P-308 Socio-ethical considerations in the recruitment of volunteers when acquiring eggs for stem cell research

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Introduction: This paper presents some initial findings from our socio-ethical evaluation of a unique scheme that provides human eggs for somatic cell nuclear transfer (SCNT) research. SCNT research has provoked controversy and criticism since its inception, a discourse compounded by the Hwang scandal and given further impetus by the development of pluripotent stem cells. During this phase a research group in Newcastle, UK, has continued work on SCNT. In late 2007 they obtained licensing and funding for a scheme they hoped would reduce the perceived bottle-neck in their research, the shortage of human eggs. The Newcastle ‘egg sharing for research’ scheme mirrors the established practice of egg sharing for treatment in offering women a half price IVF cycle in exchange for half the eggs in that cycle.

Methods: Our qualitative study uses both documentary data and semi-structured interviews with women who have been accepted onto the egg sharing scheme, some of whom went on to provide eggs for research, others of whom did not. We have also interviewed women who volunteered but were not accepted onto the scheme, as well as women who have provided eggs for the treatment of others. Finally, interviews with fertility centre staff will give us insight into the views of those professionally involved in the scheme.

We aim to identify the major themes arising from interviews and documents through the hermeneutic analysis of transcripts and texts, using constant comparison and category building procedures. This will be followed by category mapping and deviant case analysis.

Results: We have completed an analysis of the unusual strategy the researchers adopted in order to recruit women for the scheme. The clinic hoped to avoid legitimate ethical concerns about directly recruiting women to provide eggs, by adopting a ‘passive recruitment’ strategy. That is, the existence of the scheme was drawn to the attention of potential volunteers through media coverage (newspapers and radio / television programmes) in the hope that women would come forward to volunteer for the scheme. Our analysis had a dual focus; the way in which the media reported the scheme and the way in which the media were used to recruit volunteers to provide eggs.

Our analysis focused on a series of press releases issued over nine months which formed the basis of many stories in the UK media. This exposure kept the scheme in the public domain over the first year of its operation. In general the stories were framed in a similar way to the press releases, although most also included some critical voices. The stories initially focused on how the scheme could offer a reduction in the cost of IVF treatment and later reported positive aspects of the scheme, such as the number of women coming forward and the number of successful pregnancies. However, a clear omission from the reporting (and the criticism) was the views of women seeking IVF treatment.

Conclusions: It is premature to discuss any findings from the interview analysis as this is not yet complete. However, analysis of the media reporting suggests that the researchers chose a successful strategy to bring the scheme to the attention of the public they wished to recruit from.

In contrast to the findings of previous research, this particular human cloning story was not framed only in terms of controversy but was generally reported positively. This was achieved by focussing on the women volunteers and minimising attention on the research. Further work on our analysis will help to understand whether the ethical concerns about direct recruitment of providers of reproductive tissue to stem cell research were adequately addressed by adopting this ‘passive recruitment’ strategy.

P-309 A descriptive study of oocyte, blood, and organ/tissue donation features among fertility patients in Ireland

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Introduction: A landmark December 2009 Supreme Court decision addressing the advanced reproductive technologies in Ireland has focused needed attention on the need for legislation to regulate fertility services in Ireland. To inform this public policy challenge, the current investigation examined features of anonymous oocyte donation and participation in organ and blood/tissue donation programmes among fertility patients in Ireland.

Material and Methods: Demographic and clinical information was prospectively collected from 337 female infertility patients by anonymous questionnaire to determine their status as a registered organ/tissue donor. Data from patients who themselves had been blood donors (or who had ever received an anonymous blood transfusion) were recorded; patient perceptions about anonymous donor oocyte compensation were also tabulated.

Results: At study entry, 56.7% of patients had no children, and none had participated in a donor oocyte programme either as donor or recipient. Before initial consult, 19.6% had previous in vitro fertilisation (IVF) experience (range of prior cycle number = 1-12). In our sample, more than one third (35.9%) of patients had donated blood anonymously, 19.9% were organ/tissue donors, and 52.2% indicated that anonymous oocyte donors should receive some compensation. Average recommended compensation for anonymous oocyte donors was €2177 (range €200-€9500), with most patients (77.2%) supporting confidential protections for recipient and donor identity.

Conclusions: This is the first study to assess blood and organ/tissue donation features among fertility patients in Ireland, and frame such findings in the context of anonymous oocyte donation. We found the rate of blood donation among fertility patients to be more than ten times higher than the rate measured in the general Irish population. Prior blood donor experience was more common than self-designated status as an organ/tissue donor, but neither donation type correlated with age, education or other characteristics. Protection of anonymity for both donors and recipients was favoured by most patients, even those antagonistic to compensated anonymous donation. These observations, if corroborated by future studies in Ireland, will be crucial as regulations on anonymous donor gamete treatments are crafted here. Further studies should clarify patient perceptions about oocyte donation as a function of their involvement in organ/tissue procurement programmes and blood banks.

P-310 Serum anti-müllerian hormone and inhibin B levels as predictive markers of ovarian hyperstimulation syndrome (OHSS) in IVF patients

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Introduction: Ovarian Hyperstimulation Syndrome (OHSS) is a severe health complication observed in some patients undergoing controlled ovarian stimulation (COS) during IVF.

However prediction of OHSS prior to controlled ovarian stimulation in an individual IVF treatment cycle using only age and body mass index(BMI) remains a difficult task. Monitoring the serum E2 level and number of follicles have been effective in reducing the incidence of OHSS but these are determined during IVF.

The aim of this study was to find whether serum concentration of anti-müllerian (AMH) and inhibin B in patients undergoing IVF treatment may serve as a predictor of OHSS.

Material and Methods: A cohort of 106 women undertaking the IVF program was investigated prospectively, to evaluate the predictive value for OHSS by means of certain risk factors, including age, body mass index (BMI), day 3 serum E2 FSH, LH, AMH, inhibin B and antral follicles count (AFC) at Istanbul University Cerrahpasa Medical Faculty, IVF unit. Patients were included in the study if they presented moderate or severe OHSS criteria described by Navot.

Women participating in this study followed a long or short GnRH agonist protocol that began with daily s.c. injections of 0.1 mg leuprolide acetate on day 21 of the prestimulated cycles (long protocol) or on day 1 of the stimulation.
cycle (short protocol). HMG or recFSH was started at day 3 and luteal support was done with progesterone.

**Results:** Characteristics of patients with normal ovarian response (group A) were compared to OHSS (group B) Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (n = 74)</th>
<th>Group B (n = 32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH (ng/ml)</td>
<td>1.25 ± 1.24</td>
<td>6.26 ± 3.33</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>77.23 ± 44.87</td>
<td>99.15 ± 56.76</td>
<td>0.038</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>7.31 ± 5.95</td>
<td>5.49 ± 1.49</td>
<td>0.013</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>3.70 ± 2.54</td>
<td>4.72 ± 1.14</td>
<td>NS</td>
</tr>
<tr>
<td>AFC</td>
<td>6.88 ± 4.34</td>
<td>11.69 ± 6.93</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gonadotropins HMG</td>
<td>60 (54)</td>
<td>17 (53)</td>
<td>NS</td>
</tr>
<tr>
<td>Gonadotropins Rec FSH</td>
<td>44 (54)</td>
<td>55 (67)</td>
<td>NS</td>
</tr>
<tr>
<td>Long agonist protocol</td>
<td>46 (64)</td>
<td>26 (68)</td>
<td>NS</td>
</tr>
<tr>
<td>Short agonist protocol</td>
<td>26 (36)</td>
<td>6 (19)</td>
<td></td>
</tr>
</tbody>
</table>

A multivariate logistic regression model was used for further analysis the predicting factors of OHSS. (Table 2) Only AMH level appeared to be a more efficient predictor of OHSS than age, BMI, total AFC, Inhibin B and LH.

**Table 2:** Variable | Coefficient | Odds ratio (95% CI) | p value |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH</td>
<td>0.047</td>
<td>2.58 (1.51-4.39)</td>
<td>0.001</td>
</tr>
<tr>
<td>FSH</td>
<td>-0.621</td>
<td>0.537 (0.31-0.93)</td>
<td>0.027</td>
</tr>
<tr>
<td>Age</td>
<td>0.041</td>
<td>1.04 (0.89-1.23)</td>
<td></td>
</tr>
<tr>
<td>Inhibin B</td>
<td>0.000</td>
<td>1.00 (0.98-1.01)</td>
<td>NS</td>
</tr>
<tr>
<td>AFC</td>
<td>0.008</td>
<td>1.000 (0.86-1.18)</td>
<td>NS</td>
</tr>
<tr>
<td>LH</td>
<td>0.259</td>
<td>1.30 (0.79-2.13)</td>
<td>NS</td>
</tr>
</tbody>
</table>

The ROC curve for the predicting factors for OHSS were compared and the results are presented in Table 3.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area under ROC (95% CI)</th>
<th>Cutoff value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH</td>
<td>0.90 (0.84-0.97)</td>
<td>3.645</td>
<td>0.84</td>
<td>0.865</td>
<td>0.73</td>
<td>0.93</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>0.620 (0.50-0.74)</td>
<td>84</td>
<td>0.62</td>
<td>0.605</td>
<td>0.40</td>
<td>0.79</td>
</tr>
<tr>
<td>AFC</td>
<td>0.79 (0.70-0.88)</td>
<td>7.5</td>
<td>0.77</td>
<td>0.66</td>
<td>0.49</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**Abstracts of the 26th Annual Meeting of ESHRE, Rome, Italy, 27 June – 30 June, 2010**

P-312 Oocyte donation is a risk factor for first trimester bleeding and pregnancy induced hypertension but without effect on the perinatal outcome

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**Introduction:** Oocyte donation (OD) has become well established. As with any other reproductive techniques, assessment of possible associated obstetric and perinatal risk remains paramount. To evaluate the pure obstetrical and perinatal impact of conceiving through OD we compared an OD-group with a matched control group of autologous oocytes (AO). Secondly, we evaluate differences in obstetrical and perinatal outcome between OD-pregnancies for which an appropriate AO-match was available, versus OD-pregnancies at an advanced maternal age and therefore without a match.

**Materials and Methods:** All singleton (n = 210) and twin (n = 87) pregnancies beyond 20 weeks of gestation conceived after OD at our centre between 1999 and 2008 were considered for this study. Controls were selected from the patient population undergoing in-vitro fertilization with AO. As for the OD-pregnancies, all controls were conceived with the intra cytoplasmic sperm injection (ICSI) technique. A match for maternal age (± 12 months), parity, plurality (singleton or twin) and gender of the children could be found for 205 OD-pregnancies resulting in 262 live births. Obstetrical outcome data from these 205 OD-pregnancies and their matched control pregnancies were analysed by multivariate analysis. Matched groups were compared using paired t tests for continuous variables and McNemar test for categorical variables. We performed conditional logistic regression analyses, further adjusting for maternal age, of the oocyte donor and for the number of embryos transferred on the obstetric and perinatal outcomes. Obstetrical and perinatal outcome of OD-pregnancies without available match were analysed using Student t tests for continuous variables and Fisher’s exact test for categorical variables. We also conducted linear and binary logistic regression analyses adjusting for baseline characteristics of interest.
Results: OD is associated with a increased risk of first trimester bleeding (OR:1.493 CI:1.036-2.15) and pregnancy induced hypertension (PIH) (OR:1.502 CI: 1.024-2.204) as seen by conditional logistic regression of the matched groups after further adjusting for maternal age, age of the donor and number of transferred embryos. No differences in gestational age, mean birth weight and length, head circumference or Apgar scores were observed between the two matched groups.

Logistic regression of obstetrical outcome parameters in all OD-pregnancies (n = 297) found a significant association between preterm labour, the incidence of caesarean section and the presence of a twin pregnancy.

Conclusions: This is the first large follow-up study that demonstrates that OD is associated with an increased risk for first trimester bleeding and PIH independent of the recipients' parity, plurality of the recipient, donor or partner. Hence, no difference in perinatal outcome is observed after OD. A shorter gestational age and a lower birth weight and length were observed in OD-recipients of an advanced maternal age as compared to OD-recipients at an age were ICSI-pregnancies with AO available.

P-313 In vitro culture affects the birthweight of human singletons

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Introduction: Children from singleton pregnancies resulting from assisted reproductive technologies (ART), have a significantly worse perinatal outcome as compared to spontaneously conceived infants. They are at higher risk of low birthweight, very low birthweight or small-for-gestational-age and have a two-fold risk of a preterm delivery. Children born preterm and of low birthweight after ART are at risk of cardiovascular disease and diabetes in later life. Animal models have shown that culture media for in vitro culture of embryos can affect the birthweight of newborns. In a previous study from our group we established a significant relationship between culture media and birthweight in the human. In that prospective study we analyzed the birthweight of all singleton live births and studied the effect of two commercially available culture media. Only singletons that resulted from the first IVF treatment cycle of a subfertile couple were included. In the present study, we enlarged our dataset by including all singletons that resulted from either the first, second or third IVF treatment cycle in order to investigate the effect of different culture media used during IVF treatment on the perinatal outcome of newborns.

Methods: In the present analysis, we included all singleton live births after fresh embryo transfers in the period July 2003 to December 2006. During this period, our laboratory technicians strictly alternated between two commercially available culture media for the IVF procedure. The allocation to a given culture media was concealed from the clinicians involved in the IVF program. Oocytes and embryos were cultured in either a sequential medium from Vitrolife AB (Göteborg, Sweden) or from Cook (Brisbane, Australia). Besides the media, all other procedures in the IVF treatment were equal in either group. Data from only one child per couple were included. At delivery, the perinatal outcome was recorded.

Results: A total of 293 singletons were examined. The 167 live born singletons in the Vitrolife group were compared with the 126 singletons in the Cook group. Birthweight + SEM (3440 ± 44 vs. 3252 ± 50g, P = 0.005, Student’s t-test) and birthweight adjusted for gestational age and gender (mean z-score + SEM: 0.06 ± 0.08 vs. -0.27 ± 0.08, P = 0.005, Student’s t-test) were both significantly lower in the Cook group. Furthermore, the proportion of low birthweight (<2500 g) in the Cook group was 9.5% (12/126) which was significantly higher than that in the Vitrolife group which was 2.4% (4/167). Also, the proportion of low birthweight in term newborns was significantly higher in the Cook group, 6.4% (8/126) vs. the Vitrolife group 1.2% (2/167). Analysis by multiple linear regression together with other variables that could possibly affect birthweight as covariates, showed that the type of culture medium was significantly (P = 0.037) associated with birthweight.

Conclusions: Our results indicate that the type of medium used for culturing embryos during the first few days after fertilization significantly affects the birthweight of the resulting human newborns. This may explain the higher risk of low birthweight in IVF singletons. It is therefore of pivotal importance to optimize and control culture media for IVF procedures to prevent low birthweight and the associated increased risk of disease in later life.

P-314 Male age doesn’t impacts embryo implantation and clinical pregnancy rate in donor oocyte assisted reproductive technology cycles

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2Instituto Verum, Human Reproduction, Brasilia, Brazil

Introduction: There is a controversy about male’s age influence on the assisted reproductive results. Usually, the studies show the influence of the wife’s age. Therefore, oocyte donation provides an efficient model to evaluate the potential of paternal effects on clinical outcomes, since all oocytes used come from young donors, excluding wife’s age bias. The objective of this study was to determine the effect of male age on implantation, clinical pregnancy and miscarriages in recipients in an egg sharing donation program.

Materials and Methods: Retrospective data were collected from August 2003 to December 2008, in an egg sharing donation (ESD) program, from a Brazilian reproductive center. One hundred and ninety recipients in 239 cycles, undergoing oocyte donation were analyzed in this study. The recipient’s husbands were divided into two groups based on age: man age < 40 (Group A) and ≥ 40 years (Group B). We considered that there was a male factor when a sperm analysis was different from the normal results (WHO criterion). No testicular biopsy or sperm aspiration procedures were included. The selection criteria for donors included: infertile woman being between 18-35 years old, genetic screening, basal FSH < 10 U/I, and normal karyotype. The selection criteria for recipients were: need of oocyte donation for IVF cycle, being in good health and less than 51 years old. Pituitary down regulation was done in donors and recipients with ovulation, with GnRH agonist in the mid-luteal phase. Ovarian stimulation was done with FSH and/or HMG. Recipients started using estradiol valerate in increasing doses, from 2 mg to 6-8mg/day at the same day that donors started stimulation. The harvested eggs were divided equally between donors and recipients. Luteal support was done with progesterone intramuscular or vaginal. Embryo transfer was performed on day 2 or 3. Statistical analysis was performed by Statistical Package for Social Science (SPSS) version 10.0, test t for independent test, qui squared test (χ2), level of significance p < 0.01.

Results: The average age of the donors was similar in both groups: 29.09 ± 3.09 (Group A) vs 29.57 ± 3.52 (Group B) (p = 0.27). The percentage of men with seminal anomaly (24.77% in Group A and 30.15% in group B) did not show significant difference (p = 0.35). The average number of eggs used by the recipients in both groups (Group A 8.82 ± 4.18 vs 8.84 ± 3.98 in Group B, p = 0.97) as well as the difference in the average number of transferred embryos (Group A 3.16 ± 0.86 vs Group B 3.14 ± 0.98) was not significant (p = 0.35). Equally, the implantation rates (17% in Group A vs 20% in Group B), the clinical pregnancy (35% and 40% in groups A and B respectively) and miscarriages (12.82% in Group A and 16.66% in Group B) did not show significant statistical difference (p = 0.23, p = 0.50 and p = 0.64, respectively). The age of the recipients was higher in Group B (39.82 ± 5.60) than in Group A (43.43 ± 4.16) (p = 0.001).

Conclusions: The oocyte donation model used in this study allowed the exclusion of wife’s age bias on the analyzes of male age on IVF outcomes. Men over 40 years of age doesn’t have poorer outcomes on implantation, clinical pregnancy and miscarriages when the woman use oocyte donation from younger donors.
P-315  Is performing viral screening within 30 days of oocyte collection justified?

