question: Whether AMH or AFC should be used as a predictor of oocyte yield and in ovarian stimulation protocol planning in patients with discordances between measured AMH levels (AMHm) and AMH expected (AMHe) according to AFC (AMH-AFC discordances)?

Summary answer: It was demonstrated that AMH was superior to AFC as a predictor of oocyte yield, in patients with AMHm higher than AMHe. Conversely, in patients having AMHm lower than assessed by AFC, AFC should be used for IVF stimulation protocol planning.

What is known already: AFC represents a gonadotrophin responsive follicular cohort measuring 2-9 mm. AMH is predominantly secreted by preantral, ultrasonically invisible, and small antral follicles. AMH and AFC are both used for prediction of ovarian response. Although they are strongly related to each other, AMH-AFC discordances are encountered in certain number of patients. In such ambiguous circumstances, it is not clear to which ovarian response predictor the clinician should rely on when deciding on the IVF treatment strategy.

Study design, size, duration: This is a retrospective study on medical records of 1088 patients aged 20-44 years who underwent the first IVF cycle with GnRH antagonist protocol between October 2010 and July 2012. AMHe was calculated as: $\text{Log}(\text{AMHe}) = -0.2616 + 1.2089\text{Log}(\text{AFC})$. AMH-AFC discordance was defined as AMHm more than residual standard deviation away from AMHe.

Participants/materials, setting, methods: Participants with AMH-AFC discordances ($n = 281$) were divided in two groups: DG1 ($n = 143$) and DG2 ($n = 138$) comprising patients with AMHm > AMHe and AMHm < AMHe, respectively. Univariate and multivariate linear regression analysis were applied to assess the value of AMH and AFC for the prediction of oocyte yield in each group.

Main results and the role of chance: AMH and AFC were found to be predictive for oocyte yield on univariate analysis in both study groups ($R^2 = 0.198$, $P < 0.001$ and $R^2 = 0.163$, $P < 0.001$ in DG1 for AMH and AFC, respectively; $R^2 = 0.300$, $P < 0.001$ and $R^2 = 0.305$, $P < 0.001$ in DG2 for AMH and AFC, respectively). However, using multivariate regression analysis (MRA) with AMH and AFC as independent variables, significant predictive value was demonstrated only for AMH in DG1 ($R^2 = 0.104$, $P < 0.001$) and for AFC in DG2 ($R^2 = 0.314$, $P < 0.001$). The same was found even when MRA was applied after stratification by initial gonadotrophin dose and age.

Limitations, reason for caution: The main limitations of this study are the retrospective design and relatively low number of patients in both study groups. The analysis included only IVF patients treated with the GnRH antagonist protocol and cannot be extrapolated to other ovarian stimulation protocols.

Wider implications of the findings: AFC and AMH are the most significant predictors of the number of oocytes retrieved. However, in patients in whom discordance between AMH results and AFC is observed, it is important to establish which test should be used for prediction of ovarian response and for the decision on starting dose of gonadotrophins. This study results, therefore, provide additional information which could improve optimization of ovarian stimulation strategy in IVF cycles.

Study funding/competing interest(s): No funding was used since all data were collected during the routine infertility work-up. The authors have no competing interest(s) to declare.

Trial registration number: Not applicable.

P-527 Decreased ovarian reserve in female Sprague-Dawley rats induced by isotretinoin exposure

R. Abali¹, A. Yuksel², C. Aktas³, C. Celik¹, S. Guzel⁴, G. Erfan⁵, and O. Sahin⁶

¹Namik Kemal University, Gynecology and Obstetrics, Tekirdag, Turkey,

²Istanbul University Cerrahpasa Tıp Fakültesi, Gynecology and Obstetrics,

Istanbul, Turkey, ³Namik Kemal University, Histology and Embryology, Tekirdag,

Turkey, ⁴Namik Kemal University, Biochemistry, Tekirdag, Turkey, ⁵Namik Kemal

University, Dermatology, Tekirdag, Turkey, ⁶Istanbul University, Neuromuscular Pathology Unit, Istanbul, Turkey

Study question: Is there any toxic effect of isotretinoin on the rat ovary?

Summary answer: These data are the first to identify that exposure of isotretinoin may be responsible for the decreased ovarian reserve and toxic effect on the rat ovaries.

What is known already: Isotretinoin (13-cis retinoic acid) is a retinoid that is widely used for the treatment of severe nodulo-cystic acne that affects mostly young people. Although it has broad side effects profile, there is no well-designed study about its effects on the ovary.

Study design, size, duration: The rats were divided into 3 equal groups according to the dose of isotretinoin to be administered for 30 days: 0 mg/kg/day (group 1), 7.5 mg/kg/day (group 2) or 15 mg/kg/day (group3).

Participants/materials, setting, methods: Ovarian effects of isotretinoin were evaluated with serum anti-Mullerian hormone (AMH) concentrations, apoptosis by TUNEL assay, immunohistochemical observations by PCNA. The ratio of atretic follicles was calculated at each stage of folliculogenesis.

Main results and the role of chance: The serum AMH concentrations were found lower in both isotretinoin groups. The ratio of atretic follicles in both isotretinoin groups were higher than the control. There was a dose response increase in the percentage of all stage of atretic follicles with the increasing doses of isotretinoin administered ($p < 0.001$). The number of PCNA-positive granulosa cells were decreased in the isotretinoin groups. The number of ovarian follicles with apoptotic granulosa cells was increased in experimental groups.

Limitations, reason for caution: Our study results are limited to only rat ovaries and it did not investigate whether the effects of isotretinoin on the ovary is irreversible or not.

Wider implications of the findings: Our results showed that isotretinoin has detrimental effect on the rat ovaries. However, future studies investigating the effect of isotretinoin on human ovaries should be reevaluated.

Study funding/competing interest(s): This study was supported by Namik Kemal University Research Founding.

Trial registration number: N/A.

P-528 Expression of transforming growth factor- β 1 in ovarian follicular fluid from women with polycystic ovary syndrome and its effect on miR-224 in ovarian luteinized granulosa cells

H. Zhongying, L. Shangwei, M. Qianhong, F. Wei, L. Lei, X. Zhun, and W. Yan
West China Second Hospital Sichuan University Hospital, Obstetrics and Gynecology, Chengdu, China

Study question: Is there any relationship between PCOS and local TGF- β 1, and any effect of TGF- β 1 on miRNA expression in luteinized granulosa cells?

Summary answer: There may be a connection between local TGF- β 1 and PCOS. TGF- β 1 up-regulated the expression of miRNA-224 in human luteinized granulosa cells

What is known already: The quality of oocytes from women with polycystic ovary syndrome (PCOS) tends to decline, which is partly attributed to the disturbance of cytokine networks in the local micro-environment of ovary. Transforming growth factor(TGF)- β has been considered as one of the key regulators in the development of follicle and growth of granulosa cells. A recent research reported that TGF- β 1 increased the expression of miR-224 and improved the proliferation of pre-antral granulosa cells in mouse.

Study design, size, duration: This case-control study was performed among 32 women aged under 35 years undergoing in vitro fertilization (IVF) in our University hospital IVF centre during Sep 2011 to Feb. 2012.

Participants/materials, setting, methods: Follicular fluid (FF) from ovarian follicles (≥ 17 mm) in 16 PCOS women and 16 non-PCOS women was sampled and TGF- β 1 level was detected using ELISA. Luteinized granulosa cells obtained from FF were treated with TGF- β 1 for 48h and the expression of miR-224 was quantified using real-time PCR.

Main results and the role of chance: Thirty one FF samples were obtained from non-PCOS women, of which 16 from follicles with eggs and the other 15 without. Thirty two FF samples were obtained from PCOS women, of which 17 from follicles with eggs and the other 15 without. The concentration of TGF- β 1 in FF from follicles without eggs showed no significant difference between women with PCOS and those without (166.16 ± 12.28 vs 206.67 ± 16.19 ng/ml, $P > 0.05$). However, in women with PCOS, the concentrations of TGF- β 1 in FF from follicles with eggs were significantly lower than that of women without PCOS (201.56 ± 6.45 vs 404.90 ± 51.29 , $P < 0.01$). Quantitative real-time PCR showed that treatment with TGF- β 1 caused significantly increase of miR-224 expression in luteinized granulosa cells collected from PCOS women ($P < 0.05$) and from women without PCOS ($P < 0.01$).

Limitations, reason for caution: The limitation is that we observed TGF- β 1 up-regulated the expression of miR-224 in luteinized granulosa cells only in vitro.

Wider implications of the findings: In this study, the significant difference in levels of TGF- β 1 of FF between PCOS women and women without PCOS was detected, suggesting the possible relationship between PCOS and local TGF- β 1. The significance of the stimulatory effect of TGF- β 1 on miR-224 expression in human ovarian luteinized granulosa cells is still to be elucidated.

Study funding/competing interest(s): None.

Trial registration number: None.

P-529 A randomized controlled trial to evaluate the effect on oocyte yield of prolongating the follicular phase in IVF/ICSI cycles with not-elevated progesterone

F. Vandekerckhove¹, A. De Baerdemaeker¹, J. Gerris¹, K. Tilleman¹, S. Vansteelandt², and P. De Sutter Pr.¹

¹University Hospital Ghent, Reproductive Medicine, Ghent, Belgium, ²Ghent University, Department of Applied Mathematics and Computer Science, Ghent, Belgium

Study question: We evaluated the effect of delaying the moment for triggering oocyte maturation by 24 hours on the number of (mature) oocytes at retrieval in cycles with not-elevated progesterone.

Summary answer: If 30-50 % of the measurable follicles (≥ 10 mm) have a diameter > 18 mm, delaying the decision to trigger oocyte maturation by 24 hours has a favourable effect on the number of oocytes retrieved and the number of mature oocytes.

What is known already: Since the beginning of IVF, the moment for triggering oocyte maturation has differed between centres. The leading follicles have to reach diameters of 16 to 18 mm and elevated progesterone levels are known to have a deleterious effect on the endometrium. Recent studies show better results in terms of number of oocytes collected and cycle outcome when waiting for larger follicles. They are underpowered and do not exclude patients with an elevated serum progesterone level.

Study design, size, duration: Patients with 30-50 % of their follicles > 18 mm and with a serum progesterone < 1 ng/ml were randomized to receive 5000 hCG the same day or 24 hrs later.

The primary endpoint was the number of mature oocytes.

For a mean difference of 4, a sample size of 23 in each group was required.

Between January 1st 2011 and December 31st 2011, 59 patients were included.

Participants/materials, setting, methods: 189 patients with > 3 large follicles (mean diameter > 18 mm) and with a serum progesterone < 1 ng/ml were eligible for further evaluation. In 59 patients 30-50 % of the follicles were large enough and they were allocated for randomisation. 28 patients received hCG the same day, the others 24 hours later.

Main results and the role of chance: Both groups were comparable for age, gravidity, parity, duration of infertility, ovarian reserve as determined by AMH, diagnostic criteria before treatment, stimulation protocol, the number of embryos transferred and the experience of the performer of the embryo transfer.

There was a higher number of (mature) oocytes in the group with delayed triggering of oocyte maturation.

Results in terms of cycle outcome (pregnancy rate, ongoing pregnancy rate, live birth rate, implantation rate) were not different in both groups.

Limitations, reason for caution: Although significantly different results in both groups are obtained for outcome variables describing the oocyte yield, much larger study groups are required to evaluate cycle outcomes.

Wider implications of the findings: Prolongating the follicular phase in cycles with an elevated progesterone is a next point to examine. In specific clinical situations such as oocyte donation or oocyte freezing for later use, only the number of good quality oocytes at retrieval is important. So further studies are useful to evaluate the optimal moment for triggering oocyte maturation in these cases.

Outcome	Mean difference	95% CI	P
Oocytes	2.97	0.45 to 5.49	0.021
Mature oocytes	2.41	0.22 to 4.61	0.031
Fertilized oocytes	1.80	-0.15 to 3.76	0.071
Good quality embryos	1.18	-0.53 to 2.88	0.18

Study funding/competing interest(s): This study was not funded and there were no competing interests.

Trial registration number: This study was approved by the Ethical Committee of our University Hospital (B67020108975).

P-530 Evaluation of a new ovarian response prediction index (ORPI) for individualised controlled ovarian stimulation

J.B.A. Oliveira¹, R.L.R. Baruffi², C.G. Petersen¹, A.L. Mauri², A.M. Nascimento³, L. Vagnini³, J. Ricci², M. Cavagna⁴, F.C. Massaro², A. Pontes⁵, and J.G. Franco Jr.¹

¹Centre for Human Reproduction Prof. Franco Jr / Paulista Center for Diagnosis Research and Training / Department of Gynecology and Obstetrics Botucatu Medical School São Paulo State University - UNESP, Research, Ribeirao Preto, Brazil, ²Centre for Human Reproduction Prof. Franco Jr / Paulista Center for Diagnosis Research and Training, Research, Ribeirao Preto, Brazil, ³Paulista Center for Diagnosis Research and Training, Research, Ribeirao Preto, Brazil, ⁴Centre for Human Reproduction Prof. Franco Jr / Paulista Center for Diagnosis Research and Training / Women's Health Reference Center Perola Byington Hospital, Research, Ribeirao Preto, Brazil, ⁵Department of Gynecology and Obstetrics Botucatu Medical School São Paulo State University - UNESP, Research, Botucatu, Brazil

Study question: Does a new ovarian response prediction index (ORPI) based on anti-Mullerian hormone (AMH) levels, antral follicle count (AFC) and age reliably predict ovarian stimulation response?

Summary answer: The ORPI accurately predicted low ovarian response (

What is known already: Knowledge regarding a patient's potential ovarian response can assist IVF clinicians in adjusting the dosage of medication to reduce adverse effects of an excessive/low ovarian response. Despite the predictive power of individual markers, they all have errors associated with estimation. Therefore, predicting ovarian response using a single biomarker may not be sufficient for formulating a precise treatment plan.

Study design, size, duration: This prospective cohort study included 101 infertile women who underwent ICSI from March/2012 to August/2012. ORPI values were calculated by multiplying the AMH level (ng/ml) by the AFC (2-9mm). The result was divided by the patient's age (years) [ORPI = (AMH x AFC) / Age]. Spearman's test, logistic regression and receiver operating characteristic (ROC) curve were used to analyse the data.

Participants/materials, setting, methods: AMH and AFC were evaluated during the early follicular phase of a previous cycle. The ORPI values did not influence the clinical decision regarding ovarian stimulation. Patients were stimulated with 150/225IU recombinant FSH and a gonadotropin-releasing hormone analogue based on age.

Main results and the role of chance: Spearman's test revealed significant correlations ($P < 0.0001$) between the ORPI and the number of oocytes collected and the number of follicles. Logistic regression revealed that ORPI values were significantly associated with the likelihood of collecting ≥ 4 oocytes (OR = 49.25), ≥ 4 MII oocytes (OR = 6.26) and ≥ 15 oocytes (OR = 6.10; $P < 0.0001$; Figure 1). The odds ratios associated with the ORPI were consistently higher than the odds ratios associated with all other prognostic factors (i.e., age, AMH and AFC). Based on the ROC curves, the ORPI accurately predicted a low ovarian response (< 4 oocytes retrieved; area under the curve (AUC): 0.91), collection of ≥ 4 MII oocytes (AUC: 0.84) and an excessive ovarian response (≥ 15 oocytes retrieved; AUC: 0.89).

Limitations, reason for caution: Despite recruiting all eligible participants during the study period, the sample size was limited. Although we based the response definitions on the results of published studies, different reproduction clinics may prefer alternative definitions of low/excessive ovarian responses, which could modify the cut-offs used in this study.

Wider implications of the findings: The combination of different variables in the ORPI resulted in a more precise index for predicting ovarian response. The ORPI could be used to improve the cost-benefit ratio of ovarian stimulation regimens by guiding the selection of medications and tailoring the doses and regimens to actual patient needs.

Study funding/competing interest(s): The authors declare that they have no competing interests.

Trial registration number: Not applicable. The study was authorised by the local ethics committee.

P-531 Addition of luteal estradiol pre-treatment in women treated with GnRH antagonist for in vitro fertilization: a systematic review and meta-analysis

W. El-khayat and M. Elsadek

Faculty of Medicine Cairo University, Obstetrics & Gynecology, Cairo, Egypt

Study question: To evaluate the effect of luteal estradiol (E2) pre-treatment with GnRH antagonist protocol for in vitro fertilisation.

Summary answer: Estradiol pre-treatment with GnRH antagonist cycles for in vitro fertilization not associated with statistically significant difference in live birth rate nor clinical pregnancy rate but there is a statistically significant increase in duration and dose of gonadotropins among the E2 pre-treatment group.

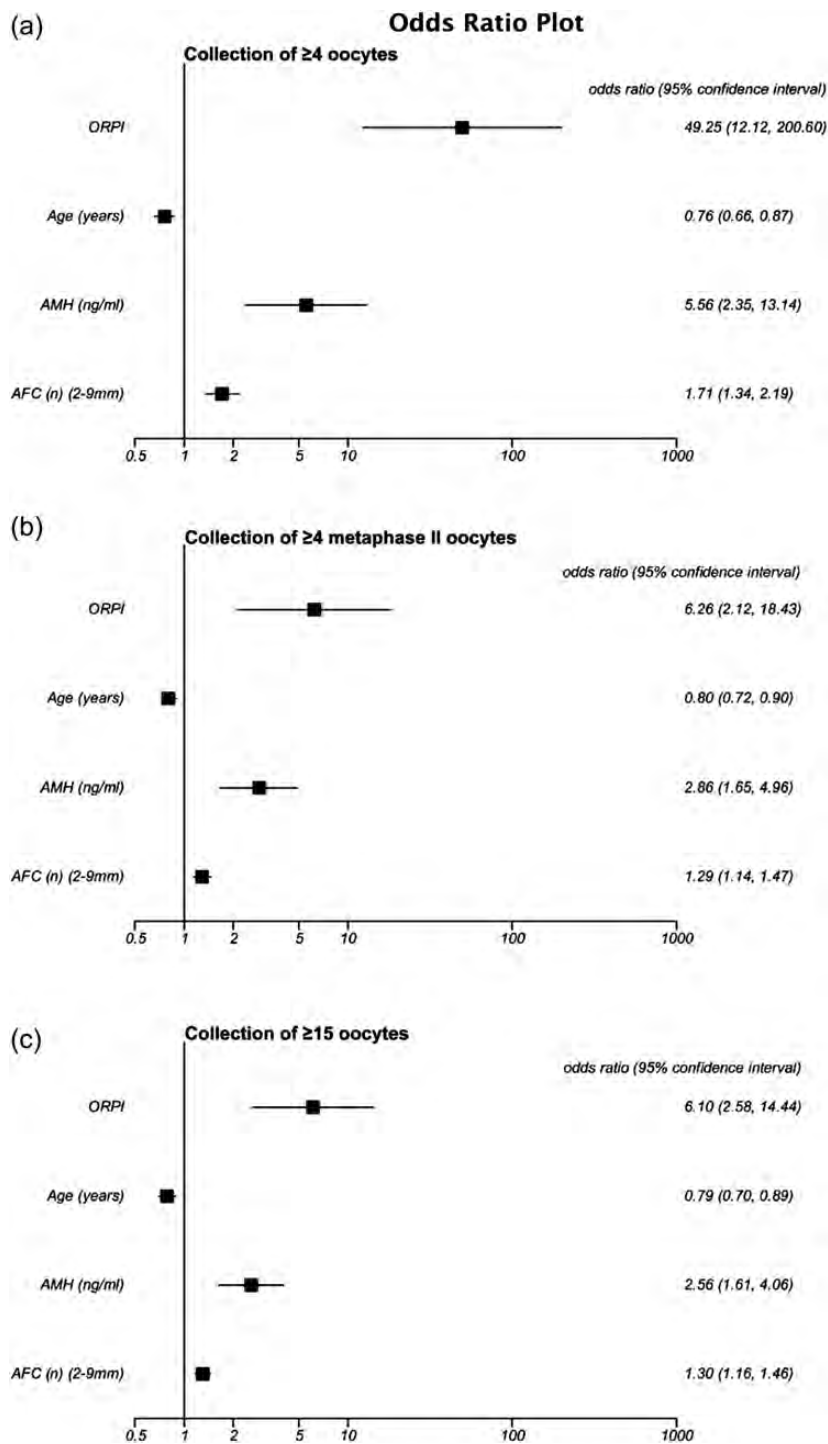


Figure 1 Logistic regression analysis for the prognostic factor regarding the collected oocytes and pregnancy occurrence. A. Collection of ≥ 4 oocytes B. Collection of ≥ 4 MII oocytes C. Collection of ≥ 15 oocytes

What is known already: This modified GnRH-antagonist protocol leads to a reduction of oocyte retrievals during weekend days, without deleterious impact on the number of oocytes and the clinical pregnancy rates.

Study design, size, duration: systematic review and meta-analysis Participants/materials, setting, methods Computerized literature search was performed independently by two reviewers until December 2012 in order to identify randomized controlled trials (RCTs) evaluating estradiol pre-treatment with GnRH antagonist cycles for in vitro fertilization. The main outcome measures were live birth rate and the clinical pregnancy rate.

Main results and the role of chance: Three studies eligible for this systematic review including 1511 patients. By synthesizing the results of these studies, it was shown that no significant differences were present between the group receiving E2 and the control group regarding live birth rate, clinical pregnancy rate. In subgroup analysis between studies comparing between E2 pre-treatment in antagonist protocol and no E2 treatment in long agonist protocol there was a statistically significant increase in the long agonist protocol group with (weighted mean difference-WMD: -0.80, 95% CI: -1.49 to -0.11) also there was a statistically significant increase in dose of gonadotropins consumption with (WMD +136 IU, 95% CI: +73 to +199), and duration of gonadotropins stimulation with (WMD +0.45 days, 95% CI: +24 to +0.66) in E2 pre-treatment group.

Limitations, reason for caution: Need more randomized controlled trials to be included in a later meta-analysis

Wider implications of the findings: we can generalize to the populations, our results is new to the literature

Study funding/competing interest(s): no funding

Trial registration number: No trial registration number

P-532 Pregnancy rate following luteal phase support in polycystic ovarian syndrome using combination therapies for ovulation induction: a randomized clinical trial

F. Foroozanfar¹, H. Saberi², A. Moravvegi³, and M. Kazemi⁴

¹Kashan University of Medical Sciences, OBS & GYN - Infertility & IVF Fellowship, Kashan, Iran, ²Kashan University of Medical Sciences, Department of Occupational Medicine, Kashan, Iran, ³Kashan University of Medical Sciences, Department of Community Medicine, Kashan, Iran, ⁴Kashan University of Medical Sciences, Student, Kashan, Iran

Study question: Can luteal phase support (LPS) with intravaginal progesterone (P) in polycystic ovarian syndrome (PCOS) using letrozole or clomiphene citrate (Cc) in combination with HMG improve pregnancy rate?

Summary answer: Administration of vaginal P for LPS may be improved the pregnancy rate in women with PCOS using letrozole or Cc in combination with HMG for ovulation induction.

What is known already: Luteal phase defect has been reported in patients with PCOS. Previous studies have produced conflicting results regard with effect of LPS with P on pregnancy rate. On the other hand, there is no prospective trial investigating the need for P administration in the combination stimulation protocols in PCOS.

Study design, size, duration: A randomized clinical trial with parallel design was employed in patients with PCOS (n = 198) between Aprils to Jan 2011.

Participants/materials, setting, methods: Patients from infertility center of Kashan, Iran that met following criteria were eligible: non existence of male factor for infertility; had normal uterine cavity and patency of fallopian tube as demonstrated by either hysterosalpingogram or diagnostic laparoscopy and hysteroscopy and having 2 of the Rotterdam diagnostic criteria. Patients underwent ovulation induction either Cc (n = 98) or letrozole (n = 100). Cc (100 mg/5day) or letrozole (5mg/5day) in combination with HMG (150 IU/5day) were used for ovulation induction. After hCG administration (5000IU), patient received intravaginal P (Cyclogest, 400mg daily) according to randomization. The outcome was the comparison of chemical pregnancy rate between the groups.

Main results and the role of chance: LPS was associated with a 10% higher pregnancy rate than in non-P cycles, although this difference did not reach statistical significant (p = 0.08). LPS improved pregnancy rate in both CC (4%) and letrozole (6%) groups. In addition, Patients who used letrozole for ovulation induction has higher pregnancy rates when using intravaginal P support than CC group.

Limitations, reason for caution: lack of statistical significance of difference between groups in present study may be a result of not having the number of cycles required to reach appropriate statistical power. Perhaps the failure to observe a significant effect of P on pregnancy rate in the different studies may be explained, in part, by either small study sizes, inadequate statistical power to detect a significant difference or the use of different drugs for ovarian stimulation, as well as different types and dosages of P for LPS.

Wider implications of the findings: our results suggest that LPS with P may be improved pregnancy rate in patients with PCOS who treated with either CC or letrozole in combination with HMG.

Study funding/competing interest(s): The authors declare that they have.

Trial registration number: IRCT138810132967N1

P-533 GnRH agonist for triggering final oocyte maturation versus IVM in patients at risk of OHSS

Y.S. Gidoni, A. Razieli, S. Friedler, D. Strassburger, D. Hadari, E. Kasterstein, I. Ben-Ami, D. Komarovsky, B. Maslansky, O. Bern, and R. Ron-El
Assaf Harofeh Medical Centre, IVF Unit, Zerifin, Israel

Study question: To evaluate the clinical outcome of both treatment modalities in patients with high risk to develop OHSS

Summary answer: This prospective randomized study shows no prevalence of one of the two methods used in patients at high risk for OHSS.

What is known already: Severe OHSS continues to be the most frequent serious complication of controlled ovarian hyperstimulation (COH) in IVF. GnRH agonist (GnRHa) for triggering final oocyte maturation in a GnRH antagonist COH protocol and in vitro maturation (IVM) of retrieved oocytes from small follicles can significantly reduce the risk of OHSS. However, there is an ongoing controversy about the superiority of each method

Study design, size, duration: Prospective randomize study. From Aug 2011 till Dec 2012 both treatments were performed after randomization in 50 patients with unovulatory polycystic ovaries. Of the GnRHa triggering group, 23 patients have been completed the treatment. One cycle was cancelled due to premature luteinization, another patient has no embryo for transfer

Participants/materials, setting, methods: The IVM protocol included 3 days of gonadotrophin administration, oocyte retrieval was performed when endometrium thickens was ≥ 6 mm. Mature oocytes were injected immediately after denudation. Oocytes maturation was examined after 24 h and those which matured were injected. The GnRH antagonist protocol was with flexible GnRH antagonist protocol and gonadotrophins with individual adjustment. GnRHa was administered when the leading follicle was of ≥ 18 mm diameter.

Main results and the role of chance: No significant difference was observed in the demographic characteristics. The total number of mature oocyte was significantly higher (P = 0.0002) in the GnRHa triggering group compared with the IVM group (16.6 ± 7 and 8.2 ± 4.3 respectively). Fertilization rates were similar ($68.5 \pm 20.9\%$ and $74 \pm 23.2\%$ respectively; NS), and so the cleavage rates ($96.1 \pm 12.4\%$ and $93.3 \pm 21.1\%$; NS). A similar number of "Top Quality" embryos were observed in both groups ($22.9 \pm 22\%$ and $27 \pm 22\%$ respectively; NS). The number of the transferred embryos was significantly higher in the IVM group compared to the GnRHa triggering group (2.4 ± 0.7 and 1.95 ± 0.47 respectively, P = 0.03). Six pregnancies were achieved in the triggering group (24% per cycle) compared with 11 pregnancies in the IVM group (44% per cycle) (P = 0.234). A total number of 47 embryos (mean 1.88 ± 3) were cryopreserved in 10 (40%) of 25 IVM patients. Six frozen-thawed embryo cycles were performed with one pregnancy (16.6%). Ninety eight embryos (mean 4.1 ± 4.6) were cryopreserved in 15 of 23 GnRH triggering patients (65%). So far 3 frozen-thawed cycles performed yielded no pregnancy. In none of the cases in either group OHSS developed.

Limitations, reason for caution: No

Wider implications of the findings: The higher pregnancy rate in the IVM group compared to the one found in the triggering group has to be confirmed in larger series

Study funding/competing interest(s): No prevalence of one of the two methods used in patients at high risk for OHSS

Trial registration number: No

P-534 Should testosterone be used for more patients than only low ovarian responders

M. Pérez Izquierdo¹, J. Ten², J. Guerrero², F. Araico¹, J. Llácer², and R. Bernabeu²
¹Instituto Bernabeu Cartagena, Medicina Reproductiva, Cartagena (Murcia), Spain, ²Instituto Bernabeu Alicante, Medicina Reproductiva, Alicante, Spain

Study question: To assess whether the administration of testosterone prior to a controlled ovarian stimulation (COS) treatment improves the clinical outcomes in patients with a low number of oocytes retrieved.

Summary answer: Poor ovarian response patients (POR) with androgen supplementation before COS show higher rates of positive β -HCG, clinical pregnancy and implantation than low responders patients with standard protocols.

What is known already: Androgens play a key role in the follicular growth by acting through specific receptors located in the human ovary (Meldrum D. *et al*, Fert. and Sterility, 2013). Several publications suggest that a pre-treatment with androgens may improve the number of oocytes retrieved and fertilized, the embryo quality and the implantation and pregnancy rates (Chung-Hoon K. *et al*, Fert. and Sterility, 2011)

Study design, size, duration: Retrospective study that included 184 IVF/ICSI treatments in which 5 or less oocytes were retrieved. 87 had been classified as POR according to Bologna criteria, and were supplemented with androgens before COS. The 97 remaining ones were unexpected low response patients that had underwent standard non-androgen-supplied protocols.

Participants/materials, setting, methods: We compare the rates of positive β -HCG, clinical pregnancy and implantation between androgen supplied patients (A) and patients with standard protocols (S). Student *t* test and Chi-square test were used when appropriate for comparison using SPSS software. Statistical significance was defined as $P < 0.05$.

Main results and the role of chance: Statistical differences between androgens-supplied (A) and standard protocols (S) were found in implantation rate (24,8 % vs 12,3 %, $P = 0.029$). We also found higher positive β -HCG rate (42,6 % vs 26,8 %, $P = 0.085$) and clinical pregnancy rate (29,6 % vs 18,3 %, $P = 0.20$) although they were not statistically significant. The improved results in A group observed were got in spite of a higher maternal age (38,1 vs 36,21, $P = 0.001$) and a lower mean of oocytes retrieved (2,79 vs 3,35, $P = 0.011$). Similar amount of gonadotropins used for both groups (2.850 IU vs 2.827 IU $P = 0.896$)

Limitations, reason for caution: The retrospective nature of the study and the different protocols used for stimulation.

Wider implications of the findings: Considering patients with low number of oocyte retrieved, our results show a clear improvement of IVF/ICSI clinical outcome in those treated with androgens prior to ovarian stimulation. This finding leads us to consider the possibility of using androgens for a wider range of patients – not only POR – in order to improve the oocyte's quality and the implantation of resulting embryos.

Study funding/competing interest(s): Conflicts of interest and source of funding none declared

Trial registration number: 0

P-535 IVF outcomes and embryo morphokinetics in women with polycystic ovarian syndrome

O. Somova¹, O. Feskov², I. Feskova², I. Bezpechnaya², I. Zhylykova¹, and O. Tishchenko¹

¹Centre of Human Reproduction Sana-Med, Laboratory of Assisted Reproductive Technologies, Kharkiv, Ukraine, ²Centre of Human Reproduction Sana-Med, IVF Department, Kharkiv, Ukraine

Study question: To examine the association of morphokinetics of embryos selected for transfer, with IVF outcomes in women with PCOS and with progesterone levels during ovarian stimulation

Summary answer: Morphokinetics of the best embryos from women with PCOS, who had positive IVF outcome is faster than in non-pregnant women and does not differ from embryo morphokinetics of regular cycling women. The high maternal progesterone level on the first day of ovarian stimulation adversely influences the dynamics of embryo development.

What is known already: Despite receiving a large number of oocytes from women with PCOS in IVF cycles, the quantity and quality of the embryos suitable

to implant remains significantly lower than in regularly cycling women. Time-lapse technology provides more informative evaluation criteria of embryo development that can improve embryo selection.

Study design, size, duration: Retrospective study of 104 embryos from 54 women with PCOS and with regular menstrual cycles (control) using time-lapse monitoring system (PrimoVision). Only top quality embryos selected for the transfer according to the morphokinetic parameters were analysed (1 or 2 embryos of Grade A and B from each patient).

Participants/materials, setting, methods: Four groups of women were compared: 1) 9 pregnant PCOS women; 2) 15 non-pregnant PCOS women; 3) 16 pregnant control 4) 14 non-pregnant control. The precise timing of embryo divisions, duration of cell cycles between divisions, progesterone levels on the first and the final days of ovarian stimulation were assessed.

Main results and the role of chance: Some kinetic parameters of the embryos with high morphological quality had significant differences depending on whether the woman became pregnant or no. Times to 4, 5 and 8 cell divisions were shorter in pregnant women with PCOS than in non-pregnant women with PCOS ($P < 0.05$), and did not differ from corresponding values in the control (group 3). In non-pregnant patients with PCOS these parameters had no any differences compared to their control (group 4). The duration and synchrony of the first cell cycles, times to 2, 3 cell divisions, progesterone level on the day of HCG administration were similar among all groups. Progesterone level on the first day of ovarian stimulation was higher in both non-pregnant groups compared to the groups of pregnant women ($P < 0.05$).

Limitations, reason for caution: All examined embryos were obtained after oocyte fertilization with ICSI. Only women of 30-37 years old, which were treated with gonadotropins and GnRH antagonists were included in this study.

Wider implications of the findings: This study indicates the advantage of time-lapse technology for assessment an early embryo development. Morphokinetics of successfully implanted embryos from women with PCOS is similar to morphokinetics of embryos from regular cycling women. The high progesterone level in the beginning of ovarian stimulation in women with PCOS may be a predictor of a significantly slower dynamics of embryo development.

Study funding/competing interest(s): Supported by private IVF clinic "Sana-Med".

Trial registration number: This study does not an RCT, no registration number.

P-536 Is anti-mullerian hormone (amh) significantly related with hyperandrogenemia or insulin resistance in women with pcos

S. Kraljic Oguic¹, D. Pavicic Baldani², L. Skrgatic², V. Simunic², H. Vrcic², D. Rogic¹, J. Juras², and M. Sprem Goldstein²

¹Clinical Hospital Center Zagreb, Department for laboratory diagnostics, Zagreb, Croatia, ²Clinical Hospital Center Zagreb, Department for human reproduction, Zagreb, Croatia

Study question: Concentrations of AMH, androgens and insulin are elevated in women with PCOS. The question is if there is a correlation between levels of AMH and androgens or between AMH and insulin resistance estimated by HOMA-IR (homeostasis model of assessment – insulin resistance).

Summary answer: In women with PCOS we found a statistically significant positive correlation between levels of AMH and free and total testosterone and androstenedione. There is no such correlation in healthy women without PCOS. No statistically significant correlation was found between AMH and insulin and HOMA-IR in both groups investigated.

What is known already: It is well known that AMH, testosterone (total and free), androstenedione, DHEAS, insulin and HOMA-IR are elevated in women with PCOS. Some studies found positive correlation between levels of AMH and androgens, but some didn't. The same situation is with relationship between AMH and insulin resistance in women with PCOS. Depending on age and body mass index (BMI), there are studies with conflicting results.

Study design, size, duration: A prospective study was conducted from 2009 to 2012 to investigate levels and the relationship of AMH with androgens and AMH with insulin resistance in 198 women with PCOS diagnosed according to the Rotterdam criteria and 170 healthy women without PCOS aged from 18 to 35 years.

Participants/materials, setting, methods: Venous blood was taken during early follicular phase and concentrations of AMH, total testosterone, androstenedione,

DHEAS, sex hormone binding globulin (SHBG), insulin were measured by routine immunochemical methods and glucose enzymatically with hexokinase. Free testosterone and HOMA-IR were calculated. Results were evaluated statistically using SPSS 17 (IBM, NY, USA).

Main results and the role of chance: A significant positive correlation of AMH was established with total testosterone ($r = 0.447$, $p < 0.001$), free testosterone ($r = 0.508$, $p < 0.001$), androstenedione ($r = 0.397$, $p < 0.001$) only in women with PCOS. Multiple regression method showed that the greatest effect on the AMH level was exerted by free testosterone and androstenedione. Although concentration of insulin and insulin resistance assessed by HOMA-IR was significantly higher in women with PCOS along with AMH ($p < 0.001$), there was no significant correlation between them. Concentration of SHBG was expectedly lower in women with PCOS, as a result of insulin resistance and hyperandrogenemia, but no correlation was found with levels of AMH.

Limitations, reason for caution: Number of participants was limited to 368 women because of limited funding, although almost 200 women with PCOS, and 170 in the control group is still adequate for getting qualitative conclusions. The age distribution was similar in both groups but it is important to emphasize that AMH level is age-dependent.

Wider implications of the findings: Our results are in agreement with other studies that confirm positive correlation between levels of AMH and degree of hyperandrogenemia in women with PCOS. These findings support the belief that intraovarian hyperandrogenism may cause follicular arrest and follicle excess followed by increased intraovarian AMH level. Based on the results of our study, free testosterone and androstenedione are the most influential factors for increased AMH levels in women with PCOS.

Study funding/competing interest(s): This investigation was part of a scientific project approved and funded by Ministry of science, education and sport, Republic of Croatia.

Trial registration number: 0

P-537 Experience on the use of Corifollitropin-alfa (Elonva) for controlled ovarian hyperstimulation in ovum donor cycles

L. Garcia De Miguel, M. Caballero Campo, A. Gurria, J. Alonso, A. Serrano, E. Marbán, and P. Caballero Peregrín
FundacionTambre, Gynecology, Madrid, Spain

Study question: The aim of this study was to evaluate the differences between the use of Corifollitropin-alfa (Group A) and daily gonadotropins (Group B) in terms of IVF results, pregnancy outcomes and cost per cycle for stimulation, in egg donor cycles in usual practice.

Summary answer: Our results show an increased number of both total and mature oocytes retrieved with Corifollitropin-alfa, but no differences in pregnancy outcomes. Nevertheless, the use of Corifollitropin-alfa is associated with an increment in the cost per cycle.

What is known already: Long term FSH, Corifollitropin-alfa (Elonva), has been developed as an alternative to daily administration of gonadotropins. Up to this moment evidence in scientific literature does not show any difference with the rest of gonadotropins except an increment in the ovarian hyperstimulation syndrome rate. Experience and results on donors have not been yet published.

Study design, size, duration: A single centre, retrospective observational study including all donation cycles in our center from March 2012 to January 2013.

Participants/materials, setting, methods: We studied a total of 213 IVF donor cycles. 83 stimulated with Elonva which represents a 39% of all cycles and 130 (61%) were stimulated with either recombinant and urinary gonadotropins with the following distribution: 43 cycles (20.2%) with Follitropin alfa (Gonal), 66 cycles (31%) with Urofollitropin (Fostipur) and human menopausal gonadotropin (Menopur) in 21 cycles (9.9%).

The dosage was chosen following our egg donor ordinary protocol. It is important to mention that we work on a shared egg donor program. Both hormonal analysis and ultrasound controls were used to control the cycle. Triggering was realized not only with Coriogonadotropin-alfa (Ovitrelle) but also with GnRh agonists.

The primary target was to evaluate the differences in cycles concerning number of oocytes, number and percentage of mature oocytes and ratio recipient per donor. Low responses and cancellation rates were also assessed.

As a secondary objective we calculated pregnancy rate per cycle and pregnancy rate per donor.

Main results and the role of chance: The descriptive statistical analysis evinces homogeneity between both groups regarding all items studied except for age. The mean age of donors within the Elonva group was 24.88 and within the daily gonadotropins group was 26.02, this difference was statistically significant but medically irrelevant.

The total number of oocytes retrieved was 17.24 in Group A and 14.38 in Group B and the total number of mature oocytes was 14.20 in group A and 11.51 in group B. Therefore we obtained 2.82 total oocytes (CI 95% 0.596 to 5.117) and 2.71 mature oocytes (CI 95% 0.822 to 4.562) more, using long-term FSH.

With respect to the number of recipients assigned per donor, there was a higher number in the corifollitropin-alfa group 1.72 versus 1.38, with a mean difference of 0.338 (CI 95% 0.090 to 0.586).

Concerning pregnancy rates, there was no evidence of an increased number of pregnancies within the Elonva group (67.9% vs 73.3%). Among these results, we found no differences on the number of pregnancies per donor cycle.

Finally, evaluating costs, corifollitropin-alfa cycles were more expensive than daily gonadotropins (694.84 € vs. 560.9 €). Hence the average cost of Elonva per cycle is 133,92 € higher than using daily gonadotropins ($p < 0.05$), (IC 95% 93; 174).

Limitations, reason for caution: This is an observational retrospective study.

The control group includes cycles stimulated with both urinary and recombinant gonadotropins

Wider implications of the findings: Our data supports the use of long-term FSH (Corifollitropin alfa) as a good alternative in oocyte donor cycles, easing drug administration on hyperstimulation cycles. In the future, that could facilitate us the correct use of Gonadotropins decreasing treatment cancellations due to incorrect administration of medication. Economical aspects may be considered. Farther investigation needs to be done.