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Introduction: Since the introduction of the EU Directives 2004/23/EC, 2006/17/EC and 2006/86/EC, relating to the procurement, processing and storage of tissues and cells, there have been various interpretations of the directive throughout Europe. One of the main areas of misinterpretation or dispute is in relation to the frequency of viral screening for partner donation. In 2007, after several consultations between the Irish Fertility Society and the appointed regulator, the Irish Medicines Board (IMB), it was agreed that as stated in the Directive 2006/17/EC Annex III, in relation to viral screening the “time of donation” for reproductive cells was re-defined and accepted as “within 30 days of procurement for couples undergoing treatments such as in-vitro fertilisation (IVF) and intracytoplasmic (ICSI)”, while for couples undergoing intra-uterine insemination (IUI), it was agreed to carry out viral screening on a 6 monthly basis. However, the overwhelming opinion in most EU countries is that viral screening at this level is excessive and not scientifically justified. From this perspective we set to clarify the risk of seroconversion in patients that attended the Human Assisted Reproduction Ireland (HARI) for ART therapy or oncology cryopreservation.

Material and Methods: In 1998, the HARI unit commenced cryopreservation and storage of gametes and embryos, implementing a policy of viral screening prior to and > 180 days post storage (quarantine). For the years 1998 to 2008, each partner in the couple had viral screen testing performed for HIV 1 and 2, Hep B Surface antigen and Hep C antibody. Similarly, all oncology patients (control group, reflecting general population) attending for sperm cryopreservation had a pre storage screen and an end of quarantine one. We performed a data base analysis of all viral screening results available and measured the seroconversion risk at our unit over a 10 year period (1998-2008). We excluded couples where one partner showed positive after screen and all positive male oncology screens. We further analysed the patients who attended for viral screening since the introduction of the testing from May 2008 until November 2009.

Results: Of the total number of couples that had IVF/ICSI and surplus embryos cryopreserved between the years 1998-2008 at the HARI Unit, 1023 returned for the scheduled 180 days end of quarantine testing. No seroconversion was identified following retesting. Of all seronegative male oncology patients attending in this 10 year period, 555 men returned for 180 day follow up testing. No seroconversion was identified following retesting. Since the introduction of the testing under the EU legislation, 17,494 individual viral screen tests were performed at the HARI clinic, all either before therapy or within 30 days of oocyte collection, in all fresh cycles. No seroconversions were noted.

Conclusion: Our 10 years study shows the risk of seroconversion from a seronegative status to be negligible in a large and representative population of infertile couples attending for ART or males requiring oncology cryopreservation. This supports the opinion that current legislation which requires screening within 30 days of procurement in the Assisted Reproductive setting does not offer any patient benefit and should be revised. The ART environment is different from the organ donation or blood transfusion one and laws need to reflect these differences based on current evidence as reported here.

P-316  Does recipient’s age change the clinical pregnancy rate in an egg sharing donation program?

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2Cenajer, Human Reproduction, Salvador, Brazil

Introduction: The influence of the recipient’s age in the results of egg donation cycles has been recently questioned. The objective of this study was to compare pregnancy rates in in vitro fertilization (IVF) cycles between recipients < 45 and ≥ 45 years old.

Materials and Methods: All egg sharing donation (ESD) cycles from January 1993 to December 2004, in a Brazilian reproduction center were analyzed. One hundred and sixty-one donors were stimulated, resulting in 216 transfers among 160 recipients. The selection criteria for donors included: being between 18-36 years old, genetic screening, basal FSH < 10 UI/L, and normal karyotype. The selection criteria for recipients were: the need for oocyte donation for IVF cycle, being in good health and less than 51 years old. Pituitary down regulation was done in donors and recipients with ovarian function, with GnRH agonist in the mid-luteal phase. Ovarian stimulation was done with FSH and/or HMG. Patients commenced using estradiol valerate in increasing doses, from 2 mg to 6-8mg/day at the same day that donors started stimulation. The harvested eggs were divided equally between donors and recipients. Luteal support was done with progesterone intramuscular or vaginal. Embryo transfer was performed on day 2 or 3. The recipients were divided in group 1, < 45 years and group 2, ≥ 45 years old. Recipient’s and donor’s age, number of oocytes injected, embryos transferred, implantation and pregnancy rates (presence of heart beat at ultrasonography) and abortion rates were analyzed. Statistical analysis was performed by Statistical package for social science (SPSS) version 10.0, Mann Whitney U test and Student’s t-test.

Results: The median age of recipients in group 1 was 39.5 ± 3.8 vs 47.3 ± 2.4 years old in group 2. Donors in group 1 was 29.6 ± 3.2 vs 29.9 ± 3.3 years old in group 2. The median of oocytes injected and embryos transferred for group 1 and 2 were respectively 9.0 vs 9.1 and 3.7 vs 3.8. The implantation and clinical pregnancy rates were respectively for group 1 and 2: 18.4% (97/527) vs 12.9% (38/293) and 41.7% (58/139) vs 35% (27/77). Abortion rates were 18.9% (11/58) in group 1 and 22.2% (6/27) in group 2. Only the differences in clinical pregnancy (p = 0.033) and implantation rates (p = 0.028) were statistically significant at 95% confidence interval.

Conclusions: We divided the recipients in two groups according to the age in order to analyze the impact of recipient’s age on IVF outcomes. The recipient’s age ≥45 years old, negatively affected the implantation and clinical pregnancy rates, in an egg sharing donation program.

P-317  Embryo in vitro culture results in long term phenotypic changes in mice

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Background: One of the most important questions in ART is the long term safety of the technique. The Developmental origin of health and disease hypothesis holds that the developing individual is sensitive to the environment. The preimplantation stage is particularly sensitive to epigenetic changes. In fact, recent evidence suggests that IVF children may be predisposed to rare imprinting disorders and may have metabolic abnormalities. IVF could therefore represent a novel stressor and induce long term phenotypic changes. In prior work, we have shown that in vitro culture in suboptimal conditions (Whitten’s medium) results in adult mice with glucose intolerance and reduced growth. This study is devoted to uncover long term metabolic effects in mice conceived in vivo or in vitro using optimized culture conditions.

Materials and Methods: C57Bl6/J mice were conceived in vitro and cultured in optimized conditions (KSO medium with amino acids and 5% Oxy). Resulting blastocysts were transferred to CFI foster mothers (IVF group). Control embryos were fertilized in vivo, flushed out of the uterus at the blastocyst stage and immediately transferred to foster mothers (Flushed blastocyst, FB group). Morphometric parameters were measured at birth and wean. Adult animals (n = 23 IVF; n = 16 FB) had intraperitoneal glucose tolerance test (IPGTT) performed at 13, 21, and 29 weeks; DEXA scan to assess fat content was performed at 8, 16, 21 and 29 weeks. Food intake was recorded monthly. Fasting insulin levels were measured using ELISA and pancreatic beta cell function was assessed in vitro after isolation of beta cells. Parametric tests were used as appropriate.

Results: A sexual dimorphic effect was noted. Male mice did now show differences in parameters measured. Birth weight of female IVF mice (n = 8; 1.36 ± 0.4mg) was lower (p < 0.05) than FB mice (n = 8; 1.65 ± 0.2 mg). Female IVF mice showed increased food intake compared to FB at 7 (p < 0.05) and 20 weeks (p = 0.001) but not at 29 weeks. IVF female mice display catch up growth, and are heavier starting at 17 weeks of life (p < 0.05). DEXA scan revealed that IVF female mice have initially decreased fat content at 8 weeks, up growth, and are heavier starting at 17 weeks of life (p < 0.05) than FB mice (n = 8; 1.65 ± 0.2 mg). A sexual dimorphic effect was noted. Male mice did now show differences in parameters measured. Birth weight of female IVF mice (n = 8; 1.36 ± 0.4mg) was lower (p < 0.05) than FB mice (n = 8; 1.65 ± 0.2 mg). Female IVF mice showed increased food intake compared to FB at 7 (p < 0.05) and 20 weeks (p = 0.001) but not at 29 weeks. IVF female mice display catch up growth, and are heavier starting at 17 weeks of life (p < 0.05). DEXA scan revealed that IVF female mice have initially decreased fat content at 8 weeks, up growth, and are heavier starting at 17 weeks of life (p < 0.05). DEXA scan revealed that IVF female mice have initially decreased fat content at 8 weeks, up growth, and are heavier starting at 17 weeks of life (p < 0.05). DEXA scan revealed that IVF female mice have initially decreased fat content at 8 weeks, up growth, and are heavier starting at 17 weeks of life (p < 0.05).
Introduction: The ovarian hyperstimulation syndrome (OHSS) is a significant complication in in-vitro fertilization (IVF) cycles, characterised by exaggerated ovarian response.

Methods: Retrospective analysis of all IVF cycles performed in a single centre from September 1993 to June 2009. All cycles were classified according to whether their gestational order remained consistent (true singletons or twins) or spontaneously reduced (e.g. a derived singleton, or a derived twin). Neonatal measures such as birth weight and gestational age were also recorded.

Results: Of 17,415 ICSI cycles were analyzed in women of 36.9 ± 5 yrs (range 18-49). Following an overall fertilization rate of 73.4%, some 48,708 good quality embryos were obtained. In most patients (92.9%) an average of 3.0 embryos were replaced. The clinical pregnancy rate (presence of 1 FHB) at 6-7 weeks compared to the number of offspring delivered. The latter were then classified according to whether their gestational order remained consistent (true) or spontaneously reduced (e.g. a derived singleton, or a derived twin).

Conclusions: Maternal and placental cues induce prenatatal development to ensure the survival of both mother and fetuses, particularly in monochorionic species. The present data suggest that the implanted fetal poles and not sacs dictated the growth pattern of multiple gestations. Even when a spontaneous reduction occurs, the derived conceptus retains the growth characteristics expected according to the number of implantation sites. The data presented is in agreement with the observation that ART offspring coming from multiple gestations are comparable to those conceived spontaneously. Our study repudiates the claim that ART procedures, IVF and ICSI, are directly responsible for generating offspring with low birth weights

P-319 Maternal constraint and preemptive adaptation influence placental plasticity in ART offspring

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Introduction: A major concern for assisted reproduction, in spite of its popularity, is the increased incidence of offspring with low birth weight or are small for gestational age. This has been observed mainly in multiple gestations but also in singleton births, and remains largely unexplained.

Materials and Methods: To address these questions, we grouped ART cycles performed at our Center from September 1993 to June 2009 according to their gestational order. We assessed the number of embryos replaced, the implantation site(s), and the number of livebirth(s). The course of pregnancy was evaluated by determining the number of embryos with fetal heart beat(s) (FHB) at 6-7 weeks compared to the number of offspring delivered. The latter were then classified according to whether their gestational order remained consistent (true) or spontaneously reduced (e.g. a derived singleton, or a derived twin).

Conclusions: Maternal and placental cues induce prenatatal development to ensure the survival of both mother and fetuses, particularly in monochorionic species. The present data suggest that the implanted fetal poles and not sacs dictated the growth pattern of multiple gestations. Even when a spontaneous reduction occurs, the derived conceptus retains the growth characteristics expected according to the number of implantation sites. The data presented is in agreement with the observation that ART offspring coming from multiple gestations are comparable to those conceived spontaneously. Our study repudiates the claim that ART procedures, IVF and ICSI, are directly responsible for generating offspring with low birth weights.

P-320 Prognostic value of first IVF cycle on success of a subsequent cycle

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Introduction: State funding for IVF in the UK is sparse, and where available usually limited to a single cycle per couple. Couples who wish to have further IVF treatment would usually have to self-fund. As IVF remains physically, emotionally and economically expensive, couples embarking on further treatment, seek guidance from clinicians to determine prognosis before investing in another cycle. Earlier studies suggest that women, who had a live birth or...
miscarriage in their first IVF cycle have better prognosis for success in a subsequent cycle compared to women who had a negative pregnancy test. This may not be true for women of all ages.

**Objective:** To determine whether a live birth or miscarriage in a previous IVF cycle is predictive of success in a second cycle, and to determine if this is true for older women as well as for young women.

**Material and Methods:** 1141 second IVF cycles where patients had first & second cycles at the Lister fertility clinic from January 2005-December 2008. Three groups were identified; Group I: women who had a live birth in the first cycle, (n = 75), Group II those who had an early miscarriage in the first cycle (n = 223), & Group III, women who had a negative pregnancy test in their first cycle (n = 843). The three groups were similar in age, aetiology of infertility, basal serum FSH level, number of oocytes retrieved and number of embryos transferred. Chi-squared test was performed to evaluate statistical significance for subsequent live birth and miscarriage rate in the second cycle. Data was further analysed to determine these outcomes for women age < 40 and those ≥ 40.

**Results:** 1141 women embarked on a second IVF cycle; 441 got pregnant (Pregnancy rate (PR) = 38.7%). Women in groups I & II had a higher PR than those in Group III. (57.3% (43/75), vs 48.0% (107/223), vs 34.5% (291/843) respectively; (p = 0.00). There was also a significant difference in the live birth rate (LBR) between the 3 groups. Women in Groups I & II had a higher LBR in their second cycle compared to those in III; (38.7%(29/75) vs 31.8% (71/23) vs 22.9% (193/843) respectively (p = 0.01). The rate of miscarriage in the second cycle was not significantly different in the 3 groups (32.6% v 33.6% v 33.7% respectively).

**When only women age less than 40 were considered:** Of 1141 women, 793 were age < 40 years. In this younger cohort, the PR was 46.4% (368/793), miscarriage rate was 29.9% and the LBR was 32.5% (258/793). Women in groups I & II had a statistically higher PR than those in group III (63.3 % v 55.2% v 41.9% respectively (p = 0.00). Similarly the LBR was higher (45% v 37.8 v 29.6% respectively, p = 0.015). There was no difference in the miscarriage rate in the 3 groups. (28.9% v 31.6% v 29.4% respectively).

**When only women older than 40 years were analysed:** Of 1141 women, 348 were age ≥ 40 years. The PR in this older cohort = 21.0% (73/348), miscarriage rate = 52.1% (38/73) and the LBR = 10.1% (35/348).There was no significant difference in PR among women in groups I, II & III (33.3 v 23.3 v 19.9% p = 0.44 (NS)).The LBR was similar in the 3 groups (13.3 v 11.8 v 9.6 % respectively). There was also no difference in the miscarriage rates.

**Conclusion:** Young women who had a live birth and those who experienced an early miscarriage after IVF have a greater likelihood of achieving a live birth in a second cycle compared with women who failed to conceive in their first cycle. A miscarriage in the first IVF cycle does not increase the risk of miscarriage in the next cycle. Outcome of first IVF cycle however does not seem to predict subsequent IVF success in older women.

**P-322** Endogenous LH levels do not affect pregnancy rates in an rFSH/ GnRH antagonist protocol: combined analysis of individual patient data from 6 RTCs

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**Introduction:** The role of endogenous luteinizing hormone (LH) levels during controlled ovarian stimulation (COS) in normogonadotropic women is not completely understood and contradictory evidence exists as to whether high or low endogenous LH levels have an association with pregnancy likelihood. The current analyses were undertaken in large data sets derived from 6 randomized controlled trials to examine the association of endogenous LH levels during the follicular phase with ongoing pregnancy rates among patients treated with recombinant follicle-stimulating hormone (rFSH) and a gonadotropin-releasing hormone (GnRH) antagonist for IVF or ICWI.

**Material and Methods:** Data from the Engage trial, Ensure trial, Xpect trial, European Ganirelix trial, North American Ganirelix trial, and European-Middle East Ganirelix trial were retrospectively analyzed. Patients were normogonadotropic women with an indication for IVF. Stimulation with 150–225 IU rFSH started on day 2 or 3 of spontaneous menses after a natural luteal phase. Endogenous LH levels were measured by a central laboratory. First, stepwise logistic regression analysis was applied to a large trial of 1506 patients (Engage) to identify predictors of ongoing pregnancy. The identified significant predictors of the reference group of the Engage trial (200 IU rFSH stimulation in a GnRH antagonist protocol) were then included as covariates in a subsequent combined analysis of LH levels measured in Engage and 5 other trials (total 1764 pa-
The incidence of OHSS in phase 3 trials of corifollitropin alfa for controlled ovarian stimulation

P.323 Combined incidence of OHSS in phase 3 trials of corifollitropin alfa for controlled ovarian stimulation

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Introduction: Corifollitropin alfa is a new recombinant gonadotropin with sustained follicle stimulating activity that has been proven to be as safe and efficacious as recombinant follicle stimulating hormone (rFSH) in achieving ongoing pregnancies after controlled ovarian stimulation for assisted reproductive technology in a gonadotropin-releasing hormone (GnRH) antagonist protocol. To further ensure that corifollitropin alfa treatment is equally as safe in terms of the incidence of ovarian hyperstimulation syndrome (OHSS), a serious complication that may become life-threatening, all cases of OHSS collected during phase 3 trials of corifollitropin alfa have been evaluated.

Material and Methods: In total, 2 randomized controlled trials and 1 open uncontrolled trial were included in the phase 3 program: Engage (comparing 150 μg corifollitropin alfa with daily 200 IU rFSH), Ensure (comparing 100 μg corifollitropin alfa with daily 150 IU rFSH), and Trust (comparing 150 μg corifollitropin alfa with daily 200 IU rFSH), respectively.