Study funding/competing interest(s): There is short clinical experience in the use of corifollitropin-alfa since it is a new gonadotropin marketed not long ago. This is a wide study that introduces the role of Elonva in donors.

Trial registration number: This was not a clinical trial.

P-538 Dysregulation of gene expression in cumulus cells of in vitro matured oocytes

A. Hourvitz¹, L. Shalev¹, Y. Yung¹, G. Yerushalmi¹, C. Giovanni², M. Dal Canto², R. Fadini², J. Has¹, and E. Maman¹

¹IVF Unit and Reproduction Lab Sheba Medical Center, Obstetrics and Gynecology, Ramat Gan, Israel, ²Biogenesi Reproductive Medicine Centre Istituti Clinici Zucchi, Obstetrics and Gynecology, Monza, Italy

Study question: To study gene expression profile in cumulus cells of in vitro matured oocytes and compare it to cumulus cells obtained from oocytes matured in vivo

Summary answer: During oocyte in vitro maturation (IVM), cumulus cells failed to expand and cumulus expansion gene expression as well as other important genes were found to be dysregulated compared to expression in cumulus cells of in vivo matured oocytes.

What is known already: IVM technology was introduced in recent years. However, the use of IVM in ART remained limited due to: low maturation rate, reduced developmental potential, low fertilization rate and lower pregnancy and live birth rates compared with conventional IVF. It was suggested that in vitro maturation process results in less competent oocytes

Study design, size, duration: Study was conducted between January 2012 and December 2012. Ten patients undergoing IVM procedure and 10 patients undergoing IVF procedure participated in this study. All patients were normo-ovulatory and their age < 37 years.

Participants/materials, setting, methods: CC were collected from metaphase II (MII) oocytes six hours and 30 hours after IVM oocyte retrieval. A third group consisted of CC from MII oocytes obtained during oocyte denudation for intracytoplasmic sperm injection after IVF procedure. Cells were subjected to RNA extraction and quantitative Real-time PCR of selected genes

Main results and the role of chance: Cumulus cells collected 6 hours after IVM and IVF procedure were all expanded. Cumulus cells from all oocytes that have matured in vitro (30hr incubation) did not expand and remained in compact stage.

Respectively, genes involved in the process of cumulus expansion expressed differently between the groups (*PTSG2*, *HAS2*). Significant differences in gene expression was observed in genes considered related to oocytes quality (*ADAMTS1*, *GREM-LINE*). Furthermore, we also observed significant differences in genes known to be downstream to the LH signal (*SFRP4*, *SFRP5*). Although not significant, but a clear difference in gonadotropins receptors expression was observed (*FSHR*, *LHR*). No differences were found in genes related to steroidogenesis

Limitations, reason for caution: Relatively low number of participants in the study due to the use of precious biological material less likely to be available

Wider implications of the findings: IVM is an important procedure that has many advantages, yet its use is limited due to the low efficiency. In order to improve the results of IVM, we need first to recognize the abnormal processes and identify markers that are optional for following during improvement efforts. Success in creating cumulus expansion in vitro with appropriate expression of genes involved in the process may lead to more competent oocytes. Genes expressed differently in CC of in vivo matured oocytes, can be used as monitoring markers

Study funding/competing interest(s): Non

Trial registration number: Not a RCT study

P-539 Molecular expression of androgen and FSH receptors after follicular priming prior to ICSI cycles - an interim analysis of the iFOLL-PRIM randomized controlled trial

M. Monterde¹, R. Gómez², A. Marzal³, O. Vega⁴, J.m. Rubio⁴, C. Diaz-García³, and A. Pellicer⁵

¹Health Research Institute La Fe University Hospital, Reproductive Medicine, Valencia, Spain, ²Fundación IVI/INCLIVA, Reproductive Medicine Research, Paterna Valencia, Spain, ³Health Research Institute La Fe University Hospital-Department of Obstetrics Gynecology and Assisted Human Reproduction La Fe Hospital, Reproductive Medicine, Paterna Valencia, Spain, ⁴Department of Obstetrics Gynecology and Assisted Human Reproduction La Fe Hospital, Reproductive Medicine, Valencia, Spain, ⁵Fundación IVI/INCLIVA- Department of Obstetrics Gynecology and Assisted Human Reproduction La Fe Hospital, Reproductive Medicine, Valencia, Spain

Study question: To evaluate whether follicular priming improves ovarian response to controlled ovarian hyperstimulation (COH) protocols by increasing FSH receptors (FSHR) and/or androgen receptors (AR) expression in luteinized granulosa cells (LGCs) of previously diagnosed poor responder patients (POR).

Summary answer: The improvement of ovarian response due to follicular pretreatment with androgens, estrogens or gestagens, during luteal phase of the previous cycle prior to COH is not associated with an increase of neither the percentage of LGCs expressing FSHR and/or AR nor the density of any of these receptors on LGCs.

What is known already: Hormonal supplementation during luteal phase of the preceding cycle is believed to increase the number of retrieved oocytes and synchronize follicular growth in POR. Concurrent injections of estrogens have been described to synergistically increase FSHR in human LGCs. Androgens may influence ovarian follicular growth by acting as a metabolic precursor for steroid production. Indeed ligands of AR have been described to augment follicular FSHR expression in rhesus-monkey LGCs.

Study design, size, duration: Since April 2011, patients diagnosed as POR after an ICSI cycle, were randomized to undergo one of three different follicular priming protocols (estradiol, testosterone or estro-progestatives) prior to a new ICSI treatment. The previous unprimed COH was used as self-control to compare the effect of treatments.

Participants/materials, setting, methods: Recruited POR were pretreated with transdermal testosterone (T) (N = 18), estradiol (E) (N = 10) or a combination of estrogens-progestins (EP) (N = 10). Ovarian response (attending to Bologna criteria) parameters and the amount of FSHR and AR in follicular fluid derived LGCs (quantified by flow-cytometry) were compared between follicular primed vs unprimed COHP.

Main results and the role of chance: Patients allocated to E group had a higher number of recovered oocytes (4.30 ± 2.05 ; $p = 0.01$) respect to the unprimed phase but there were no significant differences in T and EP pretreatments. The percentage of LGCs expressing FSHR (%LGCsFSHR⁺/CD45⁻IP⁺) was not statistically different in T (13.78[2.42-45.59]), EP (11.77[2.63-24.52]) or E

(12.79[6.11-44.7]) groups when compared to its unprimed cycle (18.81[2.21-43.33], 17.71[2.19-31.82] and 17.90[6.92-49.70] respectively). Likewise the amounts of LGCs positive for AR (%LGCsAR⁺/CD45⁻IP⁺) were not significantly different in the primed vs the unprimed cycle of T(4.95[0.01-45.58] vs 6.83[0.30-51.27]), E(12.39[2.25-53.96] vs 5.17[1.00-50.97] or EP (6.90[0.64-30.18] vs 2.71[0.23-61.62]) groups. XMean fluorescence intensity of LGCsFSHR⁺/CD45⁻IP⁺ was not statistically different in T(87.10[20.60-415.00]), EP (53.30[22.70-171.00]), E (63.55[37.20-484.00]) respect unprimed COHP. Density of LGCsAR⁺/CD45⁻IP⁺ of pretreatment groups were not different compared to unprimed COHP.

Limitations, reason for caution: The main measurement taken to avoid bias is the randomization of the poor responders in this clinical trial. A power analysis was performed assuming significance level of 0.05 and statistical power of 80%. Despite is not a yet end study no significant differences were detected in each follicular pretreatment

Wider implications of the findings: Estrogen pretreatment slightly improves ovarian response but this phenomenon is not associated with increased expression of FSHR and/or AR in LGCs. Assuming that FSHR and AR are responsible for appropriate ovarian response, the increases in oocyte quality and ovarian response promoted by estradiol pretreatment are not expected to act through increasing expression of these receptors but through alternative mechanisms allowing the improvement of its sensibility to their specific ligands.

Study funding/competing interest(s): Health Research Institute Hospital La Fe of Valencia (Spain) was the promoter of this clinical trial and was partially financed by Valencia Health Board (AP-199/10).

Trial registration number: Identifier of the present prospective, unicentric and randomized clinical trial is NCT01310647.

P-540 Comparison of two strategies to prevent ovarian hyperstimulation syndrome in high-risk patients: GnRH agonist trigger and a mild stimulation regimen

A. Eapen, A. Datta, A. Kurinchi-selvan, H. Birch, and G.M. Lockwood
Midland Fertility Services, Obstetrics and Gynaecology, Aldridge, United Kingdom

Study question: A 'mild stimulation' protocol comprising of sequential clomiphene citrate(CC), FSH and hCG trigger or a FSH, GnRH antagonist and GnRH agonist trigger for final oocyte maturation with enhanced luteal phase support(LPS) - which protocol in IVF/ICSI is more effective in preventing ovarian hyperstimulation (OHSS) and yields better clinical outcome?

Summary answer: A trend of higher clinical pregnancy rate (CPR) and live-birth/ongoing pregnancy rate (LB/OPR) are found with GnRH agonist 'trigger' and also associated with low incidence of mild to moderate OHSS and no severe primary or secondary OHSS

What is known already: GnRH agonist for final oocyte maturation in GnRH-antagonist protocol is shown to eliminate the risk of severe OHSS in IVF/ICSI treatment. Different 'mild stimulation' protocols have also been used in women at high risk of OHSS. There has been anxiety concerning low CPR/LPR with both these strategies, although adequate LPS has been shown to yield improved treatment outcomes. Direct comparison of these two strategies in women at high risk for OHSS has not been undertaken.

Study design, size, duration: Retrospective study (period: July 2011 - Oct 2012) of 62 consecutive IVF/ICSI cycles on GnRH antagonist protocol with busarelin trigger (agonist trigger group). The results were compared with a matched historical control group (Sept 2010 - Sep 2011) comprising 18 cycles of sequential CC, FSH and hCG trigger (mild stimulation group)

Participants/materials, setting, methods: Women with high (>25 pmol/l) anti-mullerian hormone (AMH) levels who had GnRH antagonist (FSH and cetorelix) protocol for IVF/ICSI in a tertiary level fertility clinic. The continuous and categorical variables between the agonist trigger and mild stimulation groups are compared by t-test and Fisher's exact/ χ^2 tests respectively.

Main results and the role of chance: No difference seen in mean age, B-MI, FSH, LH levels & proportion of PCOS between agonist trigger & mild stimulation groups. Mean AMH levels was higher (58.4 v/s 46.6, $p = 0.008$) & starting/total FSH dose significantly lower (120.5 v/s 151.2, $p < 0.001$ & 856.7 v/s 1554.5 iu/l, $p < 0.001$ respectively) with mild stimulation. Mean number of oocytes (12.1 v/s 17.9, $p = 0.02$) or embryos (5.8 v/s 9.8, $p = 0.007$) were also lower with mild

stimulation. Fertilisation & implantation rates were no different. Study suggested a trend towards higher CPRs (42.6% v/s 33.3%, $p = 0.58$) & LB/OPRs (40.7% v/s 25%, $p = 0.15$) per embryo transfer with agonist trigger; 15.7% higher LB/OPR being clinically significant. The incidence of mild to moderate OHSS was similar (agonist: 16.2% v/s mild stimulation: 11.8%, $p = 0.70$) & no severe OHSS with either group. FAE due to risk of secondary OHSS was 9.7% with agonist trigger & 17.8% with mild stimulation, $p = 0.39$

Limitations, reason for caution: A retrospective study with historical controls and no prior power calculation and smaller number of women in mild stimulation group. Adequately powered randomised control trials are required to confirm these findings.

Wider implications of the findings: This comparative analysis suggests that both GnRH agonist trigger and mild stimulation are equally effective in minimising the risk of OHSS. A mild stimulation protocol may be more cost effective. The recent improvement in LPS following GnRH agonist trigger with combined dose of estrogen, progesterone and a single dose of hCG improves the otherwise low CPR/LBR.

Study funding/competing interest(s): The study did not require any funding and there was no competing interests involved.

Trial registration number: Not applicable

P-541 Clinical pregnancy rates of late HCG administration to trigger ovulation in clomiphene citrate induced cycles

M.C. Ornek¹, U. Ates², T. Usta³, and C.P. Goksedef²

¹Kalamis IVF Center, IVF, Istanbul, Turkey, ²Haseki Research and Training Hospital, Gynecology, Istanbul, Turkey, ³Bagcilar Research and Training Hospital, Gynecology, Istanbul, Turkey

Study question: To verify better ovulation and clinical pregnancy rates with late hCG administration (leading follicle sizes within 20.0-23.0 mm in diameter) compared to earlier hCG administration (leading follicle sizes smaller than 20.00 mm in diameter).

Summary answer: In the CC induced ovulation cycles, HCG administration after the leading follicle reaches to 21mm in diameter to trigger ovulation results better pregnancy rates.

What is known already: CC is used widely for OI, especially in infertile PCOS patients, because it provides low cost, safe and effective therapy. In the CC induced cycles HCG is administered later (leading follicle sizes bigger than 18.0-20.0 mm in diameter) than gonadotropin induced cycles.

Study design, size, duration: 124 patients from January 2010 and May 2012 collected. Patients, infertile for 1 year, OI with CC is planned, HCG administered when follicle size bigger than 21mm. Ovulation and pregnancy data were recorded by USG. 124 patients in the control group is triggered when follicle sizes between 18-20mm.

Participants/materials, setting, methods: Age, BMI, infertility time, menstrual period, hormones, spermogram, tubal patency, previous operations, endometrial thickness on the hCG day, classified as predictive data.

100mg CC is used for OI, between 5-9 day, followed by USG periodically. 10,000 IU HCG administered, when dominant follicle reach 21mm. Ovulation and CPR confirmed with USG.

Main results and the role of chance: Proportional data were compared by using Z test. P value <0.05 was considered statistically significant. Ovulation rate and CRP are in HCG administration when follicle sizes within 18.0-20.0 mm in diameter, and hCG administration when follicle sizes bigger than 21mm in diameter (60.4%; 75 / 124) and (67.7%; 84 / 124) ($p = 0.23$) (9.7%; 12 / 124) and (18.5%; 23 / 124) ($p = 0.04$) respectively. Endometrial Thickness on the HCG day mean \pm S.D. (7.8 ± 3.1) and (9.2 ± 2.9) ($p = 0.0002$).

Limitations, reason for caution: Because of wellknown 'bias's of retrospective studies, results should be confirmed with further randomized controlled trial (RCT).

Wider implications of the findings: If increase CPR with Late HCG triggering is achieved and confirmed with RCT then the low cost and simplicity of the procedures in ovulation induction with CC can reach to widespread use then ever

Study funding/competing interest(s): None declared.

Trial registration number: It is a case-control study.

P-542 The value of I generation AMH measurements kits for prediction of live birth in women undergoing assisted reproductive technology

A. Bruszczynska¹, J. Glowacka¹, M. Kunicki², G. Jakiel³, T. Wasniewski⁴, K. Jaguszewska⁴, J. Liss¹, and K. Lukaszuk⁵

¹INVICTA Fertility Clinic, IVF Laboratory, Gdansk, Poland, ²INVICTA Fertility Clinic, Fertility Clinic, Warsaw, Poland, ³The Medical Center of Postgraduate Education, I Department of Obstetrics and Gynaecology, Warsaw, Poland, ⁴Univeristy of Warmia and Mazury, Department of Gynaecology and Obstetrics, Olsztyn, Poland, ⁵INVICTA Fertility Clinic, Fertility Clinic, Gdansk, Poland

Study question: To evaluate the clinical value of basal anti-Müllerian hormone (AMH) measurements in the prediction of live birth.

Summary answer: The basal serum concentration of AMH is substantial prognostic factor regarding live birth.

What is known already: AMH is produced by granulosa cells of preantral and small antral follicles. Multiple studies revealed relationship between serum AMH level and ovarian response to gonadotrophins ovarian stimulation, thus predict the effect of assisted reproduction treatment outcome— both the poor and the hyper-response. Constant value of AMH during the menstrual cycle indicates AMH as an independent novel biomarker of ovarian response during controlled ovarian stimulation in ART methods.

Study design, size, duration: During the study period, 603 cycles were investigated. We assessed the results of all first cycles per subject performed from 05/2007 until 12/2008.

Participants/materials, setting, methods: Six hundred and three women undergoing IVF with long protocol for controlled ovarian hyperstimulation. Serum level of AMH was measured on the first three days of the menstrual cycle prior to the beginning of stimulation. AMH level was measured by enzyme immunoassays ELISA.

Main results and the role of chance: In multivariable logistic regression we found the AMH and age as the independent parameters that correlated with the chance of live birth ($p < 0.0001$ and $p < 0.001$ respectively). We found the live birth rates 46.2% (<35 years), 44.7% (35-37 years), 32.1% (38-39) and 15.3% (>39) were associated with AMH >1.4 ng/ml. For AMH range 1.4-0.6 ng/ml the live birth rates were 29.3%, 12.5%, 5.6% and 2.7%, respectively and for AMH below 0.6 ng/ml- 7.1%, 8.3%, 0% and 5.8%, respectively. We noticed that the chance of live birth was greatest when AMH level was >2 ng/ml, significantly lower when AMH concentration was about 1 ng/ml, and 0% when AMH concentration was ~0.1 ng/ml.

Limitations, reason for caution: To reduce error caused by male factor infertility, we excluded cases with more than 30% sperm DNA fragmentation and cases where spermatozoa from testicular biopsies were used. We included cases with DNA fragmentation between 15 and 30%, as we have not found any elevated miscarriage rate related to this group.

Wider implications of the findings: These results remain a great promise to have an impact on treatment strategies and will be crucial for decision-making among couples seeking assistance.

Study funding/competing interest(s): There are no commercial associations of the author or any coauthors that might pose a

P-543 Predictive factors for ovarian response in a corifollitropin alfa/GnRH antagonist protocol for controlled ovarian stimulation

S. Oehninger¹, S. Nelson², P. Verweij³, and B. Stegmann⁴

¹The Jones Institute for Reproductive Medicine, Reproductive Endocrinology and Infertility, Norfolk Virginia, U.S.A., ²University of Glasgow, School of Medicine, Glasgow, United Kingdom, ³MSD, Biostatistics and Research Decision Sciences (BARDS), Oss, The Netherlands, ⁴Merck Sharp & Dohme Whitehouse Station, Clinical Research for Women's Health, NJ, U.S.A

Study question: Can predictors of low (<6 oocytes retrieved) and high ovarian response (>18 oocytes retrieved) be identified in patients undergoing controlled ovarian stimulation with corifollitropin alfa in a gonadotropin-releasing hormone (GnRH) antagonist protocol?

Summary answer: Anti-Müllerian hormone (AMH), antral follicle count (AFC), and age on stimulation day 1 were prognostic for both high and low ovarian response in a corifollitropin alfa/GnRH antagonist protocol, in addition to follicle-stimulating hormone (FSH) for high ovarian response and menstrual cycle length for low ovarian response.

What is known already: The majority of studies on predictors of ovarian response have analyzed patients treated with recombinant(r) FSH in long GnRH agonist protocols. Systematic reviews have identified AMH, AFC, and basal FSH as predictors of low ovarian response and AMH and AFC as predictors of high ovarian response in these protocols. There is, to date, no information on predictors in a corifollitropin alfa/GnRH antagonist protocol.

Study design, size, duration: In this retrospective analysis, statistical model building for high and low ovarian response was based on the corifollitropin alfa treatment group of the Pursue trial (n = 694). Multivariable logistic regression models were constructed in a stepwise fashion ($P < 0.10$ for entry).

Participants/materials, setting, methods: Infertile women aged 35–42 years received a single injection of 150 µg corifollitropin alfa, followed by ≤ 300 IU/d rFSH starting on stimulation day 8 if needed. 0.25 mg/d ganirelix started on day 5 until final oocyte maturation with 250 µg recombinant human chorionic gonadotropin (rhCG).

Main results and the role of chance: 14.1% of subjects were high, and 23.2% were low ovarian responders. The regression model for high ovarian response included 4 independent predictors, with high AMH levels and AFCs increasing the risk while high FSH levels and increasing age decreased risk. The apparent area under the curve (AUC) of the receiver operating characteristic (ROC) curve for this model was 0.888. Sensitivity and specificity were 84% and 80%, respectively. The regression model for low ovarian response also included 4 independent predictors. Older age increased, while higher AMH, higher AFC, and longer menstrual cycle length decreased the risk of low ovarian response. The apparent AUC of the ROC curve for the complete model was 0.886. Sensitivity and specificity were 77% and 87%, respectively.

Limitations, reason for caution: This study was restricted to women aged 35–42 years, with a body weight of ≥ 50 kg and body mass index ≥ 18 and ≤ 32 kg/m²; therefore, its generalizability may be limited.

Wider implications of the findings: In assisted reproductive technology, both very low and very high ovarian responses are associated with increased cancellation rates and lower pregnancy rates. A high ovarian response also increases the risk for development of ovarian hyperstimulation syndrome. The multivariable models in the current analyses predicted the occurrence of high and low ovarian response sufficiently well to be used in clinical practice to individualize treatment.

Study funding/competing interest(s): Financial support for this study was provided by Merck, Sharp & Dohme Corp., a subsidiary of Merck & Co. Inc., Whitehouse Station, NJ, USA.

Trial registration number: NCT01144416

P-544 Impact of ovarian endometrioma on oocyte retrieval, fertilisation, and first embryonic cleavage division in assisted reproductive technology

H. Ando, T. Takayanagi, H. Minamoto, and N. Suzuki
Toyohashi Municipal Hospital, Center for Reproductive Medicine and Surgery,
Toyohashi, Japan

Study question: How unfavourably does ovarian endometrioma affect oocyte retrieval, such as the number of mature, immature and degenerative oocytes, and subsequent early events including fertilisation and cleavage-stage embryo development in an attempt to produce a favourable outcome in assisted reproductive technology (ART)?

Summary answer: More FSH/HMG stimulation for the ovarian endometrioma-associated infertility yielded minimum mature oocytes, where increased degenerative oocyte retrieval was compensated in part by the decreased proportion of immature oocytes, and the retrieved mature oocytes maintained the rates of successful fertilisation and initial cleavage despite the local disadvantaged background.

What is known already: Ovarian endometrioma with/without surgical treatment may allow poor oocyte quality and implantation failure on a molecular level which is associated with toxicity, inflammation, and oxidative stress. However, the pregnancy outcome of ART for ovarian endometrioma-associated infertility has been conflicting in several studies.

Study design, size, duration: The present study was undertaken to analyse our records of ART with full application of time-lapse (TL) imaging incubator (TLII) system between November 2007 and September 2012. Cases/Oocytes were divided into 265 cycles/1,666 oocytes in which ovarian endometrioma had been pathologically proved and 1,274 control cycles/9,387 control oocytes.

Participants/materials, setting, methods: Oocytes were classified as normal (mature MII or immature GV/MI) or abnormal (degenerative). Normal fertilisation was microscopically defined by the two pronuclei. Evaluation of abnormal first embryonic division, which is extremely poor developmental prognosis, was based on the TL observation that a zygote was cleaved into three or more blastomeres.

Main results and the role of chance: Less oocytes (6.4 ± 5.5 vs. 7.4 ± 6.8 ; $P = .008$) including mature oocytes (4.5 ± 3.9 vs. 5.3 ± 5.2 ; $P = .005$) were retrieved in the endometrioma group, where more FSH/HMG was required ($2,460 \pm 1,507$ IU vs. $2,058 \pm 1,353$ IU; $P = .0001$) for younger ages (35.6 ± 4.3 vs. 36.9 ± 4.9 ; $P = .00002$). Degenerative (vs. normal) and immature (vs. mature) oocytes were 1.2 times ($P = .007$; 95%CI: 1.05–1.38) and 0.8 time ($P = .02$; 95%CI: 0.70–0.97), respectively, in the endometrioma group. The normal fertilised oocyte numbers showed a minimized difference (3.3 ± 3.1 vs. 3.8 ± 3.9 ; $P = .02$) between the groups with a background of similar abnormal fertilisation rate (10.8% vs. 10.5%; 1.0 time; $P = .99$; 95%CI: 0.83–1.21). Similarly, abnormal first embryonic division was not increased in the endometrioma group (26.9% vs. 25.4%; 1.1 times; $P = .38$; 95%CI: 0.91–1.28).

Limitations, reason for caution: Surgical treatment had been performed if an ovarian endometrioma of 5 cm or more existed. Individualized medical treatment had been performed for most of the endometrioma patients. Controlled ovarian stimulation protocols were also individualized. Both IVF and ICSI cycles were included for the primary analysis.

Wider implications of the findings: This is the first report on detailed clinical data which have been focused on retrieved oocytes and initial events associated with ovarian endometrioma. We have shown the increased number of degenerative oocytes for the first time. Our data on unchanged fertilisation and first embryonic cleavage division provided by TLII should be useful, suggesting that endometrioma might affect adjacent oocytes within certain limitations. We are ready for the further analysis using the same records of ART.

Study funding/competing interest(s): None/not applicable.

Trial registration number: Not applicable.

P-545 Anti Mullerian hormone receptor type II expression and regulation in human granulosa cells in late follicular development and the correlation to clinical outcome

E. Maman, N. Rubinshtein, Y. Yung, L. Shalev, G. Yerushalmi, and A. Hourvitz
Sheba Medical Center, Obstetrics and Gynecology, Ramat Gan, Israel

Study question: To elucidate the AMH receptor type II (AMHRII) expression patterns and regulation mechanism in human granulosa cells (GC) and cumulus cells (CC) from antral to preovulatory stages and its correlation to oocyte competence

Summary answer: During the late follicular development AMHRII expression is down regulated as follicles grow by direct action of pituitary gonadotrophins. Down regulation of AMHRII mRNA expression in CC may represent mature and competent oocytes

What is known already: AMH action is selectively mediated through the AMHRII. Most studies on AMH and AMHRII focused on the early stages of follicular development and showed that AMH inhibits the recruitment of primordial follicles and decreases the responsiveness of growing follicles to FSH. Recent studies proposed role for AMH and AMHRII in late follicular development. Yet, data regarding AMHRII expression and regulation in later follicular development has not been fully recovered, particularly in human

Study design, size, duration: Study was conducted between January 2012 and December 2012. Luteinized GC and CC were obtained from follicles (> 17 mm) aspirated from 75 IVF patients. Luteinized/FSH primed and non-luteinized/non FSH primed GC were obtained from follicles (< 10 mm) aspirated from 30 IVM patients. All patients were normo-ovulatory and their age < 37 years.

Participants/materials, setting, methods: GC and CC were collected from follicular fluid and either cultured or subjected to RNA purification. Hormonal pathways that regulates AMH and AMHRII were studied in cultured GC and CC by hormonal treatments and pharmacological inhibitors. mRNA levels were analyzed by qRT-PCR and protein levels were analyzed by EIA.

Main results and the role of chance: AMHRII expression was higher in CC than in GC. The highest expression of AMHRII was found in small antral follicles, and cumulus of immature (GV) oocytes. *In vivo*, AMHRII expression was higher non-

luteinized/non FSH primed GC obtained from follicles (<10mm) aspirated from IVM patients. In cultured CC and GC, both hCG and FSH down regulated AMHRII expression without any effects on AMH expression. This down regulation was rescued by U0126-(MAPK/ERK kinase (MEK) inhibitor). Increase in Estradiol levels in cultured CC did not alter AMHRII down regulation by FSH. Interestingly, higher expression levels of AMHRII were found in CC of metaphase II (MII) oocytes that failed to be fertilized compared to CC of fertilized MII oocytes.

Limitations, reason for caution: Most of the results are in vitro. The correlation to clinical results should be further studied

Wider implications of the findings: AMH activity in late follicular development might be regulated by direct action of pituitary gonadotrophins on its receptor, the AMHRII. Further studies are needed to identify the role of AMH and AMHRII in later stages of follicular development and the impact of the proposed regulation mechanism. AMHRII may be used as biomarker for oocytes and embryos quality. The correlation to embryo quality should be further studied in single embryo transfer set up.

Study funding/competing interest(s): This research was supported by Legacy Heritage Fund of the Israel Science Foundation

Trial registration number: Basic research

P-546 Assessment of relationships between the HMW Adiponectin and insulin resistance in normal weight adolescents with PCOS

S. Saltek¹, B. Demir¹, B. Dilbaz¹, and C. Demirtas²

¹*Etilik Zübeyde Hanım Women's Health Teaching and Research Hospital, Obstetrics and Gynaecology, Ankara, Turkey,* ²*Gazi University School of Medicine, Medical Biochemistry, Ankara, Turkey*

Study question: Is there any relationship between the HMWAdiponectin and insulin resistance in normal weight adolescents with polycystic ovary syndrome (PCOS)?

Summary answer: HMW Adiponectin concentration was lower in adolescents with PCOS compared to controls. HMWAdiponectin was lower in the insulin resistant cases compared to normal cases in the PCOS patients. However, significant correlation was not identified between the HMWAdiponectin and HOMA-IR.

What is known already: PCOS is characterized by chronic anovulation and hyperandrogenism. Insulin resistance is accepted to be a common feature of PCOS. HMWAdiponectin closely correlates with insulin sensitivity. The relationships between adiponectin and central obesity are clearly demonstrated. However, the relationships among the HMWAdiponectin, insulin resistance and testosterone remain unclear.

Study design, size, duration: Case control study included 40 normal BMI adolescents with PCOS and 40 control BMI- matched adolescents.

Participants/materials, setting, methods: Study group consisted of 40 adolescents with PCOS aged between 15-20 years. PCOS was diagnosed according to the Rotterdam criteria. BMI was accepted as normal between 18.5-24.9 kg/m². Control group included 40 BMI-matched adolescents. A history of diabetes mellitus was the exclusion criteria. Insulin resistance was calculated using HOMA-IR. 75 gr OGTT was applied all patients.

Main results and the role of chance: HMW Adiponectin was lower in adolescents with PCOS compared to controls ($p = 0.0001$). HOMA-IR ($p = 0.0001$), AMH (0.0001), second hour glucose concentration in the 75 gr. OGTT ($p = 0.027$), total and free testosterone ($p = 0.017$ and $p = 0.0001$) were higher in PCOS patients compared to controls. Insulin resistance rates were 69% in adolescents with PCOS and 2.5% in controls. HMWAdiponectin was lower in the insulin resistant cases compared to normal cases in the PCOS patients ($p = 0.0001$). HMW Adiponectin was inversely correlated with AMH ($r = -0.52$ $p = 0.001$). HOMA-IR was positively correlated with AMH ($r = 0.5$ $p = 0.001$). There was no correlation between HMW Adiponectin and HOMA-IR.

Limitations, reason for caution: Small sample size was a limitation of the study.

Wider implications of the findings: Although no overt metabolic disease was observed, metabolic disturbances were demonstrated early reproductive years in patients with PCOS. High rates of insulin resistance were demonstrated in normal weight adolescents with PCOS. Low HMW Adiponectin concentrations may contribute to the insulin resistance in patients with PCOS. Therefore, novel molecules playing a role glucose and lipid metabolism help us to determine the defect of these processes in earlier reproductive period.

Study funding/competing interest(s): No financial support. The authors have no competing interests to declare.

Trial registration number: Not applicable.

P-547 Elevated progesterone (>1.5 ng/mL) during the late follicular phase of ovarian stimulation with corifollitropin alfa or rFSH does not compromise pregnancy rates in older women

W. Kutteh¹, B. Shapiro², H. Witjes³, and K. Gordon⁴

¹*Vanderbilt University Medical Center, Obstetrics and Gynecology, Nashville TN, U.S.A.,* ²*The Fertility Center of Las Vegas, Fertility, Las Vegas NV, U.S.A.,* ³*MSD, Biostatistics and Research Decision Sciences (BARDS), Oss, The Netherlands,* ⁴*Merck Sharp & Dohme Whitehouse Station, Global Medical Affairs Women's Health and Endocrine, NJ, U.S.A*

Study question: In older women undergoing assisted reproductive technology with ovarian stimulation by either corifollitropin alfa or recombinant follicle-stimulating hormone (rFSH) in a gonadotropin-releasing hormone (GnRH) antagonist protocol, do elevated progesterone levels during the late follicular phase compromise the chance of ongoing pregnancy?

Summary answer: In older women (aged 35-42 years) undergoing ovarian stimulation with either corifollitropin alfa or rFSH, elevated progesterone levels (>1.5 ng/mL) on the day of administration of recombinant human chorionic gonadotropin (rhCG) were not associated with significantly reduced ongoing pregnancy rates.

What is known already: Preovulatory elevated serum progesterone levels (>1.5 ng/mL) on the day of hCG administration for final oocyte maturation have been associated with reduced ongoing pregnancy rates in younger women than those included in the current study undergoing assisted reproductive technology, irrespective of the GnRH analog applied, while other studies have suggested no association between progesterone elevation during the late follicular phase and ongoing pregnancy rates. The impact on pregnancy rates in older women is not known.

Study design, size, duration: Retrospective analysis of a Phase III, randomized, double-blind, double-dummy, active-controlled trial (Pursue) with 1390 participants (694 in the corifollitropin alfa arm and 696 in the rFSH arm) that collected data from 33 IVF centers in the United States with study medical intervention between July 2010 and June 2011.

Participants/materials, setting, methods: Women aged 35-42 years received a single injection of 150 µg corifollitropin alfa or daily 300 IU rFSH followed by ≤300 IU/d rFSH starting on stimulation day 8. 0.25 mg/d ganirelix started on day 5 until oocyte maturation with 250 µg rhCG. Two good-quality embryos were transferred on day 3.

Main results and the role of chance: In subjects who received rhCG (corifollitropin alfa arm, $n = 670$; rFSH arm, $n = 661$), the incidence of serum progesterone levels >1.5 ng/mL on the day of rhCG was 28.5% and 34.2% in the corifollitropin alfa and rFSH arms, respectively, and of serum progesterone levels >2.0 ng/mL was 10.9% in both treatment arms. The mean (SD) numbers of oocytes retrieved were higher in those with progesterone levels >1.5 ng/mL vs ≤1.5 ng/mL (corifollitropin alfa and rFSH, respectively, 13.0 [8.0] vs 10.3 [6.4], $P < 0.001$ and 14.0 [7.6] vs 8.9 [5.4], $P < 0.001$) and with progesterone levels >2.0 ng/mL vs ≤2.0 ng/mL (corifollitropin alfa and rFSH, respectively, 15.9 [9.2] vs 10.5 [6.5], $P < 0.001$ and 15.3 [8.4] vs 10.1 [6.2], $P < 0.001$). Implantation rates were similar regardless of progesterone cutoff levels. In both treatment arms, the ongoing pregnancy rates per started cycle were similar above and below a cutoff level of 1.5 ng/mL progesterone, but tended to be lower above a cutoff level of >2.0 ng/mL vs ≤2.0 ng/mL (20.5% vs 23.3% and 19.4% vs 25.5% for corifollitropin alfa and rFSH, respectively; $P = 0.26$).

Limitations, reason for caution: This study only included women with a regular spontaneous menstrual cycle, aged 35-42 years, with a body weight of ≥50 kg, and body mass index ≥18 and ≤32 kg/m². Intra- and interassay variabilities of the progesterone assays carried out at a central laboratory were <5% and <10%, respectively.

Wider implications of the findings: In older women undergoing controlled ovarian stimulation in a GnRH antagonist protocol, a possible negative impact of elevated progesterone during the late follicular phase on ongoing pregnancy rates is limited to those with serum progesterone levels higher than 2.0 ng/mL. In these subjects, freezing of embryos for later embryo transfer may be a more favorable option.

Study funding/competing interest(s): Financial support for this study was provided by Merck, Sharp & Dohme Corp., a subsidiary of Merck & Co. Inc., Whitehouse Station, NJ, USA.

Trial registration number: NCT01144416

P-548 Failure to shorten the stimulation period and induce monoovulation in women with anovulatory infertility using an individualized gonadotrophin dosage regimen. A prospective clinical trial

M.P. Lauritsen¹, A. Loft¹, A. Pinborg¹, N.L. Freiesleben¹, A.L. Mikkelsen², M.R. Bjerger², and A. Nyboe Andersen¹

¹Copenhagen University Hospital, The Fertility Clinic 4071, Copenhagen, Denmark, ²Dept. of Gynecology and Obstetrics Holbæk Hospital, The Fertility Clinic, Holbæk, Denmark

Study question: Is it possible to reach the criterion for human chorionic gonadotrophin (hCG) administration within two weeks in 75% of women with anovulatory infertility undergoing ovulation induction (OI) by using an individualized dosage regimen?

Summary answer: The individualized dosage regimen did not shorten the stimulation period compared with a standard low-dose step-up regimen, and it was considered insufficiently accurate for clinical use as we observed a high rate of cycles with multifollicular development causing multiple pregnancies and a high rate of conversion to in vitro fertilisation (IVF).

What is known already: The low-dose step-up protocol remains the standard approach to ovarian stimulation with gonadotrophins in anovulatory infertility. The disadvantage is primarily prolonged treatment periods because the starting dose in many cases is below the individual gonadotrophin threshold. Predictors of the ovarian response to gonadotrophins have been identified and dosage nomograms have been created for clinical use. We tested a dosage nomogram where women with amenorrhoea, high ovarian volume and high body mass index (BMI) received higher gonadotrophin starting doses than standard (Nyboe Andersen et al., 2008).

Study design, size, duration: A prospective study of 62 women with normogonadotrophic anovulatory infertility was performed during 2010–2012. The primary endpoint was the proportion of women reaching the hCG criterion within two weeks of stimulation with highly purified human menopausal gonadotrophin (HP-hMG). This was based on a previous study where a standard (75 IU/day) starting dose of HP-hMG was given and 50% of the women reached the hCG criterion within two weeks (Platteau et al. 2006). We hypothesized that the individualized regimen could increase this proportion to 75%.

Participants/materials, setting, methods: All women underwent OI with an individualized nomogram-based starting dose (75–150 IU/day) of HP-hMG. From stimulation Day 5, step-down was allowed if there were >3 follicles >15 mm. Step-up was allowed from Day 8 if there were no follicles >10 mm. The criterion for hCG administration was 1–3 mature follicles. This was followed by intrauterine insemination (IUI).

Main results and the role of chance: Among the 62 women in the study group, 26(42%) had amenorrhoea and 36(58%) oligomenorrhoea. Mean(SD) female age was 29.7(±3.2) years; median(IQR) BMI 20.0(19.0–22.0); median(IQR) ovarian volume 6.5 ml(4.9–9.3); median(IQR) antral follicle count 46.0 (31.0–65.0); median (IQR) anti-Müllerian Hormone 59 pmol/l (37.3–99.8). HP-hMG starting doses were 75 IU/day in 47(76%) and 112.5 IU/day in 15(24%) women. In total, 42(68%) women met the hCG criterion, 20(48%) within 14 days, 22(52%) after 14 days; 32(52%) achieved monoovulation. Conversion to IVF was performed in 15(24%) of the cycles; 5(8%) of the cycles were cancelled. Clinical pregnancy rates were 33%(n = 14) after IUI and 27%(n = 4) after IVF. Ongoing pregnancy rates were 29%(n = 12) after IUI and 20%(n = 3) after IVF. In the IUI group, 21%(n = 3) were multiple pregnancies. No multiple pregnancies were observed in the IVF group. No serious adverse events occurred.

Limitations, reason for caution: The study design would have been stronger if a larger randomized controlled trial had been performed comparing ovarian response in women assigned either to an individual nomogram-based dose of HP-hMG or to a standard dose.

Wider implications of the findings: Despite the effort to minimize multifollicular development by using a flexible step-up/step-down protocol, the rate of multiple pregnancies and conversion to IVF was high. Our results indicate that similar to the step-down approach earlier explored, the individualized dosage

regimen is not applicable in clinical practice unless strict conversion criteria to IVF are implemented. If so, the individualized dosage regimen may be used as a mild IVF regimen among women prone to develop ovarian hyperstimulation syndrome.

Study funding/competing interest(s): The study was partially supported by an educational Ph.D. grant from Ferring Pharmaceuticals. The study medication was supplied by Ferring Pharmaceuticals.

Trial registration number: ClinicalTrials.gov Identifier: NCT01250821. EudraCT-number 2010-021459-16.

P-549 Attenuated Wnt4 action impacts the fate of ovarian maturation by impeding Anti-Müllerian hormone in polycystic ovary syndrome

P. Chakraborty¹, S.K. Goswami², and B.N. Chakravarty²

¹Institute of Reproductive Medicine, Infertility, Kolkata, India, ²Institute of Reproductive Medicine, IUI, Kolkata, India

Study question: Does dys-regulated Wnt4 (wingless-type MMTV (murine-mammary-tumour virus) signalling influence follicular pool in polycystic ovary syndrome (PCOS)? If the answer is in affirmative, does Anti-Müllerian hormone (AMH) and steroidogenic acute regulatory protein (StAR) play any role to this altered pathway?

Summary answer: Down-regulation of Wnt4 in hyperhomocysteinemic rats might lower the threshold for follicular recruitment through up-regulation of StAR and attenuation of AMH. It would be possible to suggest that androgens synthesized in ovary are able to produce decrease in local production of AMH, allowing more follicles in the growth trajectory.