Results: In total, 95 out of 1705 patients (5.6%) in the corifollitropin alfa group had OHSS. Among these patients, 91 (95.8%) were treated with daily FSH, and 4 (4.2%) with the sustained follicle stimulant corifollitropin alfa. The combined incidence of OHSS in the corifollitropin alfa treated patients and the rFSH treated patients was 3.6% versus 1.9% in the corifollitropin alfa treated patients and 3.4% versus 2.6% in the rFSH treated patients. Subjects with OHSS in the 2 treatment groups were of similar age (30.7 ± 30.3 years) and body mass index (23.7 ± 24.2 kg/m²), and had a similar antral follicle count at stimulation day 1 (13.0 ± 13.9) and FSH levels at stimulation day 1 (5.8 ± 5.9 IU/L). The mean (SD) number of oocytes retrieved per started cycle was 20.5 (9.5) oocytes in all OHSS cases after corifollitropin alfa treatment and 17.0 (6.9) oocytes in all OHSS cases after rFSH treatment. The incidence of mild, moderate, and severe OHSS was 2.5%, 1.6%, and 1.1% in the corifollitropin alfa treated patients and 3.5%, 1.3%, and 1.1% in the rFSH treated patients. The combined incidence of moderate and severe OHSS was 3.0% versus 2.5%, of which 1.7% and 1.0% required hospitalization.

Conclusion: In this relatively young in vitro fertilization population, treatment with the sustained follicle stimulant corifollitropin alfa resulted in an OHSS incidence comparable to that observed in patients treated with daily rFSH.

Support: Financial support for this study was provided by Schering-Plough Corporation, now Merck & Co., Inc., Whitehouse Station, NJ, USA.

P.324 Corifollitropin alfa is safe and efficacious in IVF patients undergoing repeated gonadotropin-releasing hormone antagonist ovarian stimulation cycles

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Introduction: A single injection of corifollitropin alfa, the first in a new class of recombinant gonadotropins with sustained follicle-stimulating activity, is able to initiate and sustain follicular growth over 7 days. Although the probability of corifollitropin alfa being immunogenic is considered to be very low, the Trust trial was designed to assess the immunogenicity and safety of corifollitropin alfa in patients undergoing up to 3 controlled ovarian stimulation (COS) cycles with corifollitropin alfa in a gonadotropin-releasing hormone (GnRH) antagonist protocol.

Material and Methods: In this multicenter, open-label, phase 3, uncontrolled trial the immunogenicity and overall safety and efficacy of corifollitropin alfa was assessed in women (aged ≥ 18 and ≤ 39 years, who weighed ≥ 60 kg and had a body mass index [BMI] of 18–29 kg/m²) undergoing up to 3 COS cycles using a multiple-dose GnRH antagonist protocol. In each COS cycle, the main study end points were: antibody formation against corifollitropin alfa; hypersensitivity reactions; local tolerance at the injection site; occurrence of (serious) adverse events (SAEs); and efficacy in terms of the number and quality of oocytes and embryos and the ongoing pregnancy rates. Each cycle started with a single subcutaneous injection of 150 μg corifollitropin alfa (Elonva, N.V. Organon) on day 2 or 3 of the menstrual cycle. From stimulation day 8, treatment was continued with daily urinary or recombinant follicle-stimulating hormone, up to a maximum daily subcutaneous dose of 225 IU, until the criterion for human chorionic gonadotropin (hCG) was reached (as soon as 3 follicles ≥ 17 mm). On day 5 or 6 of stimulation, patients started GnRH antagonist (garelix or cetrotrexol acetate) to up and including the day of triggering final oocyte maturation by urinary or recombinant hCG. Following in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), embryo transfer (ET) was performed 3 or 5 days after oocyte pick up and a maximum of 3 embryos were transferred.

Results: In total, 682 patients started the first COS cycle and their mean age, body weight, and BMI were 32.9 years, 67.0 kg, and 24.2 kg/m², respectively. Of these 682 patients, 375 patients continued with a second cycle and 198 patients started their third treatment cycle. No clinically relevant immunogenicity or drug-related hypersensitivity was observed in any of the patients receiving 1, 2, or 3 injections of corifollitropin alfa. Local reactions at the injection site (itching, pain, redness, swelling) were all mild in nature with a similar low incidence across the 3 treatment cycles. In cycles 1, 2, and 3, 46.8%, 35.2%, and 31.3% of patients had at least 1 AE, respectively. Overall, procedural pain was the most frequently reported AE (17.7%), followed by headache (9.1%) and pelvic pain (7.6%). In total, 63 SAEs were reported in 47 patients and their incidence was 3.4%, 1.6%, and 1.5%, in treatment cycle 1, 2, and 3, respectively.
SAEs occurring in more than 1% of the subjects were ectopic pregnancy (1.5%) and ovarian hyperstimulation syndrome (OHSS) (1.5%). The incidence of OHSS was low, 3.5% in the first treatment cycle with 0.9% indicated as moderate and 0.9% as severe OHSS. OHSS incidence in the second cycle was 1.9% (0.5% were moderate and 0.5% severe) and 0.0% in the third cycle. The cumulative ongoing pregnancy rate after 3 COS cycles including in-between frozen-thawed embryo transfer (FTET) cycles and spontaneous pregnancies, censored for patients who discontinued treatment, was 61%.

Conclusion: A single injection of 150 μg corollfilotropin alpha can safely and effectively initiate and sustain ovarian stimulation during the first 7 days of COS prior to IVF/ICSI in patients undergoing up to 3 cycles of treatment, without concerns related to immunogenicity.

Support: Financial support for this study was provided by Schering-Plough Corporation, now Merck & Co., Inc., Whitehouse Station, NJ, USA.

P-325 Complications of assisted reproduction technologies in overweight and obese women

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Introduction: Overweight and obesity are a growing problem worldwide. The negative impact of overweight and obesity on female fertility has been demonstrated in epidemiologic studies. Based on these data, some authors argue that there should be an upper limit for a woman’s body mass index (BMI) – as there is in some countries for a woman’s age – to receive assisted reproduction technologies (ART). As we feel that such thresholds should be based on a high risk of complications or very low expected success rate, we searched the literature on the subject.

Material and Methods: We searched the literature for complications from ART in association with BMI and for studies on pregnancy and live birth rates following ART with respect to age and BMI. Articles were scored on methodologic quality. We calculated odds ratios for the association between BMI and complications. Finally, we compared the effect of BMI and female age on ART success.

Results: We detected 7 studies that reported on the association between complications and BMI, of which five reported on OHSS, three on multiple pregnancies and two on ectopic pregnancies. None of the individual studies found a positive association. We detected 17 studies that reported on BMI outcome of ART cycles or after FSH- and/or HCG priming. After maturation in vitro, oocytes showing the first polar body were inseminated by intracytoplasmic sperm injection (ICSI), and ensuing embryos were cultured for 48-72 hours. In each cycle, maximum 3 embryos were obtained and transferred without selection, as prescribed by law.

Conclusions: Between March 2005 and December 2009, 120 children from IVM cycles were born. Single and twin pregnancies were 111 and 9 respectively. Mean gestational age at birth was 38.3 weeks. Fifteen (13.5%) preterm pregnancies (< 37 weeks) were documented, of which 7 were twin. Two of the preterm pregnancies ended before 32 weeks. One case was a twin pregnancy and ended at 26 weeks with the death of one of the twins for prematurity, while the other was ended at 27 weeks for preeclampsia. The mean weight at birth was 3175.1 g. The mean Apgar score was 8.9 and 9.8 after 1 and 5 minutes, respectively. With regard to congenital abnormalities, we observed 1 case of Wolff Parkinson White syndrome, 2 of left pleietactyly, 1 of labiopatolatischosis associated with hypospadias, 1 of sinus pilonidalis, and 1 of plagiocphally in a twin pregnancy. No major abnormalities were found.

P-327 Anti-müllerian hormone and antral follicle count as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis

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Introduction: The maturation of an excessive amount of follicles during controlled ovarian hyperstimulation leads to a considerable portion of cycle cancelations in assisted reproductive techniques (Delvigne, 2002). The development of such an excessive response puts patients at risk of developing ovarian hyperstimulation syndrome (OHSS) a potentially life threatening condition which complicates 30-40% of in vitro fertilization (IVF) cycles (Delvigne, 2002). By adequate prediction of extreme responses, such complications could potentially be prevented by individualising treatment protocols. Anti-müllerian hormone (AMH) and antral follicle count (AFC) have both been implicated in excessive response prediction (van Rooij 2002, Kwee, 2007). The objective of the current meta-analysis is to assess the value of AMH and AFC in the prediction of excessive-response in IVF and intracytoplasmic sperm injection (ICSI) treatment.

Materials and Methods: A systematic review of existing literature and meta-analysis were carried out in an academic referral centre for tertiary care. After a comprehensive search, studies were included upon construction of 2x2 tables with AMH and/or AFC in relation to the outcome measure of excessive response. A total of nine studies reporting on AMH and four on AFC were included. Studies were scored on quality characteristic and data was pooled using the bivariate regression model. This model allowed for calculation of both a summary point estimate for sensitivity and specificity, as well as a summary ROC curve.

Results: For AMH the pooled sensitivity was 39% (95% confidence interval = 28% to 50%) at a pooled specificity of 94% (95% confidence interval = 91% to 97%). For the AFC, the pooled sensitivity was 43% (95% confidence interval = 31% to 55%) at a pooled specificity of 93% (95% confidence interval = 85% to 100%). The summary ROC curves indicate that for AMH a more optimal sensi-
tivity/specificity combination could be chosen than for the AFC. The area under the curve for the AMH and AFC can be seen in figure 1 which demonstrates the superior test performance for AMH.

Figure 1: Summary curve for AMH and AFC performance in excessive response prediction

Conclusions: This meta-analysis shows that both AMH and the AFC have an adequate predictive capacity for prediction of excessive ovarian response to stimulation. Both tests have a sufficient discriminatory capacity for the identification of excessive response but at the same level of specificity AMH could identify a greater number of excessive responders. In conclusion, AMH has the potential to be used to screen patients prior to starting IVF/ICSI and aid in the individualisation of treatment protocols.

P-328 Efficacy and efficiency of rFSH vs uFSH vs hMG: a randomized trial in 552 egg donors


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Introduction: Discrepancies exist when efficacy and efficiency of different gonadotropin preparations are examined. Nowadays, apart from obtaining good quality oocytes with high potential for implanting and achieving a pregnancy, simple, safe and cost-effective protocols should be chosen. This is especially important in egg donor programs. For this reason we designed a prospective, randomized trial to compare different protocols (long and antagonist) with different gonadotropin preparations (rFSH, uFSH and hMG).

Material and Methods: Prospective, randomized, controlled and multicenter trial performed in egg donors at IVI clinics between January 2009-November 2009. Inclusion criteria: first cycle of the donor, 18-35 years old, BMI 18-26 kg/m², > 10 antral follicle count (AFC). All donors received oral contraceptive pill pretreatment. The fixed starting dose was 225IU or 150IU when AFC > 14, in patients receiving rFSH or uFSH and 225IU/300IU in uMG.

In antagonist protocol, HCG was administered for ovulation triggering, and in antagonist protocol GnRH agonist was given instead. Cycle was cancelled if < 7 follicles were seen in ultrasound on the day of HCG, OHSS risk or drop in E2 levels. Our aim was to evaluate the parameters of controlled ovarian stimulation and the implantation, pregnancy and ongoing pregnancy rate in these six different stimulations protocols.

Results: A total of 552 oocyte donors were randomized: 185 with GnRH agonist (74 with rFSH, 52 with uFSH and 59 with hMG) and 367 with GnRH antagonist (139 with rFSH, 118 with uFSH, 110 with hMG). In terms of ovarian response, there was no difference among groups in mean number of oocytes retrieved: for agonists, number of oocytes was 15.7 (14.3-16.7) with rFSH, 14 (13.5-14.8) with uFSH, and 17 (15.5-19.2) with hMG; in antagonist, mean number of oocytes was 15.5 (14.4-16.0), 14.4 (13.1-15.5) and 12.8 (11.5-13.9) with rFSH, uFSH and hMG respectively (Pearson’s chi-square test: 0.522). Cancellation rate with GnRH agonists was 23%, 32.7% and 22% with rFSH, uFSH or hMG; however, under the antagonist the cancellation rate was 20.9%, 22.9% and 39%, with rFSH, uFSH or hMG. Differences were significant with the combination of GnRH antagonist protocol with hMG among the others combinations (Pearson’s chi-square test: 0.035). No differences were found among the different gonadotropins preparations regarding implantation rate, (33.0%, 30.9%, 31.3% with rFSH, uFSH and hMG respectively).

Conclusions: The combination of antagonist protocol with hMG has statistically higher cancellation rate and fewer number of oocytes. However, rFSH performs equally well whether we use GnRH agonists or antagonists.

P-329 Patient friendly local analgesi in the vaginal vault, a safe and efficient procedure for transvaginal oocyte retrieval

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Introduction: Since we introduced ultrasound guided oocyte retrieval for IVF local analgesi using a para-cervical block and sedation, there has been the main procedure for oocyte retrieval. We have further improved the method by testing a local analgesi in the vaginal vault in the position for the puncture, in which the local analgesi were placed ultrasonically guided just prior to the puncture.

Material and Methods: In a prospective observational study where one doctor continued the para-cervical block method for analgesi and sedation with Rapifen, was compared to 3 other doctors using the ultrasound guided placement of the local analgesi and Rapifen sedation. AVAS score for pain perception during the procedure and after were recorded in 100 patients (20 in the para-cervical blockage group and 80 in the ultrasound guided group).

A total of 1287 oocyte retrievals were observed from the 01.01.09 to 01.01.10, no drop out. 167 oocyte collections were done by the para-cervical blockage and 1120 using the ultrasound guided procedure.

Results: The live pregnancy rate per aspiration (week 12 of gestation) was found to be 24% (40/167) in the para-cervical blockage group and 23% (253/1120) in the ultrasound guided group. No differences in number of oocytes, fertilization rates or cleavage rate nor implantation rates were seen between the groups. However a significant lower volume of sedatives were used in the ultrasound guided group as well as lower VAS score for pain during and after the treatment.

Conclusion: Local analgesi applied under ultrasound guidance in the place for puncture of the vaginal vault during oocyte pick up in human ART is a simple and safe procedure, providing further comfort to the patient compared to traditional para-cervical blockage.

P-330 Circulating progesterone levels at the end of ovarian stimulation related to ongoing pregnancy rate in different patients subpopulations

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Introduction: Elevated circulating progesterone (P) levels at the end of controlled ovarian stimulation (COS) for In vitro Fertilization (IVF), have shown to have a negative impact on embryo implantation and therefore cycle outcome. Although there is no general agreement in the definition of the exact serum P threshold being detrimental for the prognosis of the cycle, a large study performed over more than 4,000 cycles showed that a P level > 1.5 ng/mL the day of hCG administration was the best cut-off value to define this event in unselected population (Bosch et al. ESHRE 2008). However, specific patients’ characteristics have not been related yet to the impact of P on cycle outcome.

In the present study we aimed to analyze the impact of high serum P levels on the ongoing pregnancy rate according to women’s, age, body mass index (BMI), cause of infertility, ovarian response and gonadotrophin consumption.

Material and Method: This was a retrospective cohort study in which 4,032 IVF cycles were analysed for the purposes of the study. The primary endpoint was the ongoing pregnancy rate (OPR), defined as the presence of a viable fetus beyond week 20 of pregnancy. The OPR of patients with a serum P level the day of hCG administration > 1.5 ng/mL was compared to that one on patients with P ≤ 1.5 ng/mL. This comparison was done through a stratified analysis according to patients’ age (< 30; 30-35; 36-40; > 40); BMI (≤ 25; 25-29.9; ≥ 30); infertility cause (Male, Tubal, Endometriosis, and PCO); ovarian response in terms of number of oocytes collected (1-5; 6-10; 11-15; 16-20; > 20) and estradiol (E2) levels (< 1000; 1000-1999; 2000-2999 and ≥ 3000 pg/mL) at the end of stimulation, and total gonadotrophin consumption (< 1500; 1500-3000; > 3000 IU).
Introduction: Ovarian hyperstimulation syndrome (OHSS) is a major complication of assisted reproductive treatment (ART) that can result in hospitalisation and occasionally death. The use of GnRH agonists to induce oocyte maturation after treatment with GnRH antagonists results in a reduced risk of OHSS but has been associated with an inadequate luteal phase and reduced pregnancy rates in fresh cycles (1). The aim of this study was to determine the effectiveness on ART outcome of a standardised stimulation regimen using GnRH antagonist therapy with different ovulatory triggers.

Material and Methods: Between January 2008 and December 2009, 368 couples were treated with 250µg of Cetroide from days 4 to 12 of the cycle. Follicle growth was stimulated from day 1 using Gonal F or Puregon. Ovulation was induced on day 12 using either a GnRH agonist (Synarel) or 5000 IU hCG. The choice of trigger was determined principally by the number of follicles observed at ultrasound on day 12. Thus the majority of patients with fewer than 10 follicles received hCG, whereas those with 10 or more follicles were triggered with Synarel. Oocytes were recovered 36 hours later and subjected to standard IVF and ICSI techniques. Fertilization was determined at 18h after insemination and embryos were transferred or frozen on day 2 or 3. Luteal phase support included Crinone, progesterone pessaries, estradiol valerate and Provera. Pregnancy was determined 16 days after OPU, followed by ultrasonography at 7 weeks.

Results: Of the 368 cycles in this study, ovulation was triggered with hCG in 126 cases (34.2%) and with synarel in 242 (65.8%). There was a significant difference between the two groups in mean female age (34.8 versus 32.7 years, P < 0.001), mean number of oocytes recovered (6.04 versus 10.5, P = 0.0001) and fertilization rate (69.9% versus 75.5%; P = 0.02). By contrast, there was no significant difference in clinical pregnancy rate (26.6% versus 30.8%; P = 0.467). Implantation rates were however significantly higher in the group triggered with agonist compared to those receiving hCG (20.3% Fetal Heart per embryo transferred versus 13.1%; P = 0.03). None of the patients in either trigger group developed any form of ovarian hyperstimulation syndrome (OHSS).

Conclusions: This study shows that by varying the ovulatory trigger according to the number of follicles, high clinical pregnancy and implantation rates can be achieved in younger women without the risk of ovarian hyperstimulation or the need to abandon fresh transfers and cryopreserve all embryos.