What is known already: Women with PCOS exhibit an excess of serum AMH levels that perhaps trigger increased proportion of follicles in the cohort that departs the resting follicular pool. Intra-ovarian hyperandrogenism seems to lead this follicle excess, mechanism being unknown. Recent studies document high incidence of excess serum homocysteine levels in PCOS women. Furthermore, hyperhomocysteinemia may be causally related with the pathogenesis of PCOS. We therefore attempt to map the pathway in the control of follicle dynamics.

Study design, size, duration: In this experimental study, post-pubertal Sprague-Dawley rats (n = 15) of ~55 day of age were gavaged with homocysteine (Hcy) through drinking water at a dose of ~100 mg/kg/day for a period of 1 month to induce PCOS. Comparisons were made with age-matched control (n = 10).

Participants/materials, setting, methods: Basic tenets of PCOS according to Rotterdam criteria had been evaluated by standard methods. Expression of regulatory factors concerned with ovarian steroidogenesis (Wnt4, aromatase, steroidogenic factor-1 (Sf-1) and StAR), follicular recruitment (AMH), and insulin resistance (GSK-3β and insulin growth factor (IGF - 1) were analyzed by reverse-transcriptase polymerase chain reaction.

Main results and the role of chance: The treated rats developed moderate degree of hyperhomocysteinemia (HHcy) and replicated morphologic as well as many metabolic spectra of PCOS. The treated rats had lower (P < 0.001) expression of ovarian AMH with the ovary depicting a number of cystic follicles, and decreased granulosa cell compartment along with many pre-antral follicles situated in the cortex. Aromatase and GSK-3-β significantly (P < 0.01) declined in hyperhomocysteinemic group with the occurrence of significant glucose intolerance followed by insulin resistance and dyslipidaemia. Expressions of Wnt4 was down-regulated (P < 0.001), while there was overt expression of StAR (P < 0.03) with up-regulation of Sf-1 and IGF-1 (P < 0.01) with increased (P < 0.003) serum levels of testosterone.

Limitations, reason for caution: Our results demonstrate reduced expression of AMH in PCOS in contrast with outcome of other studies. However, serum AMH has not been measured in the current study and hence consequence may be carefully looked as the per unit effect and not as the collective product in the circulating level.

Wider implications of the findings: HHcy attenuates Wnt4 signalling that inducts stimulation of StAR and inhibition of aromatase to overpower ovarian androgen synthesis. The mechanism how Hcy down-regulates AMH remains unknown; but it may be a secondary consequence of Hcy-induced hyperandrogenemia because androgens are known for its inhibitory influence on the expression of AMH. This decrement in Wnt4 signalling may promote follicle

recruitment, increasing the growing follicular pool. This new mechanism may have implications for understanding of PCOS pathophysiology.

Study funding/competing interest(s): None. No competing interests have been declared.

Trial registration number: N/A.

P-550 The evaluation of ovarian reserve in women with a haemoglobinopathy and iron overload using a dynamic FSH stress test (dFST)

M. Mittal¹, R. Bajoria², N. Narvekar¹, and R. Chatterjee²

¹King's College Hospital, Assisted Conception Unit, London, United Kingdom, ²University College London, Reproductive Medicine, London, United Kingdom

Study question: Do women with a haemoglobinopathy and iron overload have normal ovarian function?

Summary answer: Ovarian reserve is reduced amongst women with a haemoglobinopathy, particularly the women requiring hormone replacement therapy (HRT) compared to those naturally cycling, which suggests a correlation with clinical severity of iron overload.

What is known already: Multiple periodic transfusions in women with haemoglobinopathies, can lead to iron overload resulting in multiple organopathy and dysfunction. Although iron overload results in hypogonadotropic hypogonadism (HH) which can render women amenorrhoeic and/or anovulatory, they are thought to maintain age-appropriate ovarian function as illustrated by several studies of successful pregnancies after ovulation-induction with FSH.

Study design, size, duration: Prospective analysis of 7 patients with a known haemoglobinopathy and iron overload (Group A) compared with age matched controls undergoing a baseline fertility assessment (17 patients – Group B) and IVF treatment (16 patients – Group C).

Participants/materials, setting, methods: Ovarian reserve was assessed by measurement of the ovarian antral follicle count (AFC) by ultrasound, and follicle stimulating hormone (FSH), luteinising hormone (LH), oestradiol (E2), anti-müllerian hormone (AMH) and Inhibin B by blood test. All three groups underwent measurement of ovarian reserve on menstrual cycle days 2 ± 2 (baseline). In addition, Group A underwent a dFST: 1. Baseline ovarian reserve measurements; 2. Three daily doses of 300IU recombinant human FSH (rFSH); 3. Repeat ovarian reserve measurements on day's baseline + 1 and baseline + 3. Group C underwent an egg collection as part of their treatment.

Main results and the role of chance: Within Group A the baseline measure of ovarian reserve for naturally cycling women (n = 4) [mean ± sd: AFC = 5.0 ± 3.4; FSH = 9.9 ± 5.1 IU/L] was better than those on HRT (n = 3) [AFC = 2.0 ± 2.0; FSH = 1.2 ± 0.9], but lower than both control groups [Group B: AFC = 16.4 ± 10.4; FSH = 8.0 ± 3.2 & Group C: AFC = 20.2 ± 23.0; FSH = 7.3 ± 1.8]. Naturally cycling women were also more responsive to rFSH, evidenced by their steady rise in E2 and Inhibin B [natural: baseline E2 = 68.7 ± 9.0 pmol/L & Inhibin B = 13.9 ± 3.4 pg/ml; baseline + 1 E2 = 94.3 ± 37.5 & Inhibin B = 36.0 ± 25.0; baseline + 3 E2 = 270.5 ± 224.1 & Inhibin B = 92.7 ± 69.9] [HRT: baseline E2 = 111.7 ± 104.5 & Inhibin B = 4.3 ± 1.5; baseline + 1 E2 = 151.0 ± 160.1 & Inhibin B = 27.5 ± 18.9; baseline + 3 E2 = 184.3 ± 177.9 & Inhibin B = 140.0 ± 104.5]. AMH levels followed a classic pattern of an initial rise with stimulation and subsequent fall. The egg yield in Group C was 11.1 ± 7.6. This did not however, correlate (Pearson Correlation 0.4, p = 0.2) with that group's baseline AFC (20.2 ± 23.0).

Limitations, reason for caution: The numbers are small and the age matched controls were all infertile women who underwent baseline measurements only, limiting comparisons with Group A. This is pilot data and a much larger study is planned to correlate measures of disease severity and iron overload with measures of ovarian function.

Wider implications of the findings: Our data shows a reduction in baseline measures of ovarian reserve in women with a haemoglobinopathy in comparison with infertile controls which is consistent with previous studies. The dynamic test however, shows for the first time a differentiation in response albeit small, between naturally cycling women, and those on HRT. The latter group have been shown to have a more severely damaged hypothalamo-pituitary axis, and therefore the results suggest a correlation between disease severity and ovarian dysfunction. This information can be used to counsel the women about the risks and treatment options, including, social egg freezing, assisted reproductive treatment and egg donation.

Study funding/competing interest(s): None declared.

Trial registration number: N/A

P-551 Advantages of reporting plasma anti-Müllerian hormone (AMH) levels as multiples of the median (MoM) in women of reproductive age

J.G. Bentzen¹, T. Holm Johannsen², T. Scheike³, A. Nyboe Andersen¹, and L. Friis-Hansen²

¹Copenhagen University Hospital Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark, ²Copenhagen University Hospital Rigshospitalet, Department of Clinical Biochemistry, Copenhagen, Denmark, ³Copenhagen University, Department of Biostatistics, Copenhagen, Denmark

Study question: As AMH levels are highly age dependent, a study was performed to explore whether multiples of the median (MoM) of AMH could be useful in characterizing variability in AMH levels during normal reproductive ageing.

Summary answer: The median MoM values of AMH were significantly associated with important reproductive factors such as optimal reproductive profile, use of hormonal contraception, menstrual cycle disturbances, and infertility. Reporting MoM values of AMH in addition to absolute values may be of clinical use.

What is known already: Plasma AMH is a marker of the ovarian reserve. Due to its decline and large variation with age, changes in absolute AMH concentrations over time within the same patient may be difficult to interpret. These challenges may be overcome by reporting MoM values of plasma AMH. The MoM value is a measure of how far an individual's test result deviates from the median value of a normal reference interval.

Study design, size, duration: Cross-sectional data from a prospective cohort of 863 volunteers. All were female health-care workers aged 21-41 years. All underwent blood sampling at menstrual cycle day 2-5, and plasma AMH concentrations were analysed using a sandwich enzyme immunometric assay (EIA AMH/MIS kit, Immunotech A16507, Beckman Coulter, Marseille, France).

Participants/materials, setting, methods: Participants were grouped according to: A) optimal reproductive profile, i.e. regular cycle, two ovaries, normal basal plasma FSH, no use of hormonal contraception, no history of infertility, chronic disease or ovarian surgery (n = 351); B) current use of hormonal contraception (n = 242); C) irregular cycle (n = 62); and D) infertility > 12 months (n = 101). There was some overlap between groups.

Main results and the role of chance: The overall median AMH concentration was 19.3 pmol/L (interquartile range [IQR]: 10.6-31.8 pmol/L), corresponding to a MoM of 1.0 (IQR: 0.6-1.6).

Group A had a median AMH concentration = 20.2 pmol/L (IQR: 11.6-34.7 pmol/L) with a corresponding MoM = 1.1 (IQR: 0.7-1.9) including 153 (44%) participants with MoM < 1 and 198 (56%) with MoM > 1. The age-adjusted MoM for Group A was 20.9% (95% CI: 11.5-29.4%, P < 0.001) higher compared with those not having an optimal fertility profile.

Group B had a median AMH concentration = 19.5 pmol/L (IQR: 10.6-30.9) and MoM = 0.9 (IQR: 0.5-1.3; MoM < 1: n = 135 [56%]; MoM > 1: n = 107 [44%]). The age-adjusted MoM for Group B was 30.0% (95% CI: 14.0-47.9%, P < 0.001) lower compared with non-users of hormonal contraception.

Group C had a median AMH-concentration = 32.4 pmol/L (IQR: 19.7-59.1) and MoM = 1.6 (IQR: 1.1-3.4; MoM < 1: n = 13 [21%]; MoM > 1: n = 49 [79%]). The age-adjusted MoM for Group C was 43.5% (95% CI: 30.3-54.2%, P < 0.001) higher compared with those not having an irregular menstrual cycle.

Group D had a median AMH-concentration = 19.3 pmol/L (IQR: 10.6-32.2) and MoM = 1.2 (IQR: 0.6-2.0; (MoM < 1: n = 41 [41%]; MoM > 1: n = 60 [59%]). The age-adjusted MoM for Group D was 17.6% (95% CI: 1.8-30.7%, P = 0.03) higher compared with those with no history of infertility.

Limitations, reason for caution: To forecast an individual's reproductive event such as the occurrence of menopause, longitudinal follow-up data are needed to assess whether MoM values remain the same irrespective of age.

Wider implications of the findings: Converting individual patients' AMH results to MoM values has advantages over reporting in mass units when the reference interval declines with age. An important benefit of reporting AMH concentrations in MoM values is the ease in which serial measurements in the same individual over time can be followed. The center-to-center variation may additionally be reduced by using MoM-values.

Study funding/competing interest(s): Co-financed PhD scholarships where funding was covered by the Danish Agency for Science, Technology and Innovation; Copenhagen Graduate School of Health Science; and the Fertility Clinic at Copenhagen University Hospital, Rigshospitalet. No competing interest declared.

Trial registration number: -

P-552 Effectiveness of the GnRH agonist long, GnRH agonist short and GnRH antagonist regimens in poor responders undergoing IVF treatment: a three arm randomised controlled trial

S. Sunkara¹, A. Coomarasamy², R. Faris³, P. Braude⁴, and Y. Khalaf⁵
¹Guy's and St Thomas' NHS Trust King's College London, Assisted Conception Unit, London, United Kingdom, ²University of Birmingham Birmingham Women's Hospital, Academic Unit, Birmingham, United Kingdom, ³The Lister Hospital London, Lister Fertility Clinic, London, United Kingdom, ⁴King's College London, Assisted Conception Unit, London, United Kingdom, ⁵Guy's and St Thomas' NHS Trust, Assisted Conception Unit, London, United Kingdom

Study question: The aim of this randomised controlled trial (RCT) is to compare the efficacy of the controlled ovarian stimulation (COS) regimens: the GnRH agonist long versus the GnRH agonist short versus the GnRH antagonist regimens in women with a history of poor ovarian response undergoing a subsequent IVF cycle.

Summary answer: The GnRH agonist long and the GnRH antagonist regimens offer suitable options for ovarian stimulation in poor responders. The GnRH agonist short "flare" regimen is least effective for poor responders.

What is known already: The GnRH agonist long regimen is the most frequently used COS regimen in IVF treatment. The GnRH agonist short "flare" and the GnRH antagonist regimens have been suggested as alternatives to the standard GnRH agonist long regimen to improve outcome for poor responders undergoing IVF. A recent worldwide survey showed a wide variation in the GnRH analogue protocols chosen for poor responders. This is likely to uncertainty about the most effective regimen for poor responders.

Study design, size, duration: The RCT was conducted between March 2007 to May 2012. After informed written consent, 111 participants were allocated to one of the three study arms by third party, distant, internet-based block randomisation to ensure allocation concealment. Outcome assessor was blinded to the treatment allocation and the analysis was by intention-to-treat.

Participants/materials, setting, methods: A poor responder was defined as a woman who had a previous IVF cycle with daily stimulation dose of at least 300 IU of gonadotrophin and had ≤ 3 oocytes retrieved or had cycle cancellation due to development of ≤ 3 mature follicles. Participants were recruited from two tertiary fertility referral centres.

Main results and the role of chance: There was a significant difference in the mean number of oocytes retrieved between the three groups ($p = 0.04$). The number of oocytes was significantly higher with the agonist long compared to the agonist short (4.4 ± 3.1 versus 2.71 ± 1.6 , $p = 0.01$), whilst there was no significant difference between the agonist long and the antagonist regimens (4.4 ± 3.1 versus 3.3 ± 2.9 ; $p = 0.21$). There were no significant differences in cycles cancelled (3 vs. 4 vs. 6; $p = 0.82$) and fertilisation rates between the three regimens (52.4% vs. 48.6% vs. 49.4%; $p = 0.28$). Gonadotrophin consumption was significantly higher with agonist long compared to agonist short and antagonist regimens (5540.3 ± 12161 vs. 4819.4 ± 1145.5 vs. 4740.0 ± 1131.9 ; $p = 0.01$). The ongoing pregnancy rate was 8.1% with agonist long and short regimens respectively and 16.2% with antagonist regimen ($p = 0.48$).

Limitations, reason for caution: The study was powered to detect differences in oocyte numbers. It was not powered to detect differences in pregnancy rates as to power such a study would require a very large sample size because of expected low pregnancy rates in poor responders making such a study impractical.

Wider implications of the findings: First RCT comparing three alternative COS regimens in poor responders. Recent demonstration of a strong relationship and the initial linear association between oocyte number and live birth following IVF justifies use of egg number as a valid outcome variable in studies of poor ovarian response. The methodological rigour of the study and an evidence based definition of poor ovarian response, a recurring drawback with previous studies make the findings credible and applicable to clinical practice.

Study funding/competing interest(s): The study was funded by the participating fertility centres.

Trial registration number: The study was approved by the United Kingdom (UK) National Research Ethics Committee (REC reference 06/Q0403/157).

Trial registration number: Trial registration number: ISRCTN27044628

P-553 Triggering of final oocyte maturation with GnRH agonist in patients with polycystic ovaries undergoing IVF

A. Makedos, E.M. Kolibianakis, C.A. Venetis, S. Masouridou, K. Chatzimeletiou, L. Zepiridis, A. Mitsoli, G. Lainas, I. Sfontouris, A. Tzamtzoglou, D. Kyrou, T. Lainas, and B.C. Tarlatzis
 Medical School Aristotle University of Thessaloniki, Unit for Human Reproduction 1st Dept. of OB/Gyn, Thessaloniki, Greece

Study question: Does triggering of final oocyte maturation with gonadotrophin releasing hormone (GnRH) agonist and embryo transfer in a subsequent frozen thawed (FRET) cycle in patients with polycystic ovaries (PCO) result in elimination of ovarian hyperstimulation syndrome (OHSS), while maintaining a high probability of pregnancy?

Summary answer: Triggering of final oocyte maturation with GnRH agonist in women with polycystic ovaries eliminates OHSS and results in a high cumulative probability of ongoing pregnancy after transfer of embryos in subsequent FRET cycles.

What is known already: Patients with PCO frequently develop an excessive number of follicles during ovarian stimulation for in-vitro fertilization (IVF), leading to a high probability of OHSS, after triggering final oocyte maturation with human chorionic gonadotropin (hCG).

Replacement of hCG with GnRH agonist is known to eliminate the occurrence of OHSS and thus, might be an attractive alternative in these patients. Agonist triggering, however, results in a decreased chance of pregnancy after fresh embryo transfer.

Study design, size, duration: This is a prospective, observational, proof of concept study (September 2011-December 2012), aiming to assess the safety and efficacy of triggering final oocyte maturation with GnRH agonist and performing embryo transfer in a FRET cycle in 111 PCO patients, at high risk for OHSS (≥ 14 follicles ≥ 11 mm).

Participants/materials, setting, methods: Ovarian stimulation was performed with recombinant FSH and GnRH antagonists. Triggering of final oocyte maturation was performed with 0.2mg triptorelin, when at least three follicles of ≥ 17 mm were present at ultrasound. All resulting 2pn oocytes were frozen and embryos were transferred in subsequent FRET cycles at the cleavage stage.

Main results and the role of chance: The mean age of the patients included was 32.4 ± 4.8 years and the mean number of antral follicles was 24.3 ± 9.7 . Mean levels of estradiol on the day of agonist triggering was 4107 ± 1450 pg/ml and the mean number of follicles ≥ 11 mm was 26.1 ± 8.4 . At oocyte pick-up, 19.5 ± 10.3 oocytes were retrieved and following fertilization, 847 2pn oocytes were frozen (mean number 10.1 ± 5.6).

None of the patients experienced severe OHSS or was hospitalised. Furthermore, none of the patients reported nausea, abdominal pain, oliguria or feeling of unwellness.

Ongoing pregnancy rate after the first FRET cycle (31/111 patients) was 27.9% (95%CI: 20.4-36.9%). Cumulative ongoing pregnancy rate after 158 FRET cycles was 68.3% (95% CI: 50.3-86.4%). Until today, 506 embryos have been thawed, while 341 2pn oocytes are still frozen.

Limitations, reason for caution: This is a non-comparative, proof of concept study and thus conclusions regarding the relative efficacy and safety of agonist vs. hCG triggering cannot be drawn. On the other hand, a comparative study addressing the above question might be ethically challenging.

Wider implications of the findings: Given the unanimously accepted safety of agonist triggering in patients at high risk for OHSS, the current proof of concept study provides evidence that management of PCO patients at high risk for OHSS by agonist triggering, freezing all 2pn oocytes and transferring embryos in subsequent FRET cycles eliminates OHSS, while it maintains a high probability of ongoing pregnancy. Thus, the optimal way of managing PCO patients undergoing IVF might need to be reconsidered.

Study funding/competing interest(s): None

Trial registration number: N/A

P-554 Influence of the fsh/lh ratio in ovarian stimulation in ivf results in women aged over 35 years: a randomized study

A. Fermin, L. Crisol, A. Expósito, B. Prieto, R. Mendoza, and R. Matorras
Cruces Hospital, Reproduction Unit, Vizcaya, Spain

Study question: To assess the ivf results in women aged 35-40. A FSH/LH ratio of 2 is associated with different IVF outcomes than a ratio of 4.

Summary answer: Similar IVF results were obtained with both stimulation regimens.

What is known already: Although there is general agreement on the importance LH activity in IVF stimulation in women aged over 35, the precise amount of LH and the optimal FSH/LH ratio are not well known.

Study design, size, duration: 456 women aged 35-40 years undergoing IVF treatments at our center from January 2009 to december 2010. They were subjected to a randomized trial analysing two FSH/LH ratios. All of them were on long agonist protocol. Group A received a daily stimulation of 300IU of rec FSH and 150 of rec LH and group B, 300 IU or rec FSH and 75 of LH.

Participants/materials, setting, methods: 456 women were recruited at our University center. Main inclusion criteria were: age 35-40 years, no previous IVF cycles, FSH under 10mUI/ml. Both embryologists and doctors were blinded to the stimulation protocol. Randomization was performed according sealed envelopes.