Reference:
Therefore, the aim of this study is to evaluate pregnancy outcomes of frozen-thawed embryo transfer (FET) cycles with blastocysts.

**Materials and Methods:** Retrospective analysis of FET cycles with blastocysts between Jan 2007 and June 2009 were performed. Age-matched FET cycles with cleavage stage embryos of same period were collected as control group. A total of 58 frozen-thawed blastocyst transfer cycles were compared with 172 FET cycles of cleavage stage. The results of FET cycles were compared with fresh embryo transfer cycles (143 cycles with blastocyst transfer vs. 430 cycles with cleavage stage embryo transfer) and embryo transfer cycles of frozen-thawed blastocyst were also compared with those of post-thaw extended cultured blastocysts (PTEC) from frozen pro-nucleus (PN) stage embryos. (22 cycles) Clinical pregnancy and ongoing pregnancy were defined as the presence of gestational sac on trans-vaginal ultrasound at 5th to 7th gestational weeks and the existence of fetal heart motion at approximately 12 weeks gestation. We compared patients' characteristics and pregnancy outcomes between two groups.

**Results:** There was no difference in age (33.3 ± 3.9 yr vs. 33.4 ± 3.8 yrs), BMI (22.3 ± 3.5 vs. 21.5 ± 3.2 kg/m²), and basal FSH (10.6 ± 5.5 vs. 10.9 ± 6.5 mIU/mL) between cleavage stage FET and blastocyst FET. The survival rate of embryos after thawing was similar (90.8% vs. 93.3%) in both groups. No difference was found in implantation rate (IR, 21.5% vs. 19.5%), clinical pregnancy rate (CPR, 43.1% vs. 44.2%), and ongoing pregnancy rate (OPR, 39.7% vs. 40.7%) between two groups. In blastocyst FET, the mean number of transferred embryos was lower (2.0 ± 0.7 vs. 3.1 ± 0.8, P < 0.001) than in cleavage stage FET, however multiple pregnancy rate of clinical pregnancies (MPR, 15.0% vs. 26.3%) was similar in two groups. By additional analysis, fresh cycles using blastocysts presented higher IR (33.8% vs. 20.7%, P < 0.001), CPR (53.1% vs. 40.0%, P = 0.006), and OPR (45.5% vs. 34.2%, P = 0.017) than cycles using cleavage stage embryos without any difference of patients' characteristics and MPR. On the other hand, blastocyst transfers after PTEC of frozen PN stage embryos did not show any difference of pregnancy outcomes compared with blastocyst FET.

**Conclusions:** Although fresh ET cycles using blastocysts show better pregnancy outcomes, in FET cycles, the blastocyst transfers did not present any benefit of pregnancy outcomes compared with cleavage stage embryo transfers. These results showed from not only frozen-thawed blastocyst transfer but also PTEC blastocyst transfer, therefore it may not arise from compromised technique of blastocyst freezing and thawing.

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**P-334 E2 support for luteal phase in GnRH antagonist IVF/ICSI cycle**

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**Introduction:** The role of progesterone for luteal support in stimulated cycles for IVF is pretty well established. However the benefit of additional supplementation with estradiol (E2) in GnRH antagonist cycles is less certain. Data for its role GnRH antagonists regime is insufficient.

**Objective:** To compare the clinical pregnancy rates of stimulated IVF cycles with rFSH/GnRH antagonist in patients who received micronized progesterone for luteal phase support, with or without the addition of E2.

**Methodology:** An observational study was conducted at Medically Assisted Centre, HUKM and IUM from 1st of Jan 2008 until 31st of Dec 2009. We compared the pregnancy rates for total of 320 patients who underwent ovarian stimulation with a dose of rFSH 100-300 IU and GnRH antagonist with or without 6mg of estrogen supplementation for the luteal support. Patients received either 600 mg of micronized progesterone vaginally (n = 150, progesterone group) or 600 mg of micronized progesterone and 6 mg of E2 valerate orally (n = 170, progesterone/E2 group). Clinical pregnancy rates were the main outcome measures.

**Results:** Demographics, stimulation parameters and embryological data were comparable for the two groups compared. Forty (40) clinical pregnancies were achieved in the progesterone (26.7%) and sixty eight (68) in the progesterone/E2 group (40.0%) (p < 0.005).

**Conclusion:** It appears that the addition of E2 to progesterone in the luteal phase after stimulation with rFSH and GnRH antagonist may enhance the probability of pregnancy. Further larger scale randomized study is needed to confirm this finding.

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**P-336 Optimizing outcomes of IVM treatment cycles-results of a new and improved protocol**

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**Introduction:** Traditionally, pregnancy rates for in-vitro maturation (IVM) cycles in patients with polycystic ovaries (PCO) and polycystic ovarian syndrome (PCOS) have been reported to be lower than those for in-vitro fertilization (IVF). In 2009, we made two major changes to our IVM protocol for treatment of PCO-related infertility. Firstly, HCG trigger was given when the leading follicle reached 10-12 mm because it was demonstrated in a retrospective study that this optimizes outcomes. Secondly, we began to administer the HCG 38 hours prior to retrieval, rather than 36 hours prior. The purpose of this study was to analyze and report on the outcomes of IVM cycles after the implementation of these changes at the McGill Reproductive Centre in Montreal, Canada.

**Materials and Methods:** Fifty four (54) patients with PCO or PCOS, with a mean age of 32.6 ± 3.8, underwent a total of 39 IVM collections at our centre between January 1st and Dec 31st, 2009. The results of all cycles in terms of total...
number of mature oocytes (MII) collected, maturation rate of immature oo-
cytes, fertilization and cleavage rates, as well as embryo quality, were recorded in
a detailed database. Implantation and pregnancy rates were also recorded. For the purposes of this study, results were then analyzed separately for those patients below age 35 and those at or over age 35.

Results: Out of 59 cycles, 39 (67%) were in women below age 35 and 20 (34%) were at or above age 35. Mean dominant follicle (DF) diameter on the day of HCG administration was 10.8 ± 2.3 and mean endometrial thickness at the time of oocyte retrieval was 8.7 mm ± 2.4. A total of 922 oocytes were retrieved, on average 16.2 per patient in the group below age 35 and 14.5 in those over 35.

In 44 out of 59 cycles (75%), at least one in vivo matured (MII-stage) oo-
cyte was collected. In those younger than age 35, 88 were MII at collection, which represents 13.9% (88/633) of the total collected. In the group over age 35, 34 MII oocytes were collected, representing 11.8% (34/289) of the total. Maturation rates were 30.2% on day 1 of culture with an additional 15.3% by day 2. A total of 541 oocytes out of 922 (58.7%) reached MII stage.

Of the 541 MII oocytes that reached MII stage, 375 fertilized normally (69.3 %) and 350 were cleaved (93.3% of zygotes). Embryo transfers were performed in 58 out of 59 cycles. Only one patient did not undergo embryo transfer. The average number of embryos transferred was similar in both groups at 3.3 for patients younger than 35 and 3.4 for those 35 or older. After transfer, the implantation rates were 20% in both groups and the clinical pregnancy rate was 56.4 % in the group below age 35 (22 out of 39 embryo transfers) and 57.9 % (11 out of 19 transfers) in those over age 35. These pregnancies represented 28 singletons (84%), 4 twins (12.5%), and 1 triplet (3.1%).

Conclusions: Our results suggest that implementing the above changes to our IVM protocol resulted in improved pregnancy rates. The finding that results did not differ between patients above and below age 35 is most likely due to the small numbers of older patients. The favourable results reported above should be confirmed and, if they are, JVM could be offered as an alternative treatment to conventional IVF for patients with PCOS, while eliminating the risk of OHSS.

P-337 Is routine screening for coagulation abnormalities prior to ova
estration needed?
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Introduction: Ultrasound guided trans-vaginal oocyte pickup (OPU) is used in
the process of in-vitro fertilization. As bleeding is a recognized complication, routine screening for coagulation abnormality is performed prior to this procedure.

Objective: In this cross-sectional retrospective study we assess the utility of this screening practice and whether pre-testing clinical assessment is valuable.

Methods: The hospital recorded of 2160 OPU procedures in 1132 women were reviewed for their ethnic origin, results of the coagulation screening tests and procedure-related bleeding events (BEs). All women with abnormal coagula-
tion tests and a randomized control group (1:3) were asked to complete a bleed-
ing questionnaire (BQ) through a telephone interview.

Results: Abnormal coagulation tests were found prior to 78 OPU (3.6%, 95%
CI 2.9%-4.5%). Significant BEs occurred in 8 OPU (0.4%), 6 with normal co-
agulation tests (0.3%) and 2 with abnormal coagulation tests (2.9%) (P = 0.029).
Telephonic BQ was completed in 108 women (46%). The mean bleeding score or the frequency of other BEs were not different between those with abnormal coagulation tests and controls.

Conclusions: Although abnormal coagulation tests were associated with a BE, the practice of screening is questioned. First, most BEs occurred with normal coagulation tests and second, more than 1000 screening tests were needed in order to prevent one case of bleeding. In this study neither the classification by ethnic origin nor the BQ were found useful in predicting occurrence of a BE.

P-338 Poor adherence to the guideline on recurrent miscarriage: identification of barriers
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Introduction: Several national and international guidelines have been published on recurrent miscarriage to bridge the gap between data obtained in clinical research and daily clinical practice. These guidelines are poorly implemen-
ted in daily clinical practice [1]. To ensure proper implementation, the aim of our study was to identify existing barriers and facilitators for guideline ad-
herence according to professionals and patients.

Methods: Qualitative focus group interviews were performed with 17 gyna-
ceologists (2 groups), 13 registrars in Obstetrics and Gynaecology and six
fertility doctors (2 groups) and 6 clinical geneticists (2 groups). All interviews were supervised by an independent chairman. Individual semi-structured in depth qualitative interviews were performed with 10 patients. Reports from the interviews were analysed and the identified barriers and facilitators were
categorised in four domains, including characteristics of: 1) the guideline, II) professionals, III) patients, IV) organisation.

Results: 96 potential barriers, at all four domains, were identified among pro-
fessionals. The most prominent barriers per level were I) poor availability of the guideline in the consultancy room, II) professionals having difficulties refusing demands of insistent patients, III) being unable to overrule standard laboratory applications in individual patients, IV) assumed lack of knowledge by the pa-
tients about their family history. Barriers mentioned were comparable between gynaecologists and residents. An extra barrier experienced by the residents on professional level was the opinion of their supervising gynaecologist, who might overrule recommendations from the guideline.

Patients mentioned 40 barriers, of which most frequently: I) Patient informa-
tion is not up to date with guideline for professionals, II) professionals hav-
ing too little interest and motivation to solve patient’s problem, III) Too little communication between the different specialists involved, IV) Patients having the desire to try every test, even if they have not shown to be statistically ef-
f ective.

The potential facilitators identified were immediate availability of the
guideline or local protocol derived from the guideline, an electronic decision

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these women were sent a postal questionnaire about their ongoing fertility wish, pregnancies and health status. In case we failed to collect the questionnaire or when the answers were inconsistent, patients were approached telephonically. Primary end point was time to live birth. Secondary end points were miscarriage, immature delivery, extra uterine gravity, multiple pregnancy and intra-uterine fetal death/stillbirth as well as chronic illness.

**Results:** The response rate was 82% (n = 138). Of these, 69 women had originally been allocated to the electroacupuncture strategy and 69 women to rFSH treatment. After 10 years of follow-up the cumulative live birth rate was 91% in women who had undergone electroacupuncture and 84% in women treated with rFSH (P = 0.35; log rank test), resulting in a rate ratio of 1.1 [95% confidence interval: 0.96 to 1.23]. The spontaneous pregnancy rate during follow-up was higher in the electroacupuncture group (RR 1.7; 95% CI: 1.1 – 1.6). For women not using acupuncture and a pregnancy wish, no differences were observed in the birth of a second live born child, 90% after LE0 and 85% after rFSH (RR 1.1; 95% CI: 0.9 to 1.3). There was no evidence for differences in occurrence of chronic illnesses, i.e. diabetes type II, heart vessel disease, hypertension and thyroid dysfunction (17% versus 25%; RR 0.7; 95% CI 0.4 to 1.4) between the treatment groups.  

**Conclusion:** In women with clomiphene citrate resistant PCOS, electroacupuncture results in at least a comparable number of live births as primary treatment with rFSH without any negative long-term effects.

**P-340 Prediction of excessive response from baseline characteristics and several ovarian reserve tests: a multivariate approach**

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**Introduction:** In IVF, excessive response to FSH stimulation for IVF may introduce the risk of abdominal discomfort, painful follicle aspirations and cycle cancellations. Moreover, in case of an excessive response, the chances for pregnancy tend to become decreased. This decrease is most likely caused by detrimental effects on the development of large quantities of follicles and concomitant supraphysiological hormone levels on oocytes and embryo quality. The maturation of an excessive number of oocytes will bring the patient at risk of developing ovarian hyperstimulation syndrome (OHSS), a potentially life threatening condition. Up to 30% of IVF cycles are associated with complaints of mild or moderate OHSS and 3-8% is associated with the severe form of OHSS. The current meta-analysis with original, individual patient data (IPD) aims to assess the value of ovarian reserve tests, such as FSH, AFC and AMH in the prediction of an excessive response after ovarian hyperstimulation and their added value on baseline characteristics such as female age.

**Material and Methods:** We included original data of 29 studies presenting information on ovarian response to hyperstimulation, at least one ovarian reserve test (FSH, AFC or AMH) and one or more patient characteristics. An excessive response was defined as ≥ 15 oocytes retrieved. ROC curves were constructed to assess the predictive accuracy of the baseline characteristics and these ovarian reserve tests in the prediction of an excessive response. Moreover, the added value of ovarian reserve tests on baseline characteristics, especially female age, was analysed.

**Results:** Data of 5757 cases were available for analysis. Data from 3950 women were suitable for excessive response prediction, of these women 14% showed an excessive response. The prediction of an excessive response based on age alone was modest, with an AUC of 0.63. In younger or older women, the predictive capacity of age did not improve. Of all baseline characteristics, age was the strongest predictor of an excessive response. The other baseline characteristics BMI and duration of subfertility did not have any predictive accuracy in the prediction of an excessive response. Moreover, female age has a poor predictive accuracy in the prediction of an excessive response. Furthermore, the AFC has a moderate predictive accuracy and AMH is the only adequate predictor of an excessive response. Furthermore, in a model with female age, AMH is still the best predictor of an excessive response, although the accuracy does not increase from female age alone.

**P-341 Analysis of DNA methyltransferases in human oocytes and pre-implantation embryos**

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**Introduction:** We investigated the spatiotemporal expression pattern of different DNA methyltransferases (DNMTs): DNMT3L, the de novo methyltransferase regulator, and DNMT1, an enzyme responsible for maintenance of the methylation patterns during DNA replication. There are two forms of DNMT1: DNMT1o, found only in oocytes and cleavage stage preimplantation embryos and the somatic form DNMT1s. These proteins are crucial for the establishment and maintenance of epigenetic marks during gametogenesis and embryonic stages. DNMT deficiencies will lead to embryonic developmental defects, cancer and other diseases. Not much is known about the expression and regulation of these proteins in human oocytes and preimplantation embryos and we will compare our results to data obtained in mouse.

**Material and Methods:** Sparse human oocytes and preimplantation embryos from infertility treatments at our centre were donated after informed consent of the patients. Oocytes and embryos were stained by immunocytochemistry with antibodies against DNMT3L and two different epitopes of DNMT1 to distinguish both forms. One DNMT1 antibody is specific for the N-terminus, and thus binds only to DNMT1s, the other binds to the C-terminus of both proteins. Immunofluorescence was visualised with a confocal laser scanner for 3D images.

**Results:** DNMT3L was absent in oocytes (n = 13), zygotes (n = 2) and 4-cell stage embryos (n = 9). It was observed in the cytoplasm of blastomeres from the 8-cell stage onwards. In expanding and expanded blastocysts (10/18) DNMT3L was found in both the cytoplasm and nucleus of cells from trophoectoderm and inner cell mass.

DNMT1o and DNMT1s could not be detected in the majority of human oocytes (n = 37) while a weak DNMT1o expression could be detected in most zygotes (n = 7). DNMT1s was mainly found in the cytoplasm from the 2-cell stage onwards. The expression of DNMT1o was absent in most 8-cell stage embryos. This pattern seems opposite to mice, where DNMT1s is expressed in the cytoplasm and nucleus of cells from trophectoderm and inner cell mass.

DNMT1o and DNMT1s were not distinct at the time of blastocyst expansion. DNMT3L appears in the nucleus of the cells where it may assist DNMT3a and DNMT3b in de novo methylation processes. These results are in line with mice. A sharp rise in Dnmt3L was found before the time of implantation, which is the time window for genome-wide de novo methylation processes. In mice, Dnmt3l has been found at early stages of oogenesis. We have not been able to examine this in humans because we do not have access to growing human oocytes yet.

The absence of DNMT1o in oocytes of different maturity stages together with its weak expression in zygotes could reflect the superior quality of oocytes suitable for fertilization and bring forward the hypothesis that Dnm1 expression is only associated with maturation competency. Alternatively, maternally stored Dnmt7 mRNA transcripts are translated at the zygote stage. The absence of nuclear Dnmt1 expression during the first two cleavage divisions may help the passive genome-wide demethylation process that takes place in the early embryo. After the 4-cell stage, the protein was also detected in the nucleus.

The differences in spatiotemporal expression patterns between human and mouse for DNMT1 may be due to differences in the demethylation reprogramming process while the similarities found for DNMT3L point to a better homology for the de novo methylation process.
P-342  Differential regulation of vascular mediators by hCG versus GnRH agonists

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Introduction: OHSS still remains a complication of assisted reproduction treatments. hCG administration to trigger final oocyte maturation will release vascular mediators, being VEGF and other proteins such as VE-Cadherin or Angiopoietin-2. It has been shown that replacing hCG by GnRH agonists will induce a very short endogenous LH peak, potent enough to induce final oocyte maturation but no OHSS will develop. We examined VEGF, VE-Cadherin and Angiopoietin-2 modulation by hCG as well as GnRH agonists in oocyte donors undergoing controlled ovarian stimulation with antagonist protocols.