Main results and the role of chance: Groups were similar regarding: age, height, weight, basal FSH levels, duration of infertility as well as other demographic parameters. Estradiol levels were similar in the 2/1 group and in the 4/1 group (2300 ± 1179 vs 2110 ± 1161), as well as the number of obtained oocytes (9.04 ± 5.54 vs 8.55 ± 5.07), of inseminated oocytes (7.18 ± 4.89 vs 6.67 ± 4.72), and of fertilized oocytes (3.92 ± 3.21 vs 3.55 ± 2.73). The pregnancy rate was also similar in both groups 33.9% in the 2/1 ratio group and 35.5% in the 4/1 ratio group.

Limitations, reason for caution: Smaller differences could be detected in a larger sample, but with a small clinical relevance.

Wider implications of the findings: A daily dose of 75 IU of rec LH is enough to optimize IVF results.

Study funding/competing interest(s): I confirm that I have in relation to this work

Trial registration number: Not applicable

P-555 Inferred genetic ancestry versus reported ethnicity in polycystic ovary syndrome (PCOS)

Y. Louwers¹, O. Lao², and M. Kayser²

¹*Erasmus MC University Medical Centre, Division of Reproductive Medicine Department of Obstetrics and Gynecology, Rotterdam, The Netherlands,*

²*Erasmus MC University Medical Centre, Department of Forensic Molecular Biology, Rotterdam, The Netherlands*

Study question: How does inferred genetic ancestry of patients diagnosed with polycystic ovary syndrome (PCOS) correlate to their reported ethnic background and does inferred genetic background affect phenotypic characteristics of these patients?

Summary answer: Genetic ancestry is only moderately associated to self-reported ethnic background and should therefore be considered to allow quantifying for population stratification. Genetic ancestry inferred from genome-wide data was significantly associated with phenotypic differences in PCOS.

What is known already: It has been well established that ethnic background is associated to the phenotype of PCOS. We have previously shown that a set of 10 Ancestry Informative Markers is effective to map phenotypic differences in anovulatory patients at a continental level. However, a more precise geographic ancestry resolution of the patients would be desirable.

Study design, size, duration: Fine geographic genetic ancestry of 1499 multi-ethnic PCOS patients diagnosed according to the Rotterdam criteria was determined using genome-wide data and with the samples of the Human Genetic Diversity Panel from 51 populations with know bio-geographic ancestry as a reference.

Participants/materials, setting, methods: Patients were genotyped with the Illumina610K SNP array. Clustering algorithms, including Multi-Dimensional-Scaling (MDS) and ADMIXTURE, were applied to describe and recover the genetic ancestry for each individual. Ancestry assignments were compared to self-

reported ethnicity by means of the Cramer's V-statistic. Further, association of genetic ancestry with PCOS characteristics was tested.

Main results and the role of chance: Based on self-reported ethnicity subgroups were divided in individuals from European (n = 895), Middle East / North African (n = 182), South-East Asian (n = 40), Surinam Hindustani (n = 67), Sub-Saharan African (n = 99) and admixed (n = 42) descent. For 174 individuals self reported ancestry was unknown, however these patients could be included in determining genetic ancestry. The first 5 dimensions of the MDS explained approximately 7% of the genetic variation. These dimensions were significantly associated with differences in PCOS phenotype, i.e., BMI, androgen and insulin levels. Genetic ancestry inferred from genome-wide data allowed us to distinguish population structure at a sub-continental level. Based on the cluster analysis and the assumption of the presence of 6 clusters, genetic ancestry was found to be moderately correlated to the self-reported ethnicity (Cramer's V coefficient = 0.602).

Limitations, reason for caution: Although we were able to include a very large study population (n = 1499), the majority of these patients were of European descent providing power-limitations on non-Europeans. Increasing the number of non-European individuals would increase power and might even identify additional phenotypic differences.

Wider implications of the findings: Genetics in the PCOS field is evolving and genome wide association studies are starting to emerge. This study indicates that genetic ancestry inferred from genome-wide data is associated with the PCOS phenotype, especially BMI, androgen and insulin levels. By using inferred genetic ancestry instead of self-reported ethnicity misclassification based on family records or in admixed individuals can be avoided. Therefore, genetic ancestry inferred from genome-wide data should be recommended for the exact determination of biogeographic origin for research purposes as well as in a clinical setting.

Study funding/competing interest(s): NA

Trial registration number: NA

P-556 GnRH agonist (GnRHa) trigger in antagonist protocol cycles: incidence of failed trigger and impact of different GnRH agonist doses

A. Palumbo¹, V. Sanabria¹, J.P. Rouleau¹, M. Puopolo², and M.J. Hernandez¹

¹*Centro de Asistencia a la Reproducción Humana de Canarias, Fivap, La Laguna, Spain,* ²*Istituto Superiore di Sanità, Department of Cell Biology and Neurosciences, Rome, Italy*

Study question: To determine the percentage failed trigger with GnRHa in antagonist patient and donor cycles; to assess the effect of increasing doses of GnRHa on the mature oocyte yield and the percentage mature oocytes retrieved; to seek an association between the amplitude of the LH peak and the percentage mature oocytes.

Summary answer: The percentage failed trigger is 1.3% and independent of the GnRHa dose. LH levels >10 measured ten hours after the trigger are associated with successful retrieval. The only patient with failed trigger who had LH measured had a value of 2.3.

What is known already: Ovarian hyperstimulation syndrome (OHSS) is the worst complication of IVF and prevention strategies are mandatory in both oocyte donors and infertile patients. Our program implemented the administration of a GnRHa bolus instead of hCG in 2006 and none of the patients/donors triggered with GnRHa presented OHSS. The current literature supports the use of GnRHa trigger in cases at risk of OHSS and recent reports show a correlation between the LH peak and the oocyte yield.

Study design, size, duration: Observational study of all donors and patients undergoing GnRH agonist trigger in a single private IVF program from 1.5.2006 to 25.1.2013. Eightyfour infertile patients at risk for OHSS (91 cycles) and 428 donors (572 cycles) underwent ovarian stimulation with an antagonist protocol and were triggered with leuprolide acetate.

Participants/materials, setting, methods: During the study period GnRHa dose was increased from two 1.2 mg boluses to two 4 mg boluses 10 hours apart; LH and progesterone were measured at 10 hours in the last 49 cases. Number of follicles >10mm on day of trigger and number and percentage mature oocytes were determined.

Main results and the role of chance: Six of 572 egg donation cycles (1%) and 3 of 91 patient cycles (3.3%) had failed trigger with no oocytes recovered at retrieval. In 3 patients and 1 donor the procedure was stopped after aspirating one ovary and repeated satisfactorily after re-trigger with 5000IU hCG. The mature oocyte

yield (% number of mature oocytes/number of follicles >10mm) was 81.5 ± 2.9 vs 81.8 ± 2.0 vs 83.2 ± 2.9 in patients triggered with the 1.5, 2 and 4 mg boluses respectively. The percentage mature oocytes was 79.1 ± 1.3 vs 77.5 ± 1.1 vs 76.2 ± 0.9 vs 78.4 ± 1.2 with the 1.2, 1.5, 2 and 4 mg doses. Differences were not statistically significant (Student t test). No correlation was found between the percentage mature oocytes and LH and progesterone levels (Spearman's rank correlation coefficient).

Limitations, reason for caution: Limitations include the retrospective nature of the study and lack of randomization of subjects to the different GnRH α doses. Only a small group of cases have LH and progesterone levels and only one of these had failed trigger.

Wider implications of the findings: These results suggest that the efficacy of the lower doses of GnRH α is equivalent to that of the higher doses. Further randomized studies are needed to confirm these findings and to better assess the possible association between the LH peak amplitude and failed trigger.

Study funding/competing interest(s): none

Trial registration number: not applicable

P-557 Altered sensitivity rather than decreased amounts of FSH (FSHR) and androgen receptors (AR) in granulosa cells might be beneath the development of diminished ovarian response

C. Díaz-García¹, M. Monterde², A. Marzal¹, O. Vega³, J.M. Rubio³, R. Gómez⁴, and A. Pellicer⁵

¹Health Research Institute La Fe University Hospital, Department of Obstetrics Gynecology and Assisted Human Reproduction La Fe Hospital, Reproductive Medicine, ²Health Research Institute La Fe University Hospital, Reproductive Medicine, Valencia, Spain, ³Department of Obstetrics Gynecology and Assisted Human Reproduction La Fe University Hospital, Reproductive Medicine, Valencia, Spain, ⁴Fundación IVI/INCLIVA, Reproductive Medicine Research, Paterna Valencia, Spain, ⁵Department of Obstetrics Gynecology and Assisted Human Reproduction La Fe University Hospital-Fundación IVI/INCLIVA, Reproductive Medicine Research, Valencia, Spain

Study question: To elucidate whether the amounts of FSHR and/or AR in luteinized granulosa cells (LGCs) from poor responder women were decreased as compared to those of normo responders after controlled ovarian hyperstimulation (COH).

Summary answer: The density of AR and FSHR and the percentage of LGCs expressing such receptors was higher in poor-ovarian-responders (POR) than in normo-responders (NOR), pointing to decreased sensitivity rather than decreased expression of both receptors in LGCs as a potential cause of diminished ovarian response.

What is known already: Controversial studies describing decreased or similar amounts of FSHR in LGCs from POR versus NOR have been described. Given that LGCs specific knocking-out of AR on a mouse model results in POR and subfertility. It is particularly bizarre that the role of AR over ovarian response and consequences of differential AR expression in human LGCs over COH has never been assessed.

Study design, size, duration: Women (N = 75) suspected to respond poorly to COH according to Bologna criteria were enrolled in this prospective study ongoing since February-2011 to December-2012. Patients underwent a standard COH with a constant recombinant FSH dose. Premature LH surge was inhibited with cetrorelix and ovulation induction was triggered with triptorelin acetate.

Participants/materials, setting, methods: Human follicular fluids from each of the enrolled patients were pooled to pre-isolate LGCs by one step density gradient and centrifugation protocol. The percentage of AR and FSHR expressing cells and receptor density was quantified through flow cytometry in putative pre-isolated LGCs (CD45⁻)

Main results and the role of chance: According to Bologna criteria 64 out of 75 women included were confirmed as POR and 11 as NOR. In POR women the percentage of LGCs positive for AR, denoted as LGCs AR⁺/CD45⁻IP⁺, was statistically higher (12.26 ± 15.43 ; $p < 0.05$) compared with NOR (6.37 ± 5.96). Likewise, statistically significant increase was obtained in the mean fluorescence intensity (density) of AR⁺/CD45⁻IP⁺ (141.42 ± 208.72 , $p < 0.05$) respect to NOR (39.19 ± 29.76). A considerable but no significant increase was detected in the percentage of LGCs positive for FSHR (FSHR⁺/CD45⁻IP⁺), in POR (21.22 ± 16.67) versus NOR (19.52 ± 12.90) women. Similarly in POR the

density of FSHR⁺/CD45⁻IP⁺ obtained in LGCs from POR was higher (95.95 ± 96.83) than in NOR (59.35 ± 33.00).

Limitations, reason for caution: This study employed Bologna criteria to define what POR and NOR and thus results must be interpreted in the context of the heterogeneity that such criteria provide. Sample size was sufficient to detect significant differences.

Wider implications of the findings: Diminished ovarian response cannot be explained on the basis of decreased expression of FSH and/or AR. This phenomenon is more likely explained on the basis of a deregulation or desensitization of these receptors. We speculate that LGCs of POR "overexpress" FSH/AR to compensate the decreased input signal of desensitized/deregulated receptors.

Study funding/competing interest(s): The promoter of this prospective study was Health Research Institute La Fe Hospital from Valencia, Spain and was partially financed by Valencia Health Board (AP-199/10).

Trial registration number: This is a Basic Science Study. A trial registration number is only required for clinical trials.

P-558 Superovulation influences epab and pabpc1 gene expressions in mouse oocytes and early preimplantation embryos

S. Ozturk¹, B. Sozen¹, A. Yaba-Ucar², D. Mutlu³, and N. Demir¹

¹Akdeniz University School of Medicine, Histology and Embryology, Antalya, Turkey, ²Bilim University School of Medicine, Histology and Embryology, Istanbul, Turkey, ³Akdeniz University School of Medicine, Microbiology and Clinical Microbiology, Antalya, Turkey

Study question: Does superovulation with high or low doses of PMSG and hCG affect *Epab* (embryonic poly(A)-binding protein) and *Pabpc1* (poly(A)-binding protein, cytoplasmic 1) gene expressions in mouse oocytes and early preimplantation embryos?

Summary answer: Superovulation with high or low doses of PMSG and hCG differentially influenced expression levels of the *Epab* and *Pabpc1* genes in mouse oocytes at the stages of germinal vesicle (GV) and metaphase II (MII), and in zygote and 2-cell embryos.

What is known already: EPAB and PABPC1 play important functions in translational control of maternally stored mRNAs, required for oocyte maturation and early embryogenesis. *Epab* is expressed only in oocytes, zygote and 2-cell embryos. Also *Epab* knockout female mice are infertile due to abnormalities in oocyte maturation and ovulation. Many studies revealed that superovulation commonly used in ART (Assisted reproductive technology) procedure and experimental investigations adversely affects expression of certain genes implicating in oocyte maturation and early embryo development.

Study design, size, duration: Three different groups composed of control (non-superovulated), low dose superovulated (LD) (5 IU PMSG and 5 IU hCG) and high dose superovulated (HD) (10 IU PMSG and 10 IU hCG) were established from 6-week-old Balb/C female mice.

Participants/materials, setting, methods: Mouse oocytes (germinal vesicle and metaphase II) and early embryos (zygote and 2-cell embryo) (n = ≥ 50 from each one) were collected from control, LD and HD groups. Expression levels of the *Epab* and *Pabpc1* genes were determined by qRT-PCR (Quantitative real time polymerase chain reaction). ANOVA is used for statistical analysis.

Main results and the role of chance: High dose superovulation significantly increased *Epab* mRNA levels in the GV and MII oocytes, zygote and 2-cell embryos when compared to control group. However, low dose superovulation dramatically decreased *Epab* mRNA levels in GV oocyte, zygote and 2-cell embryo, whereas increased it in the MII oocytes in comparison with the control group. When *Pabpc1* expression was assayed in three groups, GV oocyte and 2-cell embryo from HD group had remarkably higher *Pabpc1* expression than control group, but zygote possessed lower *Pabpc1* expression. GV and 2-cell embryo from LD group exhibited predominantly lower *Pabpc1* expression in comparison with control group. Interestingly, there was no difference in *Pabpc1* expression in MII oocyte and zygote obtained from LD and HD groups.

Limitations, reason for caution: Absence of EPAB-specific antibody limits us to characterize EPAB protein expression and its relation with other proteins or mRNAs in the oocytes and early preimplantation embryos collected from control, HD and LD groups.

Wider implications of the findings: The present study revealed that HD and LD superovulation differently influenced *Epab* and *Pabpc1* expression, which have

important roles in translation control of the maternally derived mRNAs. The changes in the expression levels of these genes may adversely affect obtaining high quality oocytes and subsequent preimplantation embryos after superovulation. Further studies are required to characterize the impact of different superovulation protocols on the poly(A)-binding proteins or other genes to find the best superovulation protocol.

Study funding/competing interest(s): This study was supported by the Akdeniz University Scientific Research Projects (Project no: 2008.01.0103.006). The authors declare no competing interest(s).

Trial registration number: This study was supported by the Akdeniz University Scientific Research Projects (Project no: 2008.01.0103.006). The authors declare no competing interest(s).

P-559 A randomised, double-blind, active-controlled, multiple-dose trial investigating the pharmacokinetics and pharmacodynamics of a novel recombinant FSH (FE 999049) derived from a human cell line

H. Olsson, R. Sandström, and L. Grundemar

Ferring Pharmaceuticals A/S, Global Clinical R&D (Experimental Medicine), Copenhagen, Denmark

Study question: To compare the pharmacokinetic, pharmacodynamic, and immunogenicity profiles between a novel recombinant FSH derived from a human cell line (FE 999049; Ferring Pharmaceuticals) and follitropin alfa (GONAL-F; Merck Serono) in a multiple-dose trial.

Summary answer: Serum FSH reached steady state after 6-7 days with both FE 999049 and follitropin alfa. Area under the curve (AUC) and maximum concentration (C_{max}) of serum FSH were higher with FE 999049 than with follitropin alfa, while the apparent clearance was higher with follitropin alfa. Follicle development, inhibin B, estradiol and progesterone responses were all greater with FE 999049. No safety concerns were raised.

What is known already: In a previous trial, a single dose of FE 999049 in the dose range 75-450 IU was well tolerated and did not raise any safety concerns.

Study design, size, duration: This was a double-blind, randomised, active-controlled, parallel group, single-centre trial investigating the safety, tolerability, pharmacokinetics, pharmacodynamics and immunogenicity of FE 999049 and follitropin alfa given as daily subcutaneous doses of 225 IU for 7 days to healthy female volunteers. The trial included 49 women of whom 25 were exposed to FE 999049 and 24 to follitropin alfa.

Participants/materials, setting, methods: The trial population was healthy female volunteers. FE 999049 is a recombinant FSH produced from a host cell line of human fetal retinal origin while follitropin alfa is derived from a Chinese hamster ovary (CHO) cell line. Serum FSH, inhibin B, progesterone and estradiol were measured daily. Number and size of follicles were assessed daily by transvaginal ultrasound. Safety was assessed by clinical chemistry, vital signs, ECG, injection site reactions and anti-FSH antibodies.

Main results and the role of chance: Following daily administration of FE 999049 and follitropin alfa, the serum FSH concentration reached steady state after 6-7 days. After 7 days of dosing, the serum FSH concentration was significantly higher with FE 999049 compared to follitropin alfa. The AUC and C_{max} ratios FE 999049/follitropin alfa were 1.63 and 1.60, the 90% confidence intervals being [1.40; 1.90] and [1.38; 1.86], respectively. The apparent clearance was significantly higher for follitropin alfa. After 7 days of dosing, both number and size distribution of follicles showed a greater response with FE 999049 compared to follitropin alfa. Also, serum inhibin B, estradiol and progesterone concentrations were significantly higher following administration of FE 999049 compared to follitropin alfa. Repeated administration of FE 999049 was safe and generally well tolerated without signs of immunogenicity.

Limitations, reason for caution: Extrapolation of the results to other regimens should be done with caution.

Wider implications of the findings: FE 999049 is derived from a human cell line while follitropin alfa is derived from a Chinese hamster ovary (CHO) cell line. The results strongly indicate that the source of recombinant FSH is of importance with respect to the exposure and elimination after subcutaneous administration, as well as for the pharmacodynamic response. Equivalent IU doses of FE 999049 and follitropin alfa do not lead to similar pharmacokinetic and pharmacodynamic profiles.

Study funding/competing interest(s): This trial was sponsored by Ferring Pharmaceuticals A/S, Copenhagen, Denmark.

Trial registration number: Not applicable since this was a phase I trial.

P-560 Blastocyst transfer as a clinical strategy to overcome progesterone elevation on the day of hCG administration

E. Papaleo¹, L. Corti¹, E. Rabellotti¹, V.S. Vanni¹, M. Potenza¹, M. Molgora¹, P. Viganò¹, and M. Candiani²

¹*San Raffaele Scientific Institute, Obstetrics and Gynecology Unit, Milano, Italy,*

²*San Raffaele Scientific Institute, University Vita-Salute, Milano, Italy*

Study question: Does elevated serum progesterone (P) level on the day of hCG administration affect the IVF/ICSI outcomes after blastocyst transfer?

Summary answer: In patients with elevated serum progesterone level on the day of hCG administration, there is no detrimental effect on pregnancy outcomes after blastocyst transfer.

What is known already: Modest increases in serum progesterone levels ($P > 1.5$ ng/ml) on the day of hCG administration were negatively correlated to IVF outcomes after cleavage-stage embryo transfer (lower implantation and pregnancy rates). To overcome the problem, most of the studies propose cryopreservation of pronuclear/cleavage stage embryos in patients with $P > 1.5$ ng/ml; conversely, the efficacy of blastocyst transfer as a strategy to overcome the detrimental effect of progesterone rise is still debated.

Study design, size, duration: This is a retrospective cohort study included 204 women undergoing their first IVF cycle with blastocyst transfer. Data were collected from January to December 2012.

Participants/materials, setting, methods: Patients were grouped according to serum P level on the day of hCG administration: $P < 1.5$ ng/ml ($n = 165$) or $P > 1.5$ ng/ml ($n = 39$). The primary outcome was the clinical pregnancy rate. We used standardized descriptive statistics, comparing categorical variables with the Fisher's Exact test and continuous variables using the Student's *t* test.