Methods: Prospective cohort study between June of 2008 and January of 2009 was performed. Donors were recruited and allocated to receive either hCG (n = 26) or GnRH agonist (n = 32) after COH with antagonist protocol with 150 IU rFSH as starting dose for triggering oocyte maturation. The two treatments were allocated in a 1:1 ratio, but six donors from the group receiving hCG were excluded from the study. Blood was collected the day of hCG/GnRH administration as well as the day of egg retrieval, and follicular fluid from the first two mature follicles was also frozen. We collected granulosa cells (GC) from each group as well Levels of VEGF, sVE-Cadherin and Angiopoietin-2 were determined by ELISA in serum and in follicular fluid. VEGF, Ang-2 and VE-cadherin gene expression were determined by RT-PCR.

Data are expressed as mean ± SEM. Metric variables were analyzed by the independent t-test, and nominal variables by X² test. A significant difference was defined as p < 0.05.

Results: VEGF serum concentrations the day of HCG/aGnRH were not different between both groups. However, a statistically significant increase in VEGF follicular fluid concentration was found in those women that received hCG to trigger final oocyte maturation compared to women that received GnRH agonist (1395 ± 284 vs1069 ± 354 pg/ml, p < 0.01). As similar trend was observed in the VEGF mRNA expression in granulosa cells from the group that received GnRHα compared with donors that received hCG (7.9 ± 0.7 vs 6.9 ± 1.7 p < 0.05).

There were no differences between the levels of Angiopoietin 2 in follicular fluid neither in serum the day of egg retrieved and the day of HCG/aGnRH.). The expression of mRNA of Ang-2 in granulosa cells was comparable in both groups.

Conclusions: We have confirmed that by inducing final oocyte maturation with either hCG or a single bolus of GnRHa, differential regulation of different vascular mediators take place. The differential regulation of vascular proteins such as VEGF, VE-Cadherin and Ang-2 by hCG may explain the safety of protocols avoiding HCG to reduce OHSS.

P-343  Neonatal outcome of 995 children conceived after embryo biopsy compared to children born after intracytoplasmic sperm injection

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Introduction: Preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS), embryo biopsy is an invasive essential procedure. The major objective of this study was to determine if the embryo biopsy might affect health outcome of children. The applied biopsy technique through aspiration of 1 or 2 blastomeres was the same in all PGS/PGD conceptions. A control group of children born after intracytoplasmic sperm injection (ICSI) with embryo transfer on day 5, similarly to the procedure after PGS/PGD was included, to determine whether potential differences in children’s outcome could be exclusively attributed to the embryo biopsy. Data on outcome at birth are reported here.

Materials and Methods: A prospective longitudinal follow-up study on medical outcome of all children born after embryo biopsy at the Centre for Reproductive Medicine of the UZ Brussel has been undertaken since 1993 using the same protocol as for the follow-up of children born after ICSI in the same centre. Data on pregnancy and birth were obtained through written questionnaires. The children were examined and checked for possible major anomalies at 2 months of age by trained clinical geneticists whenever possible. Malformations were classified according to criteria previously defined at our centre. A major malformation causes functional impairment and/or requires surgical correction. Mean term, birthweight, major malformations, perinatal death and the number of neonatal hospitalizations were compared for both groups. Statistical analysis included the Fisher’s exact test for comparison.

Results: Data on medical outcome of 995 children (670 singletons, 308 twins and 17 triplets) born after PGS/PGS were compared with 1507 children (1059 singletons, 433 twins and 15 triplets) conceived after intracytoplasmic sperm injection (ICSI) at our centre between 1993 and December 2008. No statistically significant differences regarding mean term, prematurity (term <37w), mean birth weight, very low birthweight (<1500g), major malformations and neonatal hospitalizations in singletons and multiples were observed. Less singletons were very premature (term <32w) after PGS/PGS (p <0.001). Less multiples had a low birthweight (< 2500g) after PGS/PGS (p = 0.005). Perinatal death was more frequent in multiples born after PGS/PGS (p = 0.003).

Conclusion: Embryo biopsy is not adding risks to the health of singleton newborn PGS/PGS children. Multiples born after embryo biopsy appear to be at a lower risk for low birthweight or preterm birth compared with ICSI multiples. As it is suggested that infertility is a contributor to adverse outcomes, further research is warranted to clarify whether the better outcome of children born after PGS/PGS could be related to the absence of infertility in many couples undergoing PGS. The higher perinatal death rate in PGS/PGS multiples in comparison to ICSI multiples, needs to be confirmed in other follow-up series on further explored. Data on long-term health all PGS/PGS children are needed.

P-344  Early progesterone cessation after in vitro fertilization

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Introduction: There seems to be a general consensus on the supplementation of progesterone (P4) for luteal phase support (LPS) to all women after in vitro fertilization (IVF) treatment. However, there is no agreement about the precise duration of LPS. Also, by competitively inhibiting the conversion of testosterone to dihydrotestosterone, prenatal P4 use has been related to teratogenic effects. The objective of the study is to investigate the effect of early cessation of progesterone for LPS after IVF treatment on the pregnancy outcome, with special interest in determining the miscarriage rate and episodes of bleeding between the date of the first ultrasound (US) and up to 12 weeks of gestation.

Material and Methods: A total of 169 patients were recruited for the study from our university associated reproductive medicine private center. All of them achieved pregnancy after controlled ovarian hyperstimulation (COH) using GnRH analogues and fresh embryo transfers. All patients received P4 200 mg vaginally b.i.d. starting one day after oocyte retrieval. We only included patients for the present study that showed an intrauterine gestational sac in their first US and randomized them using a computer generated list. The study group (n = 88) stopped receiving P4 on the day of their first US at 5 weeks pregnancy, and the control group (n = 81) continued receiving P4 up to 8 weeks of pregnancy. Both groups had a mean age of 35 years.

Results: The miscarriage rate up to 12 weeks of gestation was 4.5% (4/88) in the study group and 4.9% (4/81) in the control group. On the other hand, there were more frequent episodes of bleeding in the study group (23.8% or 21/88) compared with the control group (16% or 13/81). Both groups showed more frequent episodes of bleeding during week 6 of pregnancy, and also the pregnancy loss was more frequent during week 7. There were 15 twin gestations in the study group, compared with 12 in the control group.

Conclusions: There was a higher percentage of bleeding episodes in patients who stopped receiving P4 earlier, but it does not appear to have an impact on the miscarriage rate. Thus, P4 supplementation can be safely withdrawn at the time of the first US.
P-345 Disclosure decisions in families with oocyte donation children born during a 15-year period

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Introduction: Worldwide there is an increasing number of families created by oocyte donation (OD). However, follow-up studies on these children and their families are few. Thus far, the studies available on OD parents' attitudes towards disclosure have included a small number of families from 17 to 92, and in these studies 26-81% of the parents intended to disclose information on their child's conception. The primary aim of this study was to gather information about the parents' plans for disclosure and secrecy issues, as well as satisfaction with infertility treatment decisions, in a complete cohort of families with an OD child born after 1992.

Materials and Methods: A questionnaire with separate material for each partner was sent to all parents (167 mothers, 163 fathers) that had had a child after treatment with donated oocytes at the Family Federation of Finland (Viäestöliitto Fertility Clinics) in Helsinki in 1992-2006. These parents had altogether 231 children aged 1-14 years. One mother and three fathers had died during the years. Seven mothers and nine fathers were excluded either because they had refused later contact from the clinic, there was no address available, or because of language problems. Parents were asked if they had told or intended to tell their child about his/her origin and when they had done so and about the reasons to disclose or not to disclose. Other questions were on openness regarding other people, possible difficulties accepting OD treatment, concerns about the donors' characteristics, counselling, and feelings towards the child.

Results: Response rate among the mothers was 67.7 % (113/167) and among the fathers 61.4 % (100/163). The answers provided information on 70.9 % of the children included in the study (164/231). The mean age of the women respondents was 44 years (range 25-57 years) and that of the men 45 years (range 25-61 years). Of the couples, 85% received oocytes from an anonymous donor, and 17 couples had a known donor. Of the mothers, 61.1% and 60% of the fathers had told or intended to tell the child about his/her origin and when they had done so and about the reasons to disclose or not to disclose. Other questions were on openness regarding other people, possible difficulties accepting OD treatment, concerns about the donors' characteristics, counselling, and feelings towards the child.

Conclusions: About half of the parents were satisfied with the amount of counselling they had received. The majority of the parents (81%) had received the information when they were 3-6 years of age and the rest at 7-9 years of age. There was a statistically significant difference between parental telling in different age groups of children (P < 0.05, X²). In the youngest age group (1-3 years), 83.3% of parents were inclined to disclosure compared to 44.4% in the oldest age group (13-14 years). Of the mothers, 86.7% and 71% of fathers had told other people about their child's conception. However, only one third of these parents had already informed their child. The majority of parents were not concerned about the characteristics of the donor. All mothers and fathers reported that the child felt like their own. About half of the parents were satisfied with the amount of counselling they had received. A higher proportion of the mothers (24%) compared to fathers (11%) thought that the psychological support was insufficient (p < 0.05). They thought that discussions with a psychologist should be arranged routinely after delivery or when it was time to inform the child. Forty-eight mothers (42.5%) and 22 fathers (22%) were prepared to participate in OD patient support groups.

P-346 Single embryo transfers in oocyte donation programme: should this be the rule?

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Introduction: Like in IVF/ICSI, multiple pregnancy is a risk to consider in oocyte donation programmes (OD). Recipients have excellent pregnancy rate due to donor’s age which is associated with the good quality of the oocytes and subsequent embryos. Due to the number of embryos replaced, multiple pregnancy rate is high in OD programmes and there is an increased risk of obstetric and perinatal complications due to the age of the recipients.

The aim of this study is to compare the results obtained in terms of pregnancy (PR) and live birth rates (LBR) in single embryo transfer (SET) versus double embryo transfer (DET) in our OD programme. The cumulative pregnancy and cumulative live birth rates including those resulting from frozen-thawed embryo transfer (FET) have also been analysed.

Material and Methods: Retrospective analysis of 874 fresh OD cycles performed between January 2000 through December 2007 (synchronous). Donations were anonymous and the age of the donors ranged from 18 to 35 years. The upper age limit to receive oocytes was 50 years old. In all cycles, at least three good quality embryos were available for transfer. We compared the results obtained in 58 women that carried out SET with 816 women who performed DET. The reasons to perform SET were the patients’ wishes or medical conditions.

Following fresh embryo transfers, a total of 666 FET were performed: 69 in the SET group and 597 in the DET group.

Analysed Variables: donors' and recipients' age, number of oocytes retrieved, insemminated oocytes, fertilized oocytes, good quality embryos available and frozen embryos. Clinical pregnancy rate per transfer, live birth rate per transfer, cumulative pregnancy and cumulative live birth rates were compared between the two groups.

Statistical analysis: The Chi-square test was used to compare fresh pregnancy rates, means were compared employing the t-Student test. The Kaplan-Meier survival analysis was used to estimate the cumulative pregnancy and live birth rates. The LogRank Test was used to compare these results between SET and DET groups.

Results: The mean age of the oocyte donors was similar in the SET and DET groups (26.8 ± 4.9 vs 26.7 ± 4.4), respectively. However, the mean age of recipients was lower in the SET group (38.0 ± 5.8) than in the DET group (41.0 ± 5.3) (p < 0.05).

The PR was lower in SET group (25/58, 43.1%) than in DET group (463/816, 56.7%) (p < 0.05). The multiple pregnancy rates in DET group was 38.4% (178/463) while no multiples arose as a result of SET (0/25), 0% (p < 0.05). Moreover, the LBR were comparable in the two groups (SET: 20/58, 34.5%, DET: 359/816, 44.0%, n.s.). In the FET cycles the mean number of embryo transferred were similar in the two groups (SET: 1.9 ± 0.5 vs. DET: 2.0 ± 0.7) (n.s.). In the SET group the estimated cumulative pregnancy rate in one year was 84% while 77% (n.s.) in the DET group. Similarly, the estimated cumulative live birth rate in one year was 75.9% in the SET group and 63.4% in the DET group (n.s.).

Conclusions: The estimated cumulative pregnancy and live birth rates in a period of one year are similar in SET and DET in our OD programme.

According to our results, it seems that SET policy can be applied successfully in an oocyte donation programme reducing the risk of multiple pregnancies without affecting the cumulative clinical outcome.

P-347 Measuring patient centredness, the neglected outcome measure in fertility care

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Introduction: Reproductive medicine mainly focuses on pregnancy rates and multiples as measures for quality of care. However, high-quality fertility care should not only be effective and safe, but also patient-centred. Because of the substantial emotional and physical burden of fertility treatments, patients benefit by care that is tailored to their individual needs. Patient centredness, also called ‘quality through patients’ eyes’, is ideally monitored by measuring patients’ experiences. Presently, there is no suitable measurement instrument for patient centredness in fertility care. It is thus unclear how patient-centred current fertility care is, and what care aspects need improvement. Therefore, this study aims to develop a valid, reliable, and widely usable instrument (Patient Centredness Questionnaire-Infertility: PCQ-Infertility), that can measure patient centredness in fertility care.

Material and Methods: Seven focus group discussions with a total of 54 patients were used to conceptualize patient centredness within the fertility care context. These patients originated from three Dutch regions (East, West, and North) and were undergoing various fertility treatments. Each focus group was recorded and transcribed. Using an established framework, 729 relevant quotes were extracted from the transcripts. These quotes yielded 81 care aspects that should be fulfilled in high-quality fertility care according to patients.

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Depending on their frequency and intensity, care aspects were selected for the PCQ-Infertility. For each of the 53 remaining care aspects one ‘experience item’ and one ‘importance item’ was formulated. Subsequently, 20 background questions were added to the questionnaire. The PCQ-Infertility was pilot tested among patients and care professionals. In the above-mentioned three regions, 30 fertility clinics were invited for participating in the validation study. A random sample of 1189 subfertile couples, taken of all patients with a fertility treatment between April and June 2009, received a questionnaire. The PCQ-Infertility was psychometrically tested by inter-item analyses, reliability analyses and importances scores. Experience scores and importance scores per item were calculated. Using multivariate multilevel regression analysis (p < 0.05), we examined if adjustment for background characteristics was necessary when measuring patient centredness. The discriminative power of the PCQ-Infertility was determined by calculating the intra-cluster correlation coefficient (ICC).

Results: Twenty-nine clinics participated. A total of 888 couples (75%) filled out the questionnaire. Participants’ treatment was in 51% IVF/ICSI, in 41% insemination, and in 7% ovulation induction. Their median duration of infertility was 34 months. Analysis determined there were seven domains in which patient centredness could be reliably measured: Accessibility; Information; Communication; Respect for patients’ values; Continuity of care; Autonomy; and, Competence. Seven experience items did not survive the psychometric tests, making the final PCQ-Infertility being composed of 46 experience items, apart from the background questions. Averagely, ‘Communication’ was the most positively experienced domain, ‘Accessibility’ the least. The most important care aspect was ‘Sincerity on what to expect from the fertility care service’. For improving patient centredness, ‘supplying couples with a tailored treatment plan and schedule’ should have the highest priority, as this care aspect was scored as highly important yet insufficiently met. ‘Woman’s education’, ‘partner’s gender’, ‘treatment type’, and ‘being pregnant’ were significantly associated with the experienced patient centredness. After adjustment for these factors, (quality) differences between participating fertility clinics appeared to be responsible for 15.5% of the total variance in patient centredness.

Conclusions: This large, multicenter study resulted in a valid, reliable, and strongly discriminating instrument for measuring patient centredness in fertility care. As the PCQ-Infertility can identify clinics’ main shortcomings on patient centredness, it can be adopted for improving the quality of care. And from now, quality of fertility care can not only be monitored and benchmarked on live birth and complication rates, but also on patient centredness.

P-348 Luteolysis will prevent OHSS development in IVF cycles in which ovulation was triggered with a GnRH agonist, independently of estradiol serum levels

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Introduction: In IVF cycles, E2 levels and number of oocytes recruited are considered the two main predictors of the hCG-dependent ovarian hyperstimulation syndrome (OHSS). Lately, GnRH agonist has been introduced as an alternative to hCG for final oocyte maturation (in GnRH antagonist cycles) as an effective way to prevent the development of OHSS have not been evaluated. Thus, the objective of the present study was to evaluate the potential genotoxic activity of three cryoprotectants extensively used at high concentrations for vitrification in ART: dimethyl sulfoxide (DMSO), ethylene glycol (EG), and propylene glycol (PrOH). Some established cell lines, especially those developed from Chinese hamsters, are commonly used in chromosome aberration assays with well-standardized protocols.

Material and Methods: Assays were performed on the well established CHO-K1 (Chinese hamster ovary) cell line, commonly used in genetic toxicology. This cell line, is characterized by a relatively good genetic stability and by a short generation time. Some carcinogens are biologically inactive unless they are transformed into DNA-reactive electrophilic metabolites by the cytochrome P450 oxidation systems, which are present in the liver. Thus, to mimic the in vivo transformation of cryoprotectants, we added to the assays an exogenous mammalian activation system called S9 Mix.

After exposure to different concentrations of cryoprotectants (2.5%, 5%, 7.5%, 10%, 15%), without and with S9 Mix, two tests were performed to assess the genotoxicity of each cryoprotectant:

1. Alkaline comet assay to evaluate the capacity of cryoprotectants to induce DNA strand-breaks. This technique is based on the measurements of denatured DNA fragments migrating out of the cell nucleus during electrophoresis. The resulting image obtained with this technique is a “comet” with a distinct head consisting of intact DNA, and a tail containing damaged or broken pieces of DNA.

2. Micronucleus assay. Micronuclei are defined as chromosome fragments or whole chromosomes that lag during cell division due to the lack of a centromere or to a defect in cytokinesis. Micronuclei may be produced by clastogenic or aneugenic compounds. The micronucleus assay allows the scoring of micronuclei in the cytoplasm of interphasic cells exposed in vitro or in vivo to clastogenic and/or aneugenic agents.

For each experiment, two controls were examined: a negative control in the culture medium and a positive control with CHO cells exposed to a well-known genotoxic compound.

Results: Results showed that DMSO was not genotoxic. EG did not exert a direct genotoxic activity, but EG metabolites obtained in the presence of an external cytochrome-based P450 oxidation system (S9 Mix) exhibited significant genotoxic and clastogenic activities. PrOH produced in vitro DNA damage leading to chromosome mutations in the presence and the absence of S9 Mix.

Conclusions: The use of the CHO-K1 cell line, validated in genetic toxicology, was the first step for the study of the long-term effects of the cryoprotectants. Our results have shown that PrOH may cause long-term adverse effects in eukaryotic cells, and suggest that there is a potential genotoxic risk. The genotoxicity of the PrOH gives place to contradictory publications. That’s why, additional studies on the genotoxicity of cryoprotectants and vitrification techniques must be performed on germinal cells and embryos.

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P-350 Impact of the Spanish fertility society guidelines on the number of embryos to transfer: on the right road, but still far from journey's end

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Introduction: The multiple pregnancy rate in ART cycles depends, fundamentally, on the number of embryos transferred. It is essential that patients and professionals should have good practical guidelines on the best number of embryos to be transferred in each cycle in order to obtain high pregnancy rates with minimal risk of multiple pregnancies. The purpose of this study is to analyze the impact made by the Spanish Fertility Society (SEF) guidelines on the number of embryos to be transferred, as regards the policies adopted at clinics in Spain, and the resulting financial repercussions.

Material and Methods: Data were collected from the Assisted Reproductive Technology register of the SEF and compared over three periods of time: 2002-2003, when there was not legal regulation and no SEF guidelines; 2004, when there was only legal regulation; and 2005-2006, when there was legal regulation and SEF guidelines. An estimation of financial impact was carried out. As the SEF Register only included assisted reproduction clinics, data on deliveries represented approximately 50% of the pregnancies obtained, and so for the cost calculation it was necessary to estimate the number of deliveries on the basis of the percentage of each type of delivery registered during each of the study periods, and from the total number of pregnancies registered in 2005-2006, adjusted by an estimated 18% loss of pregnancies, due to abortions, miscarriages and ectopic pregnancies. In addition, unit costs were calculated taking into account the type of delivery, according to a study carried out in Spain using data for 2004. Under these premises, a budgetary impact analysis was carried out, with the aim of extrapolating the unit cost results to the entire population to whom the recommendations were made, in this case regarding the number of deliveries obtained. The results of the budgetary impact analysis are presented in the form of a bivariate sensitivity analysis concerning the variables with greatest impact on total costs: the occurrence rates for each type of delivery. Thus, we calculated the total incremental cost for each percentage point of multiple delivery avoided.

Results: The degree of acceptance of SEF guidelines varies according to the IVF technique employed. The guidelines have led to a reduction in multiple pregnancy rates, especially concerning triplets, in patients’ own eggs and with donor eggs. Over the three periods, and considering both own and donated egg cycles, the observed percentage of single pregnancy was 69.5% in the 2002-2003 period, 71.3% in 2004, and 74% in the 2005-2006 period. With respect to twin pregnancies, the observed percentage was 28% in 2002-2003, 27.2% in 2004, and 24.9% in 2005-2006. The observed percentage of triplet pregnancy was 2.5% in 2002-2003, 1.6% in 2004, and 1.2% in 2005-2006. The reduction in the financial cost of deliveries achieved in the years 2005-2006 ranges from 890,187 to 18,593,242 euros, and the incremental cost per percentage point of multiple pregnancy avoided is 2,899,613 euros.

Conclusions: Even without full implementation, these results validate the clinical utility of the SEF guidelines. They constitute a useful tool to reduce the incidence of the principal adverse effect of the ART cycles, namely multiple pregnancies.

P-351 Reduced live birth rate in overweight patients compared to normal weight patients undergoing controlled ovarian stimulation

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Introduction: In women undergoing ART, it has been hypothesised that body mass index (BMI) could influence treatment outcome, ovarian response and gonadotrophin consumption. However, different BMI cut-offs as well as pooling of overweight and obese patients contribute to a lack of consistency in the literature.

Material and Methods: This investigation provides treatment outcome data by BMI categories from a study cohort derived from the combination of two large randomised, multicentre, multinational trials comparing highly purified menstropin (HP-hMG, MENOPUR, Ferring Pharmaceuticals) (N = 731) and recombinant FSH (rFSH, GONAL-F; Merck-Serono) (N = 727) [Nyboe Andersen et al. Hum Reprod 2006, 21, 3217; EISG, Fertil Steril 2002, 78, 520]. Patients were undergoing controlled ovarian stimulation for IVF/ICSI following the long GnRH agonist protocol. They were 18-38 years old and were mainly diagnosed with unexplained infertility, tubal disease, male factor infertility or endometriosis. The cohort of patients included in this investigation had a BMI of 18.5-29.9 kg/m²; overweight (BMI < 18.5 kg/m²) and obese (BMI ≥ 30.0 kg/m²) women were not included. The analysis population was distributed according to BMI as follows: N = 1,140 with BMI 18.5-24.9 kg/m² (normal weight) and N = 316 with BMI 25.0-29.9 kg/m² (overweight), with the mean being 21.6 ± 1.7 and 26.9 ± 1.3 kg/m², respectively, in the two categories. Number of oocytes retrieved and live birth data were available for the full cohort (N = 1,417), and embryo quality data were available for a subset (N = 709).

The probability of live birth was modelled using logistic regression including BMI (normal weight / overweight) and age as covariates.

Results: The live birth rate in the fresh stimulation cycle among patients with BMI 25.0-29.9 kg/m² was significantly (p < 0.05) lower compared to those with BMI 18.5-24.9 kg/m²: 18% versus 24%. The age-adjusted odds ratio of treatment resulting in at least one live born neonate was 0.70 (95% confidence interval 0.51-0.96) for overweight women compared to those with a BMI categorised as normal. Ovarian response was similar in the two BMI groups, with an average of 11.7 ± 7.3 and 11.2 ± 7.2 oocytes retrieved in the normal weight and overweight women, respectively. Similarly, the number of top-quality embryos was not significantly different between patients with normal weight (0.97 ± 1.5) and overweight (0.74 ± 1.2). Total dose of gonadotrophin used and duration of stimulation were significantly (p < 0.05) larger among the overweight women compared to those with normal weight, but the differences were of a small magnitude (105 IU and 0.24 days) and not considered clinically relevant.

Conclusions: Overweight women (BMI 25.0-29.9 kg/m²) undergoing IVF/ICSI treatment in a long GnRH agonist protocol appear to have a reduced probability of obtaining a live birth compared to normal weight women (BMI 18.5-24.9 kg/m²). Treatment cycles in overweight patients are associated with slightly more gonadotrophin use, with the ovarian response being similar to that in normal weight patients. The available data do not suggest that BMI influences embryo quality in women with BMI in the range 18.5 to 29.9 kg/m².

P-352 Quality of life measure as an extra tool for delivering patient centred care

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Introduction: Patient-centredness, one of the dimensions of quality of care, encompasses the quality of care through the patients’ eyes. It could be measured by asking patients about their experiences with fertility care. Because of the high emotional impact of being infertile, it would not be surprising if a person’s well being or quality of life (QoL) interferes with the way patients experience care. In this case, patients’ QoL should also be taken into account when tailoring care to their individual needs and identifying patients who need extra attention. The objective of this study was therefore to determine to what extent experiences with health care are associated with the patients’ quality of life.

Materials and Methods: In a large multi-center study, the Patient-Centredness Questionnaire-Infertility (PCQ-Infertility), along with the Dutch version of the FertiQoL-questionnaire, was sent to a random sample (n = 1089) of couples attending 29 Dutch clinics for a fertility treatment. In addition, the Dutch version of the Hospital Anxiety and Depression Scale (HADS) was included to a subset of patients (n = 785). The PCQ, a validated instrument to measure patient-centredness of fertility care, is composed of 46 questions on patients’ ex-
A total of 2487 patients attending our Reproductive Endocrinology - Women’s Reproductive and Health Lab, Hangzhou, China 1

Materials and Methods: We performed these analyses for all subscales of the FertiQoL, HADS and PCQ.

Results: In total, 875 women (74%) completed both the PCQ-Infertility and FertiQoL-questionnaire. The HADS was filled out by 595 patients. Women being pregnant were excluded from analyses (n = 167). Participants’ treatment was in 50% IVF/ICSI, in 41% insemination, and in 6% ovulation induction. Their median duration of infertility was 34 months. The mean FertiQoL total score was 70.8 (SD 13.8) on a scale of 1 to 100. The mean scores on the emotional, mind-body, relational, and social domains were respectively 59.8 (SD 18.7), 70.8 (SD 19.4), 78.2 (SD 14.5) and 74.0 (SD 16.6). QoL of infertile women was associated with all dimensions of patient-centredness, except for ‘information’. Strikingly, the mind-body domain of QoL, which consists of aspects as concentration, physical condition and fatigue, was related to all patient-centredness dimensions, in contrast with the other FertiQoL subscales. The aspects ‘communication’ and ‘competence’ were most strongly related to the mind-body, social and emotional quality of life domains. The relational domain of the FertiQoL was, on the contrary, not related to any of patients’ experiences.

Conclusions: Our study demonstrates that patients’ experiences with fertility care are related to patients’ wellbeing. Patients with a better QoL have more positive experiences with patient-centred aspects of health care (or vice versa). Importantly, the results suggest that a holistic approach to care could potentially reduce short-term effects of treatment on concentration, physical health and interference on day-to-day activities. Measuring the patient’s QoL, anxiety or depression could provide health care professionals with valuable information in tailoring care to patients’ individual needs and thus reaching a higher level of patient-centredness.

P-353 Gene chip analysis of mouse placenta derived from the first and second filial generations of gestational diabetes mellitus

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Introduction: In recent years, more and more studies confirmed the association between intrauterine environment and adult diseases, in addition to effects of genetic factors. The importance of the intrauterine environment of gestational diabetes mellitus (GDM) is highlighted by studies in the general population that indicate an association between poor fetal growth followed by subsequent risk of diabetes in adulthood. As we know, a well-established and functional intrauterine environment of GDM has potential influence on both first and second filial generations. Furthermore, since epigenetic reprogramming begin at the time of gametogenesis and epigenetic abnormality happening during this phase are closely associated with growth and development.

Conclusions: During pregnancy, intrauterine hyperglycemia environment of GDM lead to abnormal gene profiles of placenta in the first-generation offspring. Furthermore, many differential genes were found in the placenta of the second generation offspring of GDM. These differentially expressed genes are valuable for the evaluation of potential association between GDM intrauterine environment and offspring outcome. Transgenerational transmission of differential gene expression may be related to epigenetic regulation.

P-354 Optimizing embryo transfer to reduce multiple pregnancy in a Southern European country: a prospective study

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Introduction: The high incidence of multiple pregnancies is the most frequent complication of Assisted Reproductive Technologies (ART) and their obstetrical, neonatal, psychological and social implications are very important. In Spain, as well as in most western countries, there has been a significant rise in the incidence of multiple pregnancies. According to the National Institute of Statistics (INE), the incidence of twin pregnancies in 1980 was 7/1000 deliveries and in 2007 this figure was 20/1000. As far as triplet pregnancies are concerned, the incidence was 1/10000 deliveries in 1980 whereas in 2000 it was 5/10000. The objective if this study is to corroborate if our internal Multiple Pregnancy Score (MPS) based on age and number of optimal embryos available helps in reducing the occurrence of multiple pregnancy.

Material and Methods: A total of 2487 patients attending our Reproductive Medicine Service were prospectively analyzed from 2005 to 2008. According to our MPS: patients aged < 35 years should receive one optimal embryo (OE) depending upon how many OE are available. Patients 35-37 years old should be transferred always two embryos. We compared the pregnancy rate (PR) and the multiple PR of the patients who did and did not comply with the MPS.

Results: MPS transfer 1 embryo = 642 patients: 251 completed (PR = 40.6%, multiple PR = 0%); 391 patients did not comply with the MPS and 2 embryos were transferred (PR = 57.8%, p < 0.001; twin PR = 28.3%, p < 0.001; triplets PR = 1.3%, p < 0.001). MPS transfer 2 embryos = 1850 patients: 1641 completed (PR = 43.4%, twin PR = 25%; triplets PR = 0.6%); 209 patients did not comply with the MPS so three embryos were transferred (PR = 44.9%, p = not significant; twin PR = 19.6%, p < 0.001; triplets PR = 6.5%, p < 0.001).

Conclusions: The MPS is a validated score which, while allowing good PR, reduces multiple pregnancies. When the transfer of one embryo is recommend, transferring two does increase the PR, however it also increases multiple pregnancy and the two outcomes should be thoroughly discussed with patients. When two embryos are recommended, transferring three does not increase PR, but it does increase multiple pregnancy significantly so patients should be strongly discouraged not to comply with the score.
P-355 Adherence to tailored expectant management in a cohort of subfertile couples

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Introduction: Prediction models for spontaneous pregnancy are useful tools to select subfertile couples that have good fertility prospects and should therefore be counseled for tailored expectant management. Tailored expectant management, i.e. expectant management in couples with a good prognosis, is a cost-effective strategy that is recommended in the subfertility Guidelines of the Dutch Society of Obstetrics and Gynaecology (NVVOG). Not adhering to the guideline leads to overtreatment, which subsequently cause complications and unnecessary costs. The aim of this study is to evaluate the adherence to tailored expectant management when indicated.

Materials and Methods: Between January 2002 and February 2004, consecutive couples presenting at 38 fertility clinics in The Netherlands participated in a prospective cohort study. All couples underwent a basic fertility work-up according to the guidelines of the Dutch Society of Obstetrics and Gynecology. In couples with mild male subfertility or unexplained subfertility treatment independent pregnancy chances were calculated. The study-protocol prescribed tailored expectant management for 6 to 12 months if the 12-months probability of spontaneous pregnancy resulting in live birth were 40% or more. Couples with a prognosis below 40% were counseled for treatment according to the national fertility guidelines. Follow-up started at the completion of the fertility work-up and ended after 12 months. In the present study, we calculated the number of couples that qualified for tailored expectant management together with the adherence to tailored expectant management. We identified factors that may have influenced the decision to deviate from tailored expectant management (chi-square-test).

Results: We included 2,691 couples of whom 1,076 (40%) had a chance on spontaneous pregnancy of ≥ 40% within one year. There were 151 couples (14%) that started treatment within six months and 366 couples (34%) started treatment within 12 months.

Factors that were associated with an early start of treatment were previous miscarriages (RR 0.7 CI: 0.5-0.9). Female age, duration of subfertility or subfertility being primary or secondary between were not associated with early treatment

Conclusion: We found that our guideline of expectant management in couples with good prognosis, when embedded in a nationwide prospective cohort study, was relatively well implemented. We conclude that tailored expectant management is a feasible option in subfertile couples.

P-356 Modelization of growth between birth and 6 years of age in children born after ART in a French monocentric cohort compared to references growth curves

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Background: Long-term health studies of ART Children are still needed, especially to evaluate the epigenetic impact of in vitro conception. In early 2000, some reports were warning the medical community about an increase of epigenetic pathologies. The frequency of identified syndromes, as Prader Willy or Angelman, seems not to be confirmed. Nevertheless, the impact of DNA regulation can be partially evaluated by studying the growth of individuals as a reflection of good health. French health authorities recommend follow-up examinations for in vitro fertilization children. Procedures were set-up in the hospital of interest to ensure follow-ups would take place. We can now report 15 years of follow-up experience. The quality of the follow-up is a crucial condition to allow the drawing of accurate conclusions; it’s why we decided to conduct a monocentric study to avoid difficulties in data collection. Each couple in our centre is informed before the ART attempt that a medical follow-up will be conducted after the birth, on a voluntary basis.

Aim: The aim of this methodological study is to describe the tool of surveillance based on data from children’s health records and to construct the first IVF children growth curves. The procedure was initiated in 2004. The collection of existing data for every child born is gathered from the files of the attempted IVF and medical reports of the pregnancy survey. The retrospective data was collected by mail. For babies born after 2004, information regarding the importance of the study allowed us to reach a participation rate of 85% of parents who agreed to allow the use of documentation, including medical files when necessary, for the study. Parents were asked to complete questionnaires and provide their child’s personal health records. The quality of the data was medically evaluated using prenatal and postnatal analysis. The longitudinal anthropometric data of the health record was used to describe the percentiles of weights, size and the body mass index from birth to 6 years old for children in this historic population, at age-specific reference intervals. In this way, we collected a population for the study similar to those for the referenced growth curves.

Results: The follow-up included 2081 children born since 1995 with a response rate of 68.9 %. A brief summary report of the cohort was created with a group of 1053 children aged five years and above. 225 representative personal health records were provided allowing the estimation of quintiles curves for anthropometric data. We selected this specific population to study the adiposity rebound in children as a simple indicator for predicting obesity. We controlled the representation of this subpopulation in terms of sex, ART technique, multiple pregnancy rate, in particular twins, preterm parturition, percentile of birth weight, and the characteristics in age and weight of the concerned parents before the analysis. The differences between monoyzotic birth and twins were noted.

Conclusion: The procedure adopted for the in vitro fertilization children follow-up responds to qualitative health requirements that were fixed and provides many benefits without harming the children involved. Collection of information from personal health records allowed the exploitation of growth data by including the calculation of anthropometric percentiles in this IVF population. This report represents the first set of IVF child growth standards and describes the methods used to construct the standards. We found statistical differences between our population and the population of naturally conceived babies born, especially in height. The data also shows that the BMI chart was preserved leading to a final report of harmonious growth for these children. To validate these findings, we propose a comparison with an actualised control sample to eliminate a simple difference due to the ageing of the control group.