Main results and the role of chance: Ovarian stimulation cycles characteristics (days of stimulation, E_2 on the day of hCG administration and FSH total dose) and IVF outcomes (retrieved and fertilized oocytes, positive β -hCG and clinical pregnancy rate) were assessed in both group ($P < 1.5$ ng/ml and $P > 1.5$ ng/ml).

Patients with subtle increase of Progesterone had a higher number of oocytes retrieved but the same number of fertilized oocytes and equal proportion of top-quality embryos transferred.

No differences in clinical pregnancy rate after blastocyst transfer were observed in women with elevated progesterone levels compared with women with normal progesterone levels (41% vs 47.3%, $p = 0.59$).

Limitations, reason for caution: The power of the study is limited by the small population number.

Wider implications of the findings: Supporting results from some studies but contrary to others, blastocyst transfer could represent the elective strategy to overcome a progesterone elevation above a detrimental threshold level. From pathogenetic point of view, the negative impact of progesterone elevation on oocyte/embryo quality is not supported.

Study funding/competing interest(s): This study did not receive any specific grant from any funding agency in the public commercial or not-for-profit sector. The authors declare no conflicts of interest.

Trial registration number: N/A

P-561 A randomised, assessor-blind, dose-response trial in patients undergoing COS for IVF/ICSI with a novel recombinant FSH (FE 999049) derived from a human cell line

A. Nyboe Andersen¹, M. Fernández-Sánchez², E. Bosch³, H. Visnova⁴, P. Barri⁵, J.A. Garcia-Velasco⁶, P. De Sutter⁷, B.J.C.M. Fauser⁸, and J.C. Arce⁹

¹*Copenhagen University Hospital, The Fertility Clinic, Copenhagen, Denmark,*

²*IVI-Sevilla, Reproductive Endocrinology, Sevilla, Spain,*

³*IVI-Valencia, Reproductive Endocrinology, Valencia, Spain,*

⁴*IVF CUBESE, Clinic for Assisted Reproduction, Prague, Czech Republic,*

⁵*Institut Universitari Dexeus, Department of Gynaecology and Obstetrics, Barcelona, Spain,*

⁶*IVI-Madrid, Reproductive Endocrinology, Madrid, Spain,*

⁷*UZ Gent, Department of Reproductive Medicine, Gent, Belgium,*

⁸*University Medical Centre, Department of Reproductive Medicine and Gynaecology, Utrecht, The Netherlands,*

⁹*Ferring*

Pharmaceuticals A/S, Global Clinical R&D (Reproductive Health), Copenhagen, Denmark

Abstract withdrawn by the author.

P-562 Different pharmacokinetic properties between a novel recombinant FSH (FE 999049) derived from a human cell line and follitropin alfa

R. Sandström, H. Olsson, and L. Grundemar
Ferring Pharmaceuticals A/S, Global Clinical R&D (Experimental Medicine), Copenhagen, Denmark

Study question: To compare the pharmacokinetics in terms of absorption (bioavailability), distribution and clearance between a novel recombinant FSH derived from a human cell line (FE 999049; Ferring Pharmaceuticals) and follitropin alfa (GONAL-F; Merck Serono).

Summary answer: The trial clearly demonstrates a difference in pharmacokinetics between FE 999049 and follitropin alfa. There is no difference in the extent of absorption (bioavailability) or in distribution between the two recombinant FSH preparations. However, the clearance is slower for FE 999049 than for follitropin alfa leading to an overall greater exposure of 50-60% for FE 999049 when administered intravenously or subcutaneously compared to follitropin alfa.

What is known already: There is no previous trial comparing the pharmacokinetics of two recombinant gonadotropins with both intravenous and subcutaneous administration.

Study design, size, duration: This was an open-label, cross-over, parallel group trial with 25 healthy female volunteers in each group. All subjects first received a single 225 IU intravenous infusion with 7 days of blood sampling followed by a single 450 IU subcutaneous dose with 10 days of blood sampling.

Participants/materials, setting, methods: The trial population was healthy female volunteers. Subjects were down-regulated with oral contraceptives for 14 days before the first dose of gonadotropins and throughout the trial. FE 999049 is a recombinant FSH produced from a host cell line of human fetal retinal origin while follitropin alfa is derived from a Chinese hamster ovary (CHO) cell line. All pharmacokinetic parameters were estimated by non-compartmental analysis. Samples for anti-FSH antibodies were collected before and after the trial.

Main results and the role of chance: The absolute bioavailability was similar for FE 999049 and follitropin alfa, approximately 60-65%. There was no difference in volume of distribution between FE 999049 and follitropin alfa. The clearance was significantly lower for FE 999049 compared to follitropin alfa, and consequently the half-life was longer for FE 999049. The drug exposure measured as area under the curve (AUC) was 50-60% higher for FE 999049. FE 999049 was safe and generally well tolerated as assessed by adverse events, vital signs, ECGs, and clinical laboratory tests. No treatment induced anti-FSH antibodies were detected.

Limitations, reason for caution: The pharmacokinetic findings should not be extrapolated for expected pharmacodynamic responses.

Wider implications of the findings: The results of this trial show that the novel recombinant FSH (FE 999049) derived from a human cell line requires a lower dose than follitropin alfa to achieve the same drug exposure.

Study funding/competing interest(s): This trial was sponsored by Ferring Pharmaceuticals A/S, Copenhagen, Denmark.

Trial registration number: Not applicable since this was a phase I trial.

P-563 The evaluation of AMH and AMHR2 genes polymorphisms in infertile women and the correlation with AMH, FSH and estradiol serum levels and assisted reproduction outcomes

P. Peluso¹, C.M. Trevisan¹, E.B. Cordts¹, V. Cavalcanti¹, D.M. Christofolini¹, F.A. Fonseca², C.P. Barbosa², and B. Bianco²

¹Faculdade de Medicina do ABC, Gynecology and Obstetrics, Santo Andre, Brazil, ²Faculdade de Medicina do ABC, Clinical Analysis, Santo Andre, Brazil

Study question: We hypothesized that the T146G and A-482G polymorphisms of AMH and AMHR2 gene are associated with serum estradiol, FSH and AMH; controlled ovarian hyperstimulation response and assisted reproduction outcomes

Summary answer: In the present study, the polymorphisms T146G and A-482G of the AMH and AMHR2 genes were not associated with the FSH, AMH and estradiol levels or with the assisted reproductive outcomes.

What is known already: Recent studies have demonstrated that serum AMH levels reflect the size of the primordial follicle pool. Studies in *Amh* null mice showed that, in the absence of AMH, follicles are recruited at a faster rate, and are more sensitive to FSH. So, AMH can be a marker of ovarian reserve and can predict the ovarian response.

Study design, size, duration: Cross sectional study comprising 136 infertile women (n = 35 with Idiopathic infertility, n = 39 with tubal obstruction, n = 62 women with male factor involved).

Participants/materials, setting, methods: All patients were ≤38 years old, had normal PRL and TSH serum levels, both ovaries without morphological abnormalities, BMI ≤ 30, no previous history of poor ovulatory response, no evidence of endocrine disorders or endometriosis. Detection of polymorphisms were performed using *TaqMan* methodology. Dosage of estradiol, FSH and AMH was performed by Elisa assay.

Main results and the role of chance: Statistical analysis revealed that T146G and A-482G polymorphisms were not statistically significant when compared to serum FSH (p = 0,387; p = 0,363), estradiol (p = 0,208; p = 0,599) and AMH (p = 0,946; p = 0,517), respectively. The correlation between the polymorphisms of the AMH and AMHR2 genes and the results of controlled ovarian hyperstimulation also did not show a significant difference (p = 0,165 e p = 0,644), thus the genotypes do not influence in the ovarian response. Even when we separated by infertility factor and compared with embryo parameters we did not find a significant difference. However if we compared the number of cycles performed with infertility factor, patients with idiopathic infertility perform fewer cycles than patients with male factor or tubal obstruction, p = 0,001.

Limitations, reason for caution: The number of patients is low.

Wider implications of the findings: It has been suggested that polymorphisms in AMH and AMHR2 genes may influence hormone function in folliculogenesis and cause the arrest of follicular growth and so, leads to decreased of ovarian reserve. In the present study, the polymorphisms T146G and A-482G of the AMH and AMHR2 genes were not associated with the FSH, AMH and estradiol levels or with the assisted reproductive outcomes.

Study funding/competing interest(s): This work was supported by grants from FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) # 2011/08681-1 and #2011/15045-4.

Trial registration number: Not applicable.

P-564 Premature luteinization in IVF or ICSI cycles using GnRH antagonists for pituitary downregulation

P. Bakas, N. Vlahos, D. Hassiakos, D. Tzanakaki, O. Gregoriou, A. Liapis, and G. Creasas

Aretaieio University Hospital, Obstetrics and Gynecology, Athens, Greece

Study question: What is the incidence of premature luteinization in IVF or ICSI cycles with GnRH antagonists and its effect on clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR).

Summary answer: Progesterone more than 1.2 ng/ml but mainly more than 1.5 ng/ml on triggering day adversely affects reproductive outcome.

What is known already: Limited evidence that progesterone rise on the day of triggering adversely affects the delivery rates in GnRH antagonists protocols.

Study design, size, duration: Prospective observational study including 108 patients. None lost to follow up. Duration of the study 2 years.

Participants/materials, setting, methods: 108 patients that underwent controlled ovarian stimulation for IVF or ICSI along with GnRH antagonist protocol in the IVF unit of the second Department of Obstetrics and Gynecology of University of Athens. Patients had estradiol, progesterone and LH measured on day 6-7, 9-10 and on the day of triggering. All patients had a multidose, fixed GnRH antagonist protocol starting on day 5.

Main results and the role of chance: The incidence of premature progesterone rise on the day of triggering was 26.8% for progesterone > 1.2ng/ml and 16.6% for progesterone > 1.5ng/ml. The overall clinical pregnancy rate (CPR) was 31.5%, the abortion rate was 8.3% and the ongoing pregnancy rate was 23.1%. The CPR for progesterone < 1.2 ng/dl was 37.7%(26/69) and for progesterone < 1.5 ng/ml was 34.1% (28/82), while the CPR for progesterone > 1.2ng/

ml was 19.3%(6/31) and for progesterone > 1.5 ng/ml was 20% (2/10). The OPR for progesterone < 1.2 ng/dl was 31.8%(22/69) and for progesterone < 1.5 ng/ml was 30.4 (25/82) %. The OPR for progesterone > 1.2ng/ml was 16.1% (5/31) and for progesterone > 1.5 ng/ml was 0% (0/10) .

Limitations, reason for caution: The limited number of patients with progesterone rise > 1.5ng/ml.

Wider implications of the findings: Values of progesterone > 1.5ng/ml on the day of ovulation induction could raise the option a) of embryo freezing and b) seeking of alternative ways of down regulation in future IVF attempts in order to improve reproductive outcome.

Study funding/competing interest(s): none

Trial registration number: M94/22-12-2011.

P-565 Impact of FMR1 mutation on follicle responsiveness to exogenous FSH as measured by the Follicle Output RaTe (FORT)

E. Adda-Herzog¹, J. Steffann², S. Sebag-Peyrelevede¹, M. Poulain³, A. Benachi¹, and R. Fanchin¹

¹Centre Hosp. Antoine Bécélère, Dept. Ob Gyn & Reprod. Medicine, Clamart Cedex, France, ²Centre Hosp. Necker-Enfants Malades, Genetics, Paris, France, ³Centre Hosp. Antoine Bécélère, Dept. Reprod. Biology, Clamart Cedex, France

Study question: Carriers of FMR1 premutation, but not full-mutation, frequently suffer from premature ovarian insufficiency. As they often candidate to PGD for preventing Fragile-X syndrome transmission, these patients ought to respond adequately to controlled ovarian hyperstimulation (COH). We questioned whether follicle responsiveness to exogenous FSH is compromised in these patients.

Summary answer: Carriers of FMR1 premutation or full-mutation, devoid of profound premature ovarian insufficiency, display similar responsiveness to COH than controls with comparable age and antral follicle endowment.

What is known already: Whereas up to 20% of carriers of FMR1 premutation become menopausal prematurely, the remaining cycling patients may show different degrees of ovarian defects including poor response to exogenous FSH. Unfortunately, published studies indicating that non-menopausal, FMR1 premutation carriers respond weakly to COH were inadequately designed to assess their actual responsiveness to exogenous FSH. Yet, a novel index, Follicle Output RaTe (FORT), constitutes an interesting clinical approach to assess antral follicle responsiveness to exogenous FSH.

Study design, size, duration: We compared 9 FMR1 premutation carriers (20 COH cycles) and 8 FMR1 full-mutation carriers (13 COH cycles) with 2 different controls of same age and antral follicle count (38 and 27 COH cycles, respectively). All patients received the same COH protocol.

Participants/materials, setting, methods: FORT was calculated by preovulatory follicle (16-22 mm) count on day of hCG x 100/small antral follicle (3-8 mm) count at baseline (FORT-dhCG). In cancelled stimulations, FORT was determined by growing follicle (12-16 mm) count on day 8 x 100/ small antral follicle (3-8 mm) count at baseline (FORT-d8).

Main results and the role of chance: We did not observe any significant difference in antral follicle responsiveness to exogenous FSH in patients carrying or not FMR1 abnormalities. Indeed, FORT-hCG values between FMR1 premutation carriers (47.8% versus 46.9%, respectively) and FMR1 full-mutation carriers (39.0% versus 39.4%, respectively) and respective controls were comparable. Further, including cancelled patients, FORT-d8 values between FMR1 premutation carriers and controls (39.4% versus 41.1%, respectively) were also similar. FORT-d8 values are not available to FMR1 full-mutation carriers because none of these patients had their COH cancelled.

Limitations, reason for caution: Larger studies are needed to confirm the present results.

Wider implications of the findings: The present data indicate that antral follicle responsiveness to exogenous FSH in regularly-cycling patients carrying either FMR1 premutation or FMR1 full-mutation is not compromised. Since, in previous studies, FORT values have been positively related to oocyte-embryo competence, the present results are reassuring as these patients usually candidate to PGD and require COH.

Study funding/competing interest(s): None

Trial registration number: None

P-566 Does delayed endometrial shedding post OC pretreatment increase pregnancy rates in women undergoing COS with rFSH in a GnRH antagonist regimen

K. Gordon¹, D. Zhang², and A. Nyboe Andersen³

¹Merck & Co. Whitehouse Station, Global Medical Affairs Women's Health and Endocrine, NJ, U.S.A., ²Merck & Co. Whitehouse Station, Global Health Outcomes, NJ, U.S.A., ³Copenhagen University Hospital Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark

Study question: To evaluate whether delayed onset of menstrual bleeding post oral contraception (OC) pretreatment and, thus, delayed start of gonadotropin therapy increases conception rates in women undergoing controlled ovarian stimulation (COS) with recombinant follicle-stimulating hormone (rFSH) in a gonadotropin-releasing hormone (GnRH) antagonist regimen.

Summary answer: Timing of withdrawal bleed onset post OC pretreatment does not impact the pregnancy rate following fresh embryo transfer.

What is known already: OC pretreatment appears to have a detrimental effect on fresh transfer pregnancy rates in women undergoing COS with rFSH in a GnRH antagonist cycle (Nyboe Andersen et al., 2011). One possible mechanism could be inadequate length of time to clear the progestogen of the OC in order to develop a receptive endometrium.

Study design, size, duration: In this retrospective analysis of the prospective Xpect trial (Nyboe Andersen et al., 2011), pregnancy rates were evaluated according to when day 1 of menses occurred relative to cessation of OC treatment in a cohort of ovulatory women who were pretreated with OCs.

Participants/materials, setting, methods: Patients received OCs for 14-21 days and daily 200 IU rFSH starting 5 days after stopping OC (stimulation day 1) if withdrawal bleeding had occurred. With no bleeding, treatment was delayed until first day of full bleeding. 0.25 mg ganirelix was started on day 5, continuing until final oocyte maturation.

Main results and the role of chance: Day of withdrawal bleed was known for 212 of 223 patients who received OCs. Withdrawal bleed for these patients occurred with the following distribution: before day 3 (n = 13), 3 days (n = 55), 4 days (n = 98), 5 days (n = 24), and after day 6 (n = 22) after stopping OC. Pregnancy rates for the groups were 30.8%, 38.2%, 31.6%, 25%, and 36.4%, respectively. For every 1-day delay in withdrawal bleed, the odds of pregnancy is likely to decrease by 6% (odds ratio, 0.940; 95% confidence interval, 0.737-1.198; P = 0.617). This was not statistically significant.

Limitations, reason for caution: This was a retrospective analysis of data from a prospective trial. Conclusions drawn from the analyses must be viewed with caution because all of the comparisons were post hoc and should be confirmed by prospective studies in which the starting time of gonadotropin stimulation should be varied.

Wider implications of the findings: Timing of withdrawal bleed onset post OC pretreatment does not impact the pregnancy rate following fresh embryo transfer.

Study funding/competing interest(s): Financial support for this study was provided by Merck, Sharp & Dohme Corp., a subsidiary of Merck & Co. Inc., Whitehouse Station, NJ, USA.

Trial registration number: NCT00778999

P-567 Decreased estradiol level after hcg administration can be used as a predictor for poor ivf outcome

F. Aybar¹, S. Temel², and S. Kahraman¹

¹Memorial Sisli Hospital, IVF Department, Istanbul, Turkey, ²Uludag University, Embryology Department, Bursa, Turkey

Study question: What is the value of decreased estradiol levels after hCG administration in predicting IVF outcome?

Summary answer: Estradiol drop after hCG administration is associated with poor IVF outcomes in normoresponder patients.

What is known already: Many factors may influence the success of IVF treatment. Among these, the value of serum estradiol level after hCG administration is still unclear. Several mechanisms; like premature luteinization, poor oocyte quality, endometrial receptivity has been postulated to explain why an estradiol drop after hCG may effect IVF outcomes. There are contraversal studies in the literature. Our aim is to evaluate the value of serum estradiol level after hCG administration in predicting IVF outcome.

Study design, size, duration: We performed a retrospective analysis of 2812 women aged 18-47 years undergoing IVF-ICSI cycles from August 2011 to January 2013 at Memorial Sisli Hospital, Istanbul, Turkey. Patients were subdivided as poor and normo responders according to the Bologna criteria.

Participants/materials, setting, methods: We compared the implantation, biochemical pregnancy and miscarriage rates in the cycles demonstrating rise or drop in serum estradiol levels before and after hCG in the total group, normo and poor responders. Age and BMI was similar. Total gonadotrophin dose was higher in the estradiol drop patients.

Main results and the role of chance: Of the total 2812 cycles; there was estradiol rise in 2450 and drop in 362. When subgrouped; 2478 patients were normoreponders, 2197 with rising levels and 281 drop. Among the 334 bad responders, 253 demonstrated a rise and a drop in 81. In the total group; pregnancy and implantation rates were significantly higher in estradiol rise group (53.2% vs 43.4%, $p = 0.016$ and 37.8% vs 28.3%, $p = 0.001$), abortion rates show no difference (20.9% vs 25.2%, $p = 0.300$). In normoreponders, pregnancy and implantation rates were higher (54.9% vs 44%, $p = 0.002$ and 39.6% vs 28.4%, $p < 0.001$) and abortion rates were lower significantly (20% vs 27.1%, $p < 0.001$) in estradiol rise group. No difference in pregnancy, implantation and abortion rates were observed in bad responders (36.7% vs 40.6%, $p = 0.694$ and 20.7% vs 27.9%, $p = 0.288$ and 30.7% vs 16.6%, $p = 0.2747$)

Limitations, reason for caution: In bad responder patients we found no statistically significant difference, this may be because of confounding factors that may effect IVF outcome in these patients.

Wider implications of the findings: Our data indicated that post-HCG estradiol drop in sera is a valuable marker in the normoresponder group, to predict poor IVF outcome rather than that in the bad responders. By this way clinicians can consult their patients about their IVF-ICSI outcomes prior to oocyte pick up.

Study funding/competing interest(s): No funding was used.

Trial registration number: Not applicable

P-568 Simultaneous initiation of GnRH antagonist co-treatment and recFSH for ovarian hyperstimulation in IVF does not affect clinical outcome: a randomized controlled trial

O. Hamdine¹, N.S. Macklon¹, M.J.C. Eijkemans², J.S. Laven³, B.J. Cohlen⁴, A. Verhoeff⁵, P.A. van Dop⁶, R.E. Bernardus¹³, C.B. Lambalk⁷, G.J.E. Oosterhuis⁸, C.A.G. Holleboom⁹, G.C. van den Dool-Maasland¹⁰, H.J. Verburg¹¹, P.F.M. van der Heijden¹², A. Blankhart¹⁴, B.C.J.M. Fauser¹, and F.J. Broekmans¹

¹University Medical Center Utrecht, Gynecology & Reproductive Medicine, Utrecht, The Netherlands, ²University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, The Netherlands, ³Erasmus University Medical Center, Reproductive Medicine, Rotterdam, The Netherlands, ⁴Isala clinics, Gynecology & Fertility, Zwolle, The Netherlands, ⁵Maasstad Hospital, Gynecology & Fertility, Rotterdam, The Netherlands, ⁶Catharina Hospital, Gynecology & Fertility, Eindhoven, The Netherlands, ⁷VU Medical Center, Reproductive Medicine, Amsterdam, The Netherlands, ⁸Medisch Spectrum Twente Hospital Group, Gynecology & fertility, Enschede, The Netherlands, ⁹Bronovo Hospital, Gynecology & Fertility, The Hague, The Netherlands, ¹⁰Albert Schweitzer Hospital, Gynecology & Fertility, Zwijndrecht, The Netherlands, ¹¹Leiden University Medical Center, Gynecology & Fertility, Leiden, The Netherlands, ¹²ZGT Almelo, Gynecology & Fertility, Almelo, The Netherlands, ¹³Tergooi Hospital, Gynecology & Fertility, Blaricum, The Netherlands, ¹⁴St. Antonius Hospital, Gynecology & Fertility, Nieuwegein, The Netherlands

Study question: What is the impact of initiating GnRH antagonist co-treatment for *in vitro* fertilization (IVF) on cycle day (CD) 2 on live birth rate per started cycle (LBR) and on the cumulative live birth rate (CLBR) compared to initiation on CD 6?

Summary answer: Early initiation of GnRH antagonist does not appear to improve clinical outcome of IVF compared with midfollicular initiation. The currently used GnRH antagonist co-treatment protocol starting on CD 6 therefore remains the optimal choice. Further studies may still be needed to improve the overall efficacy of the GnRH antagonist treatment approach.

What is known already: During ovarian stimulation for IVF, a GnRH antagonist is usually administered from the midfollicular phase onwards. However, the

optimal protocol for routine clinical use may not yet have been identified. Earlier initiation may improve the follicular phase hormonal milieu and therefore overall clinical outcome.

Study design, size, duration: The study was an open-label multicentre randomized controlled trial, conducted between September 2009 and July 2011. A web based program was used for randomization. A total of 617 IVF/intracytoplasmic sperm injection patients were included.

Participants/materials, setting, methods: Recombinant FSH (150-225 IU) was administered daily from CD 2 onwards in both groups. The study group (CD2; n = 308) started GnRH antagonist co-treatment on CD 2, whereas the control group (CD6; n = 309) started on CD 6.

Main results and the role of chance: There were no significant differences in clinical outcome between the two groups. A non-significant trend towards a higher live birth rate per started cycle and cumulative live birth rate was observed in the CD6 group compared with the CD2 group (LBR: 24.0% vs. 21.5%, $p = 0.5$; CLBR: 29.9% vs. 26.7%, $p = 0.6$).

Limitations, reason for caution: The study was terminated prematurely because no significant difference was observed in clinical outcome after 617 inclusions. A much larger population would be needed to detect a small significant difference in favour of either study arm, which raises the question whether this would be relevant for clinical practice.

Wider implications of the findings: The clinical importance of the observed non-significant difference is probably negligible. Additionally, increasing the number of daily injections by four may increase patient discomfort as well as treatment costs and imposes the risk losing some of the benefits of a GnRH antagonist protocol. The present study findings show clearly that this additional treatment burden and cost is not justified as early initiation of GnRH antagonist does not improve live birth rates.

Study funding/competing interest(s): This study was partially supported by a grant from Merck Serono.

Trial registration number: www.clinicaltrials.gov, no. NCT00866034

P-569 A comparative study of antral follicle count with FSH and AMH in an IVF programme

J. Bhattacharya¹, A. Mitra², G.B. Dutta³, A. Kundu⁴, M. Bhattacharya⁵, and S. Kundu³

¹AHIVF & Infertility Research Centre, Infertility & IVF, Kolkata, India, ²AHIVF & Infertility Research Centre, Embryology, Kolkata, India, ³AHIVF & Infertility Research Centre, IVF Specialist, Kolkata, India, ⁴AHIVF & Infertility Research Centre, Reproductive Medicine, Kolkata, India, ⁵AHIVF & Infertility Research Centre, Ultrasonography, Kolkata, India

Study question: Which one is the best predictor for ovarian response in an invitro fertilization (IVF) programme, number of antral follicle prior to stimulation or reproductive hormones like follicle stimulating hormone (FSH) and antimullerian hormone (AMH) levels?

Summary answer: Antral follicle count prior to stimulation is the best predictor compared to endocrine parameters like basal D2/3 FSH and AMH level to assess the ovarian response in an IVF programme.

What is known already: Follicular development plays a major role in the successful outcome of IVF. Prediction of ovarian response prior to stimulation is useful in tailoring the dosage of gonadotropin. Basal FSH and AMH reflect the ovarian reserve. Ultrasound assessment of antral follicle count helps to predict number of follicular maturation.

Study design, size, duration: A prospective study was performed with hundred and six infertile patients (age 30.2 ± 7.5 years) attending the clinic at IVF & Infertility Research Centre from Jan'2011- Nov' 2012 with regular menstrual cycle. Patients with polycystic ovarian syndrome and abnormality in the other endocrine parameters were excluded from the study.

Participants/materials, setting, methods: Basal FSH (2-10mIU/ml) and AMH (2-6 ng/ml) were assessed on D2/3 for every individual. Trans vaginal ultrasound was done for all the patients and number of antral follicle were counted. Both endocrine profile and number of antral follicle were noted and correlated with the outcome of oocyte retrieval.

Main results and the role of chance: Regression analysis revealed that the antral follicle number showed the highest correlation with number of mature follicles with mature oocytes ($r = \pm 0.68$, $P = 0.001$). Other variables, FSH and AMH,

were moderately correlated with number of mature follicles and quality of oocytes. Responses of gonadotropin agonist were moderately correlated with endocrine profile, but highly correlated with the number of antral follicles (>3-4 follicles in each side). The total number of antral follicle achieved the best predictive value followed by basal FSH and AMH in women undergone IVF treatment.

Limitations, reason for caution: Age, occupational hazards, environmental effect, empty follicular syndrome, polycystic ovarian syndrome and also the dosage of recombinant FSH can affect the ovarian response.

Wider implications of the findings: The study concluded that the number of antral follicles (>3-4 antral follicles on each side of the ovary) has the closest association with chronological maturation of follicles in women. The size and number of the antral follicle can be considered one of the best single test to predict the ovarian response and quality of oocytes retrieved than the endocrine parameters among women undergoing invitro fertilization (IVF) treatment.

Study funding/competing interest(s): Study was funded by A.H IVF and Infertility Research Centre and we acknowledged Dept. of statistics, University of Calcutta for the completion of this work.

Trial registration number: It's a prospective study so no trial registration number has been obtained.

P-570 Comparative analysis of two widely-used immunoassays for the measurement of serum AMH in women

P. Pigny¹, A. Dassonneville¹, S. Catteau-Jonard², C. Decanter², and D. Dewailly²
¹CHRU Lille, Laboratoire d'Hormonologie, Lille, France, ²CHRU Lille, Reproductive Endocrinology, Lille, France

Study question: The aim of this work was to compare the 2 main immunoassays currently used in Europe (so-called Gen II and Immunotech) for the assay of serum Anti-Mullerian Hormone (AMH) in normal women and in patients with Polycystic Ovary Syndrome (PCOS).

Summary answer: In both groups, AMH levels assessed with Gen II were about 2-fold lower than those obtained with the Immunotech assay.

What is known already: Serum AMH measurement is now frequently used to assess the ovarian follicular status in women, either to identify patients with low ovarian reserve or to detect follicle excess such as in patients with Polycystic Ovaries, either alone or as part of a PCOS. This information is particularly relevant in the setting of Assisted Reproduction Techniques to minimise both the risks of hypo- or hyper-response to controlled ovarian stimulation. However, there is no consensus on predictive serum AMH thresholds, mainly because of discrepancy between assay techniques.

Study design, size, duration: Each woman had paired determination of serum AMH level using both techniques. Samples were distributed in two different series run using the same lot of reagents.

Participants/materials, setting, methods: Sera of 59 women (32 controls and 27 patients with PCOS according to the Rotterdam criteria) kept at -80°C were selected from our biological collection. Serum AMH was measured the same day on the same sample (undiluted) by the same operator by 2 methods: AMH Gen II Assay (A79765, Beckman Coulter) and AMH MIS (A11893, Immunotech, Beckman Coulter) according to the manufacturer instructions.

Main results and the role of chance: In the total population, values obtained with each assay were highly correlated ($r = 0.936$, $p < 0.0001$, Spearman test). In both groups, AMH levels assessed with Gen II were about 2-fold lower than those obtained with the MIS assay (controls: 11.9 ± 6.1 vs 24.1 ± 10.9 pmol/l; patients: 26.7 ± 11.7 vs 47.9 ± 17.9 pmol/l, respectively, $p < 0.0001$ for both, Wilcoxon test). An AMH ratio was calculated by dividing the Gen II value by the MIS value. There was a significant trend towards a higher ratio in PCOS patients than in controls (0.55 ± 0.09 vs 0.49 ± 0.1 , respectively, $p = 0.02$, Student's t test).

Limitations, reason for caution: We did not compare the 2 assays in patients with low ovarian reserve. It has thus to be shown whether the ratio between values given by each assay is constant across the whole range of AMH values encountered in clinical practice.

Wider implications of the findings: At variance with previous data, our preliminary results suggest that the Gen II assay provides AMH results that significantly differ from those obtained with the MIS assay. We thus need (i) to determine the threshold levels for low ovarian reserve and follicle excess with Gen II as we

did with MIS; (ii) to compare the clinical performance of the 2 assays using these specific thresholds.

Study funding/competing interest(s): The authors do not have any

P-571 Acceptability and utility of the CONSORT algorithm for calculating recombinant human follicle-stimulating hormone starting doses for ovarian stimulation in assisted reproductive technology: an observational study

J. Pouly¹, F. Olivennes², N. Massin³, M. Celle⁴, and N. Caizergues⁵
¹CHU Estaing, Unité de FIV, Clermont Ferrand, France, ²Clinique de la Muette, Reproductive Health, Paris, France, ³Université Paris 12, Unité d'Assistance Médicale à la Procréation, Créteil, France, ⁴SOLADIS, Department of Fertility, Lyon, France, ⁵Merck Serono S.A.S, Reproductive Health, Lyon, France

Study question: To determine the acceptability and utility in routine clinical practice of the CONSORT (CONsistency in r-FSH Starting dOses for individualized tReatmenT) calculator for choosing starting doses of recombinant human follicle-stimulating hormone (r-hFSH) for controlled ovarian stimulation (COS) for assisted reproductive technology (ART) in France.

Summary answer: An acceptability rate significantly >75% was observed (97.8%), with physicians finding the calculator friendly and easy to use; utility was limited, with CONSORT recommendations followed for 45.2% of patients; physician-planned r-hFSH starting doses were generally higher than the CONSORT recommendation and most prescribed doses were higher than selected by CONSORT.

What is known already: The CONSORT calculator was developed to help clinicians determine the optimal r-hFSH starting dose in women aged 18–34 years who were normal responders to COS in a long gonadotrophin-releasing hormone (GnRH) agonist protocol. It is based on four predictive factors: baseline FSH, body mass index, age and antral follicle count. In a pilot interventional study, individualized treatment through use of CONSORT-calculated starting doses of r-hFSH resulted in good rates of oocyte retrieval and pregnancy.

Study design, size, duration: Non-interventional, prospective study involving 45 physicians in France; each physician had to recruit 1–5 patients undergoing COS for a first ART cycle with r-hFSH and a GnRH agonist protocol. Patients ($n = 197$) were followed between February 2010 and April 2011, until 12 weeks after embryo/blastocyst transfer, according to standard practice.

Participants/materials, setting, methods: Physicians registered their planned r-hFSH starting dose for each patient, and then used the CONSORT calculator to compute a starting dose (112.5–450 IU). Acceptability was defined as reporting the CONSORT website friendly and easy to use. Utility was defined as adoption of the CONSORT-calculated dose for the prescribed dose.

Main results and the role of chance: 44/45 physicians found the CONSORT calculator moderately friendly/very friendly and easy/very easy to use for $\geq 75\%$ of their patients; the acceptability rate (95% confidence interval), 97.8% (88.2–99.9), was significantly greater than the acceptability threshold of 75% ($p = 0.0002$). Physicians stated they would follow CONSORT recommendations for 89/197 patients (45.2%). The most common reasons for not following the CONSORT recommendation were that the CONSORT dose was too divergent from the planned dose (48.1%) and that the CONSORT dose did not correspond to the patient profile (46.3%). Mean (standard deviation) starting doses of r-hFSH were: physician-planned, 163.9 (51.2) IU; CONSORT-calculated, 119.7 (20.9) IU; prescribed dose, 151.7 (51.1) IU. Pairwise comparisons of mean r-hFSH doses were significantly different between the three dose-selection methods ($p < 0.0001$ for all comparisons).

Limitations, reason for caution: As physicians had volunteered to take part in this observational study, the physician population may not be representative of all ART physicians in France. Study selection criteria mean that this patient population comprises a high proportion of women with good prognoses for COS under a long GnRH agonist protocol.

Wider implications of the findings: To our knowledge, this is the first study of how comfortable physicians are using dosing algorithms in ART and how they use the information provided. These algorithms lead to greater individualization of treatment, and this study suggests that they show potential for use in routine clinical practice. However, their utility may be limited by a current lack of trust by treating physicians. Large comparative randomized controlled studies are required.

Study funding/competing interest(s): Financial support and editorial assistance for abstract preparation were provided by Merck Serono S.A.S., an affiliate of Merck KGaA, Darmstadt, Germany.

Trial registration number: NA

P-572 Values of anti-Müllerian hormone within individual women show high consistency relative to age related populations over time, and can inform fertility planning advice

R. Fleming¹, M. Gaudoin¹, M. Messow², A. McConnachie², and S.M. Nelson²
¹Glasgow Centre For Reproductive Medicine, Reproduction, Glasgow, United Kingdom, ²University of Glasgow, School of Medicine, Glasgow, United Kingdom

Study question: One of the primary questions regarding the decline in anti-Müllerian hormone (AMH) with age is ‘how closely do individuals follow the decay curve of the population?’ The answer to this question is complicated by limited sample numbers and by evolution of the assay used to measure AMH.

Summary answer: Circulating AMH in individual women is stable over protracted time-scales when compared with their age-related normal population values.

What is known already: AMH is a reliable marker of ovarian reserve in women undergoing ovarian stimulation and low values at an early age may predict an early menopause. AMH has been shown to be consistent within a single menstrual cycle and over relatively short periods of time, but the relative consistency for individuals over larger passages of time has not been established

Study design, size, duration: The database at a single clinic (GCRM) was retrospectively explored to examine changes of AMH within individuals over periods greater than 6 months. The data were collected from 2007 - 2012.

Participants/materials, setting, methods: Women seeking fertility treatment advice (n = 189) provided an AMH sample (S1) and returned for subsequent analyses at S2 with time intervals of 6 months or greater. Within the time frame, two assays have been deployed to measure AMH (supplied by Beckman Coulter, UK), and their comparator normal ranges have been described (Nelson et al 2011, and Nelson et al, 2013). Quality control pool samples of both assays have shown reliable performance throughout their deployment.

We compared each sample with its age related and validated median value for the specific assay used. The values of S1 and S2 were then evaluated by relation with age and assay related median values.

Main results and the role of chance: The time differences between S1 and S2 for the 189 women were: mean = 28.9 months, range 6 to 63 months. The mean age at S1 was 35.0y and at S2 was 37.5y. Overall, the women tended to show higher than median AMH values in S1, showing values 51% higher than the ‘normal’ population median. The subsequent AMH value (S2) also showed higher values than the age related median: on average, with 45% higher than their age-related median value (the paired t test comparison [Wilcoxon] showed no change in relative values, p = 0.55). The correlation between the S1 and S2 median-related values was strong (r = 0.72; p < 0.0001). Of the 73 women showing AMH values below the median in sample 1, 84% showed a value below the median in sample 2. Likewise, 75% of those showing a value greater than the median in S1, showed a value higher than the median in S 2.

If the AMH median related value were a matter of chance, we would expect a more equal (50%) distribution of values.

Limitations, reason for caution: Although the coefficient of variation of the assay is very narrow (8%), ideally, the two samples (S1 and S2) would have been assayed simultaneously, and decay evaluation in absolute values would have provided more specific information.

Wider implications of the findings: These findings strengthen the value of AMH as a reliable biomarker of both current and future ovarian reserve. This supports the use of AMH for providing fertility planning advice.

Study funding/competing interest(s): Circulating AMH in individual women is stable over protracted time-scales when compared with their age-related normal values, and a single value is probably indicative of future trends within individuals.

The authors have no competing interests.

Trial registration number: There is none

P-573 Pulsatile GnRH administration in women with functional hypothalamic anovulation in presence of polycystic ovary-like anomalies

D. Dewailly, L. Vanhove, M. Peigné, P. Thomas, G. Robin, and S. Catteau-Jonard

Hôpital Jeanne de Flandre, Gynecologie Endocrinologie et Medecine de la Reproduction, Lille, France

Study question: The objective of this work was to analyze retrospectively whether physiological stimulation by pulsatile GnRH administration is appropriate in women with functional hypothalamic anovulation (FHA) in presence of polycystic ovary-like anomalies (PCO-L).

Summary answer: The ovarian response in patients with FHA and PCO-L was identical to that of patients with FHA without PCO-L. Ovulation rate was slightly lower but multifollicular response and pregnancy rates were identical between the two groups.

What is known already: We recently demonstrated the presence of PCO-L (i.e., polycystic ovary morphology [PCOM] at ultrasonography and/or elevated serum Anti-Müllerian Hormone [AMH] level) in about 40% of women with FHA (Robin G *et al.*, J Clin Endocrinol Metab. 2012;97:4236-43). This does not necessarily translate the existence of a PCO Syndrome (PCOS) that would be masked by the low serum LH level characteristic of FHA. However, previous studies in such patients have reported the possibility of PCOS-like response during unphysiological stimulation by exogenous gonadotrophins. In addition, whether the presence of PCO-L *per se* is deleterious for fecundity is poorly documented.

Study design, size, duration: This was a retrospective study in an academic center using data collected between 2002 and 2012. Forty-five patients with FHA were included.

Participants/materials, setting, methods: Inclusion criteria were: secondary amenorrhea for more than six months, low to normal basal serum LH and FSH levels, history of significant weight loss and/or intensive exercise. Other causes of gonadotropin deficiency were carefully excluded. PCO-L was defined by the presence of PCOM and/or high serum AMH level (>35 pmol/l or 5 ng/ml) (Dewailly D *et al.*, Hum Reprod. 2011;26:3123-9). Pulsatile GnRH administration was performed intra-venously in 53% patients at the initial dose of 5 µg/pulse and subcutaneously in 47% patients with the initial dose of 15 µg/pulse every 90 minutes. The ovarian response was monitored by estradiol assay and ultrasound. No patient received hCG injection to trigger ovulation.

Main results and the role of chance: Patients were divided into 2 groups according to the presence or absence of PCO-L (n = 21 and n = 24, respectively). At baseline, age and BMI were similar in both groups, as well as serum levels of androgens and LH. In contrast, the FHA-PCO-L group differed from the other group by significantly lower serum FSH levels and higher serum AMH levels and follicle counts. Administration route and doses of GnRH pulses were identical in 2 groups. The ovulation rate was higher in the FHA than in the FHA-PCO-L group (93% vs 73%, p = 0.02). However, the pregnancy rate (PR)/cycle was similar between the 2 groups (45% vs 39%, respectively) as well as the cumulated PR (75% vs 62%, respectively). Few moderate multifollicular responses were observed, with identical rate in the 2 groups (15% and 19%, respectively). 2 twin pregnancies were observed in the FHA group and none in the FHA-PCO-L group.

Limitations, reason for caution: The retrospective design and the variability of PCOM definition according to the time of inclusion limit results of this study as well as the relatively small number of patients.

Wider implications of the findings: These results suggest that the presence of PCO-L does not expose to a greater risk of ovarian hyperstimulation during physiological stimulation by pulsatile GnRH administration. This complements our previous data indicating that the presence of PCO-L in women with FHA has the same meaning as in the general population of ovulatory women without hyperandrogenism. Pulsatile GnRH administration remains the first-line treatment in patients with FHA, whether or not they have PCO-L.

Study funding/competing interest(s): The authors do not have any

Reproductive epidemiology and health economy

P-574 Sociodemographic factors and birth following assisted reproductive technology (ART) treatment in Australia, 2007-2009, a population study