P-357 Investigation of methylation and gene expression in the placenta of pregnancies conceived by assisted reproductive technologies (ART)

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Introduction: The invasiveness of the ART procedure has previously shown to have effects on the genetics of children which are procured by them. More recently, there has been heightened interest in the epigenetic consequence of ART. Although a few recent studies have looked at methylation alteration in ART conceptions, no study to date has looked at alterations in gene expression in the placenta of ART conceptions mostly because of the difficulty involved in the rapid acquisition and preservation of tissue required for such analysis. Two important imprinted genes that are involved in proper fetal growth and placental development and thus healthy pregnancy are IGF2 and H19. Here we look at changes in DNA methylation of ICR1 (a site believed to be the imprinting control region of IGF2 and H19) in the placenta of ART patients and correlate that data with expression of both IGF2 and H19 in the same patients. These results not only shed light on an expression study that has yet to be done, but also show epigenetic differences between the IVF and ICSI groups.

Materials and Methods: A total of 20 placentas were collected (8 - ICSI, 8 - IVF, 4 - natural conception). For expression analysis, placental tissue was biopsied and stored in RNAlater solution (Sigma) within 30 minutes of birth, and then stored at -80C for long term storage. RNA was extracted from these samples using the RNeasy® Mini Kit (Qiagen), and was then converted to cDNA using the GE First Strand cDNA Synthesis Kit. Expression analysis was performed on the prepared samples by quantitative real time PCR. For methylation analysis, DNA was extracted from placental tissue by standard salt-out methods, followed by bisulfite modification using the EZ DNA Methylation-gold Kit (Zymo Research). The modified DNA was amplified using HotStarTaq Polymerase (Qiagen) and then subjected to pyrosequencing using a primer
covering the distal end of ICR1. Pyrosequencing was performed in two replicates for each sample.

Results: Real-time PCR analysis revealed changes in gene expression in both the IVF and ICSI group when compared to controls. IVF and ICSI patients both showed an increase in H19 gene expression (by a factor of 1.75 ± 0.21 and 1.83 ± 0.46, respectively) while showing a decrease (by a factor of 0.92 ± 0.12 and 0.85 ± 0.08, respectively) for the expression of IGF2. Statistical analysis done via ANOVA showed the changes in H19 expression for both groups to be significantly different as compared to controls, but not those in IGF2. Furthermore, methylation analysis of the key region predicted to control the expression of these two genes (the ICR1) did not reveal a statistical difference via ANOVA (70% ± 10.2% for IVF; 63% ± 5.4 for ICSI and 64% ± 4.6 for control).

Conclusions: These results give us a first glimpse into placental expression patterns observed in individuals that undergo ART. Both IVF and ICSI placenta showed significantly higher H19 expression as compared to the controls. H19 is a noncoding RNA transcript that is implicated in the regulation of IGF2 expression. However, only slight decreases in IGF2 expression was observed in both IVF and ICSI placenta. Furthermore, although imprinting of H19 and IGF2 is thought to be controlled by ICR1, we did not observe any difference in DNA methylation in this region in IVF and ICSI placenta compared to that of controls. Our preliminary results suggest that additional factors other than DNA methylation at ICR1 may influence the expression of H19 and IGF2; these findings warrant further investigation using a larger sample size.

P-358 Evaluation of gene environmental interaction in pregnancies with intra uterine growth retardation

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Introduction: Intra uterine growth retardation (IUGR) is a complication of pregnancy often described as small for gestational age (SGA), affecting 5-10% of newborns. It is associated with substantially increased infant mortality as well as childhood and adulthood morbidities such as increased risk for cardiovascular disease, obesity and diabetes. While the etiology is poorly defined, IUGR may be a consequence of several detrimental factors occurring during pregnancy. Exposure to environmental chemicals such as organochlorine pesticides (OCPs) has been suggested as a possible etiologic factor for IUGR, but the association remains highly controversial. The glutathione-S-transferase (GST) family of enzymes, being important members of phase II detoxification pathways, catalyzes the conjugation of a variety of electrophilic substances to glutathione, facilitating their elimination from the body. To assess whether GST gene polymorphisms modulate the effect of OCPs in IUGR risk, we conducted a hospital based case-control study among pregnant Indian women.

Materials and Methods: Fifty primiparous women (study group) delivering IUGR babies (birth weight < 10 percentile for gestational age) were included in this study after their admission to Guru Teg Bahadur Hospital, Delhi. Study group was compared with same number of women (control group) delivering healthy normal birth weight term neonates. We have excluded potentially confounding factors such as women of occupational exposure to pesticides and farming communities from this study. OCPs were quantified in maternal and cord blood and genotyping was conducted for the null alleles from GSTM1 and GSTT1. We used linear regression models to measure the association between OCPs and GST gene polymorphisms in IUGR.

Results: After adjustment of potential confounding factors like weight gain during pregnancy, socioeconomic status, gestational age, we found that maternal and cord blood of mothers with IUGR babies had higher levels of \( \gamma \)-hexachlorocyclohexane (HCH) than that of mothers with normal-weight babies (OR = 1.21; 95% CI: 1.05-1.38, p = 0.006 and OR = 1.44; 95% CI: 1.13-1.84, p = 0.003 respectively). Based on genotyping, subjects were categorized into: GSTM1+GSTM1+, GSTM1-GSTM1+, GSTM1+GSTM1-, and GSTM1-GSTM1-. The odds ratio for development of IUGR was significantly higher in GSTM1-GSTM1- group (OR = 2.47, 95%CI = 1.11-5.495, \( \chi ^2 = 5.085, p = 0.024^* \)). Moreover, GSTM1-GSTM1- genotypes showed a statistically significant increased risk of IUGR in combination with high maternal and cord blood levels of \( \gamma \)-HCH.

Conclusions: The results of this study suggest an association between high blood levels of \( \gamma \)-HCH and IUGR and GST gene polymorphisms may modify the relation between environmental exposure to OCPs and IUGR risk. Our data also raised the possibility that we can identify women at high risk of IUGR by taking into account both environmental exposure and gene polymorphisms.

P-359 Shared-cycle ovum donation: having too few oocytes for a secondary recipient might be good news for the primary one

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Introduction: As a feasible solution to the limited number of oocyte donors and the increasingly high costs of third party reproduction, IVF centers often share the costs and oocytes obtained from a single donor between two recipients. As previously reported, outcomes from shared and non-shared ovum donation cycles are not different.1,2 When not enough oocytes are available for a secondary recipient, all of the oocytes are allocated to the primary recipient, who along with the program, bears the responsibility for the increased cycle costs. To date, no published data exist evaluating the outcome of primary recipients in shared donation cycles with poor oocyte yield.

Materials and Methods: In our center, if fewer than 12 oocytes are obtained from a donor in a shared cycle, the secondary recipient’s cycle is cancelled, and all the oocytes are allocated to the primary recipient. We queried our database to identify cycles in which secondary recipients were cancelled due to low oocyte yield to assess the outcome for the primary recipient. Patient demographics and cycle outcomes from these cases were analyzed and compared to our previously reported overall shared and non-shared ovum donation cycles1. Chi-square and Kruskall-Wallis tests were performed when appropriate.

Results: Between June 1st 2002 and October 31st 2009 only 24 shared cycles were identified in which the secondary recipient was cancelled due to a limited number of retrieved oocytes. The average donor and recipient ages (mean ± SD) were 26.7 ± 3.1 and 42.5 ± 4.6 years, respectively. In these cycles, 8.8 ± 1.9 oocytes were retrieved, yielding a 56.8% (120/211) fertilization rate. Transfer of 2.04 ± 0.8 embryos per patient resulted in an implantation rate of 46.93% (23/49) and a clinical pregnancy rate of 70.8% (17/24). Two primary recipient cycles were cancelled due to embryo arrest (8.3%) and 25% (6/24) of patients had optimal quality surplus blastocysts for cryopreservation. When comparing these results to completed shared and non-shared cycles respectively, although a significantly lower number of oocytes were allocated to the recipient (8.8 ± 1.9 vs. 11.8 ± 5.3 (p = 0.0019); and 17 ± 1.6 vs. 17 ± 1.8 (p < 0.0001)) clinical pregnancy rates were not different among the groups (70.8% (17/24) vs. 58.9% (240/399); p = 0.41); and 60.2% (75/128; p = 0.36).

Conclusions: In shared ovum donation cycles with properly selected anonymous donors, cancellation of the secondary recipient due to a low oocyte yield is a rare event. When this situation arises, however, primary recipients can be reassured that the anticipated outcome will not differ from overall shared and non-shared ovum donation cycles.

References:

P-360 64-row multidetector computed tomography virtual hysterosalpingography: Three years of experience

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P-361 Safety, drawbacks and advantages of hysterosalpingocontrast sonography (HyCoSy), as a first line examination for tubal patency evaluation

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Introduction: To assess the safety, drawbacks and advantages of hysterosalpingocontrast sonography (HyCoSy).

Material and Methods: We have examined with HyCoSy 700 infertile women from January 2005 to December 2009, followed at The Centre Of Andrology and Pathophysiology of Reproduction at the “S. Maria Goretti” Hospital, Latina. The patient average age was 33 (22-44) and the infertility average duration was 60 months (range 96-24). 452/632 (71.7%) had a primary infertility, the patient average age was 33 (22-44) and the infertility average duration was 60 months (range 96-24). 452/632 (71.7%) had a primary infertility, the infertility average duration was 60 months (range 96-24) and the differential diagnosis with other pathologies.

Results: Of 700 patients, 632 (90%) returned the questionnaire. The mean numeric rating scale was 4.1 (range 0-9), 15 (2.3%) patients required drug treatment for pain relief. 26 patients (4.11%) have shown mild vaso-vagal reaction, without need to administer atropine. No severe vaso-vagal reactions and late complications (haemorrhage, PID, fever) in our series has been reported. We found 79.5% (503/632) bilateral tubal patency, 8.3% (53/632) unilateral patency and 12.02%(76/632) bilateral tubal occlusion. In 56/632 (8.8%) the HyCoSy was not conclusive and we performed Hycosy to the same patients during the next menstrual cycle. During the second Hycosy was reported less pain from the patients (mean numeric rating scale 2.9 vs 6.4). In 35/56 we found other bilateral patency. 40/632 (6.3%) patients performed a laparoscopy, most for concomitant pelvic pathology (34/40; 85%). In 182/632 (28%) cases we diagnosed an associated pelvic pathology (myomas 15% (95/632); polyps 1.5% (5/632); endometritis 7.1% (54/632); congenital uterine malformations 5.8% (37/632).

Conclusions: Our data support the safety of HyCoSy. The HyCoSy is a well tolerated examination with a very low rate of complications and side-effects. Not any medication is necessary before, during and after the execution of the procedure. Introduction of this procedure, performed by skilled operators, has permitted to evaluate tubal patency as well as uterine and ovarian conditions without exposing the patient to radiation or to the risk of allergic reaction. These data, in line with recent literature, shows as the HyCoSy is an easy execution procedure, a well tolerated examination that not only gives informations on the tubal patency but also on the whole pelvic anatomy. The HyCoSy should be used as a first line examination for tubal patency evaluations.

P-362 Afterloading embryo transfer technique as an alternative to improve clinical pregnancy rate: a prospective randomized clinical trial

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Introduction: Prospective studies about pregnancy rate improvement according to the embryo transfer technique are scarce. The afterloading embryo transfer technique has been described as a refinement of the direct embryo transfer technique that may increase clinical pregnancy rate (Neithardt et al., 2005). However, little is known about the real improvement of afterload embryo transfer (AT) (in which an empty catheter is placed at or just past, the internal cervical os, previous to embryo transfer) compared to direct embryo transfer (DT) as there were no prospective studies. The purpose of this study is to evaluate these two types of embryo transfer procedures and its effectiveness according to clinical pregnancy rate, difficulty and duration of embryo transfer (ET).

Material and Methods: This prospective randomized clinical trial was performed between October 2008 and December 2009 in our IVF/ICSI program. Randomization was realized by the Department of Statistics using a computer-generated list. Allocation concealment was unknowed by clinicians and participants until the transfer procedure. The inclusion criteria were age <43 years and transfer on day 2 or 3 after oocyte retrieval. Randomized participants were 302 (152 AT and 150 DT).

The primary outcome analyzed was clinical pregnancy rate per transfer as defined by the presence of a gestational sac on ultrasound at 6-8 weeks of gestation. Secondary outcomes were difficulty of ET (embryo transfers were scored between 10 –no difficulty- and 0 –maximum difficulty-) and duration of ET (time count was registered starting when embryoologist took the embryos out from the incubator and finishing when ET was completed). Frequency distributions and means were analyzed with the Chi-square-Test or T-Test respectively. All tests were bilateral and with a statistical significance α < 0.05.

Afterload embryo transfer: an empty Wallace RCO catheter was passed to the level of the lower uterine segment under ultrasound guidance to a point where the inner catheter entered the endometrial cavity. The inner sheath was slowly removed and then a second inner sheath was loaded by an embryologist who assisted the transfer physician in threading the inner sheath into the catheter to complete the procedure.

Direct embryo transfer: embryos were loaded into the Wallace RCO catheter and the catheter was passed to the transfer physician to complete ET under ultrasound guidance.

Results: We analyzed 152 afterloaded embryo transfers (AT) and 150 direct embryo transfers (DT). Both groups were comparable according to age.
In-vitro maturation and in-vitro fertilization (IVM-IVF) has chances of pregnancy.

Acupuncture is an ancient therapeutic art, which has been given renewed attention in light of recent scientific research and current integration with modern medical practice in the treatment of a wide range of diseases, including infertility. Recently certain studies have suggested that acupuncture might have a role in increasing pregnancy rates among women undergoing IVF.

Objective: of the study was to evaluate the effects of acupuncture in a population of women with a reduced ovarian reserve.

To clarify this issue, we have set up a case-control study comparing patients who underwent acupuncture during the controlled ovarian hyperstimulation to those who did not receive acupuncture.

Material and Methods: Between May 2007 and December 2009 204 patients selected for IVF-ICSI with an unfavourable reproductive prognosis were assigned randomly to two groups. Inclusion criteria were as follow: 1) at least two previous poor responses to ovarian stimulation and/or recurrent implantation failure (for ≥ 2 cycles) 2) ovarian and/or pelvic endometriosis 3) raised early follicular phase FSH (> 10 IU/L).

The population of the study consisted of women selected for IVF-ICSI cycles who underwent acupuncture during the IVF cycle. Acupuncture sessions were given during the ovarian stimulation and immediately before and after embryo transfer in accordance to diagnostic and therapeutic criteria of Traditional Chinese Medicine. The control group did not receive acupuncture at the time of the cycle. No more than three oocytes were fertilized at one time in accordance to the 2004 italian law. The primary outcome was pregnancy rate. The secondary outcome was ovarian responsiveness to hyperstimulation.

Results: One hundred two cases and 102 controls were recruited. The two groups did not differ in terms of age, basal FSH, dosage of gonadotropins, days of stimulation, peak estradiol at hCG. No difference was observed between the study and control group in terms of oocytes retrieved (6.4 versus 4.8; p > 0.05) and number of embryos obtained (2.1 versus 2.1; p > 0.05).

Embryo transfer was not performed in 6 and 20 women in the study and control groups, respectively (p < 0.01). Overall, the number of pregnancies was 22 and 10, respectively. Pregnancy rate per starting cycle was 22% and 10%, respectively (p < 0.05). Pregnancy rate per embryo transfer was 23% and 12%, respectively (p = 0.06). The implantation rate was 13% and 8%, respectively (p = 0.17).

Conclusions: This is the first prospective randomised study that investigates acupuncture’s effects in a population of women with poor reproductive prognosis. Analysis of these preliminary data is encouraging. Even if the number of cases is small, this study demonstrates that acupuncture prior to and at embryo transfer improves the reproductive outcome in women undergoing IVF-ICSI with poor prognosis. Larger series are required to draw definite conclusions regarding the impact on the ovarian responsiveness to hyperstimulation and the chances of pregnancy.

P-365 Effect of laser-assisted removal of lysed blastomeres from vitrified-warmed embryos on development and implantation rate in human cryo-ET cycles

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Introduction: In-vitro maturation and in-vitro fertilization (IVM-IVF) has been developed as a new option for assisted reproductive technology (ART), especially for PCO patients. Other benefits of IVM-IVF are prevention of OHSS, low medication cost and less stress. However, the success rates of IVM-IVF are less than those of conventional IVF protocol. In fact, it is very difficult to decide the timing to retrieve oocyte with high developmental...
competence. Recently, it has been shown that the follicular size of leading follicle (LF) reflects sibling oocyte maturation and developmental competence. However, the relationships between the LF diameter and the outcome after embryo transfer are not well-understood. The present study is focused on whether the LF diameter on the day of oocyte retrieval affects clinical outcome or not.

**Material and Methods:** IVM-IVF with low dose FSH was performed in 125 cycles of PCO patients, either with LH surge by hCG of 10,000 IU. or GnRH agonist (GnRHa; buserelin acetate 300 μg). Follicular monitigations were started from cycle day 7. Oocyte retrieval was performed after LF reached more than 8 mm. Retrieved oocytes were cultured in IVM medium (MediCult) with 10% SSS (Irvine) for 26 hours and intracytoplasmatic sperm injection (ICSI) was performed on matured oocytes. Fertilization was confirmed 18 hours after ICSI. Day-3 embryos were transferred after assisted hatching. These cycles were divided into three groups according to LF diameter as follows; Group 1: LF was less than 10 mm (15 cycles), Group 2: LF was between 10 and 14 mm (78 cycles), Group 3: LF was more than 14 mm (32 cycles). Various parameters were compared.

**Results:** There was no significant difference in the numbers of retrieved oocytes (9.6 to 10.8), maturation rates (48.7 to 55.2%), and fertilization rates (80.5 to 83.4%) in the three groups respectively. There was no significant difference in pregnancy rate per transfer between Group 1 and Group 2 (22.2% versus 32.7%). However, pregnancy rate per transfer in Group 2 was significantly higher than Group 3 (P < 0.05, 32.7% versus 9.1%). Moreover, cancellation rate of embryo transfer in Group 1 (46.7%) and Group 3 (65.6%) were significantly higher (P < 0.01) than Group 2 (29.5%).

**Conclusions:** The present study suggests that optimal LF diameter for oocyte retrieval in IVM is between 10 and 14 mm. Embryos from groups of less than 10 mm LF might have low competence potential because of higher cancellation rate. In the case of the appearance of LF with over 14 mm, the schedule for oocyte retrieval had better be shifted from IVM protocol into natural cycle IVF protocol. The present study revealed LF diameter is one of the important factors of IVM-IVF cycles to decide the timing of oocyte retrieval.

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**P-367 ESHRE annual meeting. Are presentations eventually getting published in a peer-review journal?**

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**Objective:** To determine the rate of full publication of randomized trials presented at annual meetings of the European Society of Human Reproduction and Endocrinology, quantify bias against publishing non-significant results and results not favoring the experimental arm, and identify factors associated with time to publication.

**Design:** Survey of 155 abstracts from randomized controlled trials presented at ESHRE meetings 2003 and 2004. Trial results were classified as significant (P < 0.05 for any outcome measure) or non-significant (P > 0.05 not reported) and in favor of experimental arm (P < 0.05 for any outcome measure in favor of the experimental arm). Type of presentation, country of origin, subject and sample size were also recorded. Subsequent full publication was identified using a search of MEDLINE completed January 2010.

**Results:** Among 155 abstracts describing randomized trials 85 (55%) abstracts were published in full-text in a peer-review journal. Median time from presentation to publication was 14 months (range: 0-70). In a bivariate analysis, studies with oral presentation and studies that reported a positive outcome in favor of the experimental arm were more likely to be published compared to studies that were presented as posters or did not report a significant outcome in favor of the experimental arm, (p = 0.022 and 0.019 respectively). Multivariable logistic regression model was performed with the inclusion of 2 variables which in bivariate analysis revealed p-value < 0.05. Oral presentations (OR 2.18 95%CI 1.12-4.25, p = 0.022) and trials with a positive outcome in favor of the experimental arm (OR 2.3 95%CI 1.17-4.52, p = 0.016) were more likely to be published. Finally, Kaplan Meir curves revealed that oral presentations were published sooner than those presented as poster (log-rank test = 0.015) as well as trials favoring the experimental arm compared to all the others (log

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**P-366 A multi-faceted strategy to improve the use of national fertility guidelines; a cluster-randomized controlled trial**

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**Introduction:** Proper use of clinical practice guidelines can decrease variation in care between settings. However, actual use of fertility guidelines is suboptimal and in need of improvement. Hence, a cluster-randomized controlled trial was designed to study the effects of two strategies to implement ten national Dutch guidelines on comprehensive fertility care.

**Materials and Methods:** It is commonly known in guideline implementation research that there is no ‘magic bullet’ for successful implementation of every clinical problem. For example, literature is still inconclusive regarding the effects of multifaceted versus single interventions for guideline implementation. Therefore, a minimal, professional oriented implementation strategy of audit and feedback was compared with a professional as well as patient-oriented strategy that was both professional as well as patient-oriented. Elements of the latter strategy comprised the feedback report and discussion of it, information from the trial revealed positive patient experiences with the intervention material. Scores were lower in the maximal intervention group. Process evaluation of the trial revealed positive patient experiences with the intervention material. Patients reported an increased knowledge of potential causes (71%), treatment procedures (90%) and guidelines (51%), an increased understanding of their doctor’s treatment policy (61%), an increased ability to ask questions about the treatment (61%). The scores for an improved communication with the doctor (22%), as well as perceived increased empowerment for decision making during consultations (22%) were lower. In total, 83% would want to receive comparable leaflets in the future and 97% would recommend the leaflets to peers. Professionals’ appreciation of intervention elements varied; in both intervention arms the feedback report was highly appreciated and reported to actually contribute to the implementation of the guidelines, as was the discussion of it in the maximal intervention clinics. In those, the suggested tools for implementation was moreover highly appreciated, whereas professionals were indifferent to the leaflet on Shared Decision Making and the patient information checklists. A frequently mentioned reason for not using the offered intervention material, was that it was considered to be “not my job responsibility” to initiate practice changes.

**Conclusions:** Absence of an intervention effect may be due to the nature of the strategies, incomplete execution or flaws in study design. Process evaluation data raises the question whether professionals should be the only actors responsible for guideline implementation. Summarizing, although the tested interventions were ineffective, the results of our study can contribute to an increased understanding of the potential role of patients in clinical guideline implementation, as the process evaluation data on the patient oriented intervention showed promising results. Patients did feel empowered to act as a partner in the diagnostic and treatment process and experienced an improvement in communication. Whether professionals are also prepared to accept patients as equal partners in clinical decision-making, remains another challenging focus for further research.
P-368 Meloxicam reduces cycle cancellation rate by premature ovulation in patients with diminished ovarian reserve undergoing natural cycle IVF

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Introduction: This study was performed to determine if an oral administration of the cyclooxygenase inhibitor meloxicam can prevent premature ovulation prior to oocyte retrieval in patients with diminished ovarian reserve (DOR) undergoing natural cycle IVF.

Material and Methods: One hundred patients with DOR were randomly allocated into meloxicam group (n = 50) or control group (n = 50) by the use of sealed envelopes and a computer-generated list. Patients included in meloxicam group received 15 mg/day of oral meloxicam for 3 days from the day at 15-16 mm of lead follicle diameter. When the mean diameter of lead follicle reached 15-16 mm in natural ovulatory cycles, 250µg recombinant hCG was administered subcutaneously. Transvaginal ultrasound-guided oocyte retrieval was performed 35hrs after hCG injection. Embryos were transferred 3 days after oocyte retrieval.

Results: There were no differences in patients’ characteristics between meloxicam and control groups. Cycle cancellation rate by premature ovulation before oocyte retrieval was significantly lower in meloxicam group of 16.0% (8/50) compared with 36.0% (18/50) in control group (P < 0.039). Overall cycle cancellation rate was also significantly lower in meloxicam group (P = 0.035). There were no differences in the mean numbers of oocytes retrieved, MII oocytes, fertilized oocytes and grade I/II embryos between the two groups. The clinical pregnancy rates per initiated cycle and per ET cycle seemed to be higher in meloxicam group (12.0% vs 8.0%, 15.8% vs 14.8%, respectively), but the differences did not achieve the statistical significance.

Conclusions: Meloxicam treatment during late follicular phase is beneficial in reducing the cycle cancellation rate by premature ovulation in natural ovulatory cycle, and therefore might potentially improve IVF outcome in infertile patients with DOR undergoing natural cycle IVF.

P-369 Influence of physiological factors in homologous intrauterine insemination outcome

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Introduction: Many factors have influence on the homologous IUl outcome. Among them are female age, sterility type, diseases affecting the partners, body mass index (BMI), ejaculate parameters, semen processing techniques, survival rate of spermatozoa etc.

Material and Methods: We conducted a study of 694 women, undergone one unstimulated cycle of homologous IUI. Depending on the female age patients were distributed in two groups: Group 1 – women below age 35 and Group 2 above 35. The mean age was 32.9 (ranging from 22 to 45). Each group was divided into two subgroups according to the BMI: subgroup 1.1. (< 35 age and BMI ≥ 25), subgroup 1.2. (< 35 age and BMI range 19-25), subgroup 2.1. (>35 age and BMI > 25) and subgroup 2.2. (>35 age and BMI range 19-25).

Results: According to those groups, this study had shown that in subgroup 2.1. pregnancy rate (PR) per cycle was only 2.7%. In contrast, the subgroup 1.2. revealed five-fold higher, amounting PR of 12% (P ≤ 0.05). In the remaining subgroups 1.1. and 2.2., the PR per cycle was 9.5% and 7.4% respectively. Despite the advanced female age in group 2, the percentage of developing pregnancies was significantly higher in the normal weight subgroup 2.2. (7.4%) comparing to the overweight subgroup 2.1. (2.7%), (P ≤ 0.05).

Conclusions: To improve the outcome of homologous IUI, an appropriate diet and physical activities could be suggested to all women with obesity prior enrolment in the ART program.

P-370 How to easily improve of more than 40 % the pregnancy rate in intrauterine insemination cycles?

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Introduction: Guidelines of good laboratory practice do recommend to pre wash catheters prior to embryo transfer. Actually using non pre washed catheters result in pregnancy rate impairment. Because of the possible presence of cellular toxic chemical within the catheter tube, we decided to check the effectiveness of pre-washing intrauterine insemination (IUI) catheters on pregnancy rates.

Materials and Methods: Every other week for two years, IUI catheter (Frydman8 Classic catheter, CCD) were prewashed with culture medium (Earle’s medium, Eurobio) before IUI at Cochin Hospital (Paris). A total of 552 cycles in 322 patients (group 1 washed catheters) and 550 cycles in 330 patients (group 2 unwashed catheters) were included. IUI were performed for various causes of infertility, including cervical factors, ovulation dysfunction, cryopreserved semen (sperm donation, HIV infected male and cancer patients) and unexplained infertility. Controlled ovarian hyperstimulation was conducted using FSH or hMG; IUI was then performed 36 hours after triggering ovulation if at least one follicle measuring > 16 mm and an endometrial thickness of > 7 mm (with triple-line development) were obtained.

Results: There were no differences in age, cycle day 3 FSH, day of hCG E2 levels, number of mature follicles and total motile spermatozoa inseminated in both groups. Higher clinical pregnancy rates were observed in any IUI indication whether frozen-thawed or fresh sperm were used (25.1% vs 19.5%, P > 0.05, 13.7% vs 9.8%, P > 0.05, respectively). A total of 40% increase in the pregnancy rate was obtained (19.1% vs 13.6%, P < 0.05).

Conclusion: Guide lines of good laboratory practices should be modified and included prewashing of the catheter before IUI. More studies should be conducted on environmental toxic compounds in IVF laboratory disposable material.

P-371 The prognostic value of the postcoital test for spontaneous pregnancy: is the game worth the candle?

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Background: At present there is inconclusive evidence on the ability of the postcoital test (PCT) to predict spontaneous pregnancy in subfertile couples. The aim of this study was to assess the additional prognostic value of the PCT and evaluate its performance in a large prospective multicenter cohort of subfertile couples.

Methods: The study was designed as a prospective cohort study performed in 38 hospitals in the Netherlands. Between January 2002 and February 2004, we included consecutive subfertile couples who had not been evaluated previously for subfertility. Primary end-point in this study was spontaneous conception resulting in an ongoing pregnancy. We used three concepts to evaluate the additional
prognostic performance of PCT in comparison to the existing model for the prediction of chances of spontaneous pregnancy without the PCT: discrimination (AUC), calibration, and the net reclassification improvement (NRI).

**Results:** We included 3,021 couples of whom 592 (20%) had a spontaneous pregnancy, 55 (1.8%) a non-successful pregnancy, 1,316 (44%) started treatment within 12 months, 824 (27%) neither started treatment nor became pregnant and 289 (10%) were lost to follow up within 12 months. Discrimination improved by adding the PCT result from an AUC 0.63 (95% CI 0.60 to 0.65) to an AUC of 0.64 (95% CI 0.61 to 0.66), but this improvement was not statistically significant. Calibration did only marginally improve by adding the PCT to the existing model. The net reclassification changed for the worse if the PCT was added to the existing model was 1.1.

**Conclusion:** This study demonstrated that the postcoital test has prognostic value, but its added value is clinically insignificant.

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**POSTERS**

**FERTILITY PRESERVATION**

**P-372 Three dimensional alginate-collagen IV matrix enhances the in vitro growth of human isolated follicles**

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**Introduction:** Recently, many efforts have been done to help young patients to preserve their fertility through the storage of their own germ cells before chemo-and/or radiotherapy partially or completely destroys their follicular reserve. To this end whole ovary or ovarian cortical strip may be cryopreserved and transplanted in order to recover the ovarian function after anticancer treatment. Unfortunately, in some types of cancer these techniques are associated with the risk of transmitting malignant cells present in the cryopreserved tissue. This risk can be avoided if the follicles are isolated from the ovarian stroma and grown in vitro. On the other hand the extracellular matrix plays a key role for the development and the full competence of follicles in vivo and may be needed for in vitro follicle growth. Herein we investigated the effectiveness of three dimensional matrix alginate and alginate + collagen IV to support the in vitro growth of encapsulated isolated primary and secondary follicles.

**Material and Methods:** Human cortical ovarian tissue was dissected in pieces of 0.5mm x 0.5mm x 1mm, digested in Leibovitz L 15 medium supplemented with collagenase type 1A, 2mg/mL, DNase, 0.2 mg/mL, 1% FCS, at 37°C for 1 h. Single primary and secondary follicles were collected under the stereomicroscope and encapsulated in alginate 1% or alginate 1% + collagen IV 0.3 mg/mL. The encapsulated follicles were cultured in McCoy’s 5a supplemented with 20% FCS, transferrin (2.5 mg/mL), selenium (4 ng/mL), insulin (10 ng/mL) in 5% CO2 atmosphere at 37°C for seven days. The follicle growth was measured daily and image acquisitions and analysis were performed by means of Nikon NIS-Element Imaging AR 3.0. The viability was assessed through fluorescent labelling with Hoechst 33258. Cultured follicles were labelled with FITC-anti a tubulin and rhodamine phalloidin for confocal laser scanning microscopy analysis. Morphology was evaluated under Hoffman modulation contrast and transmission electron microscopy (TEM).

**Results:** At the end of the culture period, the average growth and viability of follicles were 20% and 55% for 1% alginate and 38% and 77% for alginate 1% + collagen IV respectively. Moreover confocal and TEM analysis showed that the three dimensional follicle architecture was better preserved after alginate + collagen IV encapsulation.

**Conclusion:** The encapsulation in a three dimensional matrix of alginate + collagen IV better supports the in vitro growth of isolated follicles. These data indicate that compounds of the extracellular matrix play a key role in the modulation of survival, growth and morphological organization of mammalian follicles in vitro and should be taken into account to improve the biotechnology for the in vitro growth of isolated human follicles.

**P-373 Co-culture of human decidua monolayer cells with frozen-thawed ovarian tissue improves the survival and follicular development**

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**Introduction:** It has been reported that Matrigel (a kind of extracellular matrix) can be used to coat on the surface of culture dishes which supports a greater proportion of viable follicles developed during culture in vitro. However, it has been concerned that Matrigel is an extract of a tumor cells. Therefore, an alternative, human decidua monolayer cells, was used instead of Matrigel in this study. The objective of this study was to establish a new culture system, using human decidua monolayer cells to restore survival and development of follicles following co-culture with human frozen-thawing ovarian tissue in vitro.

**Materials and Methods:** Ovarian tissues were obtained from 15 women who underwent gynecologic surgery for the indication of mature teratoma. The ovarian cortex was cut into small strips (10mm x 1mm x 1mm) and cryopreserved by vitrification method. Decidua biopsy was performed during induced abortion surgery. Decidua cells were cultured in 4-well dishes to generate monolayer cells in culture medium DMEM/F12 with 15% fetal bovine serum (FBS) containing penicillin and streptomycin in a 5% CO2 incubator at 37°C in high humidity. The frozen ovarian tissues were thawed and further cut into cubes, and then allocated into the 4-well dishes where decidua monolayer cells generated (10 cubes in 1 well of dish). The experiment was designed with following: Group A, frozen-thawed tissue cultured with monolayer cells; Group B, frozen-thawed tissue cultured without monolayer cells; Group C, fresh tissue cultured with monolayer cells; Group D, fresh tissue cultured without monolayer cells. Following culture, the medium was extracted every other day and added the same volume of medium up to 14 days to detect the concentration of estradiol and progesterone. End of culture, the ovarian tissues were fixed for histological analysis. Total follicle surviving rate (TFSR) and growing follicle rate (GFR) were assessed. The statistical analyses were applied for t and y2-tests.

**Results:** There were no differences in the TFSR (92.3% versus 94.5%) and GFR (59.8% versus 53.3%) between group A and C. Also there were no differences in the concentrations of Estradiol (311.7 ± 73.6pmol/l versus 328.4 ± 63.9pmol/l), and Progesterone (1.6 ± 0.7IU/L versus 1.5 ± 0.6IU/L) at the end of 14 days between group A and C. However, these numbers were significantly higher than group B and D.

**Conclusions:** Co-culture of human decidua monolayer cells with the frozen-thawed ovarian tissue improves survival and development of follicles cultured in vitro. This new culture system will be beneficial to ovarian tissue culture.

**P-374 Acute necrosis following endometrioma vaporization by plasma energy is not harmful for underlying ovarian tissue**

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**Objective:** Ovarian endometrioma cystectomy often leads to the inadvertent removal of underlying ovarian parenchyma along with the cyst wall, even when careful ovarian tissue sparing technique is performed. The aim of our study was to evaluate whether or not vaporizing ovarian endometriomas with plasma energy allows for the complete ablation of the inner layer of the endometrioma (endometrial epithelium and stroma) without extending beyond the outer layer of the cyst (fibrotic tissue).

**Materials and Methods:** We conducted a pilot study in a series of 10 consecutive ovarian endometriomas requiring surgical management. Plasma energy was used to vaporize the inner layer of the cyst wall consisting in the endometrial epithelium and stroma (settings 40, coagulation mode, and average time of application 2 seconds). Histological specimens were obtained after complete vaporization of the cyst followed by cystectomy, in order to measure the depth of necrosis induced by the procedure and to evaluate the efficacy of the endometrial tissue ablation.