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 induced 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 focussed 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 Mehtods: 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.

POSTERS

SAFETY & QUALITY (I.E. GUIDELINES, MULTIPLE PREGNANCY, OUTCOME, FOLLOW-UP ETC.)

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 heve been effective in reducing the incidence of OHSS but these are determined near the completion of COS.

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 descripted 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

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cycle (short protocol). HMG or recFSH was started at day 3 and luteal support was done with progesteron.

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

Table 1:

	GROUP A (n = 74)	GROUP B ((n = 32)	p value
AGE (yrs)	32.22 ± 4.27	29.56 ± 4.87	0.006
BMI (kg/m2)	25.57 ± 4.24	24.6 ± 3.81	NS
AMH(ng/ml)	2.35 ± 1.24	6.26 ± 3.33	0.0001
Inhibin B(pg/ml)	77.23 ± 44.87	99.15 ± 56.76	0.038
FSH (mIU/ml)	7.31 ± 3.95	5.49 ± 1.49	0.013
E2(pg/ml)	44.84 ± 23.86	43.19 ± 32.55	NS
LH(mIU/ml)	3.70 ± 2.54	4.72 ± ±3.14	NS
AFC	6.68 ± 4.34	11.69 ± 6.93	0.0001
Gonadotropins HMG	30 (%41)	17 (%53)	NS
Gonadotropins Rec FSH	44 (% 59)	15 (%47)	
Long agonist protocol	46 (%64)	26 (%81)	NS
Short agonist protocol	26 (%36)	6 (%19)	

A multivariate logistic regression model was used for futher 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.

FSH level was a weak predictor of OHSS.

Table 2:

Variable	Coefficient	Odds ratio (95% CI)	p value
AMH	0.947	2.58 (1.51-4.39)	0.001
FSH	-0.621	0.537 (0.31-0.93)	0.027
Age	0.041	1.04 (0.89-1.23)	NS
Inhibin B	0.000	1 (0.98- 1.014)	NS
AFC	0.008	1.008 (0.86-1.18)	NS
LH	0.259	1.30 (0.79-2.13)	NS

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

Table 3:

Variable	Area under ROC (95% CI)	Cutoff value	Sensitivity	Specificity	PPV	NPV
AMH	0.90 (0.84-0.97)	3,645	0.84	0.865	0.73	0.93
Inhibin B	0.62(0.50-0.74)	84	0.62	0.605	0.40	0.79
AFC	0.79 (0.70-0.88)	7,5	0.77	0.66	0.49	0.86

The ROCauc for AMH featured the largest area under the curve among the listed risk factors for OHSS than age,inhibin B, antral follicle counts.

Conclusions: The basal serum AMH level could be utilized effectively to predict OHSS A cut off value of 3.64 ng/ml was determined by the corresponding ROC curve with % 84 sensitivity, %86 specificity, %73 PPV, %93 NPV.This basal AMH level could serve a predictive marker and patient friendly protocols for COS could be utilized to prevent the detrimental effects of OHSS.

P-311 Nicotine exposure limited to the preimplantation period is associated with altered fetal and placental growth

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Introduction: Maternal tobacco smoking is one of the leading preventable causes of perinatal complications. Indeed tobacco exposure affects the development of fetal organs and tissues and results in increase in low birth weight, preterm delivery, stillbirth, perinatal mortality, and birth defects. Among the smoke products, nicotine is considered to be the major teratogenic substance that perturbs embryonic development. While it is recognized that tobacco exposure affects every aspect of reproduction, it is presently unknown if limited exposure in sensitive periods of development is associated with long term consequences. In this study, we examined if nicotine exposure limited to the preimplantation period is associated with alteration in blastocyst development, abortion rate, fetal and placental growth.

Material and Methods: To evaluate the effect of nicotine during the preimplantation embryo development we fertilized in vitro CF1 oocytes with B6D2F1/J spermatozoa. Experimental embryos were divided in 2 groups; one group was exposed to a low dose of nicotine (0.2 μM) for the whole preimplantation stage; the second group was exposed to a higher nicotine dose (1.24 μM). The lower dose corresponded to the plasma nicotine concentrations 5 min after a cigarette is smoked. The higher dose corresponds to nicotine levels found in heavy smokers. Nicotine exposure was started 5 hours after eggs and sperm were mixed together and continued until the blastocyst stage. Control embryos were not exposed to nicotine. Embryos were cultured using optimal culture conditions (KSOM medium with amino acids and 5% Oxygen concentration). Preimplantation embryos cultured in vitro and exposed to 1.24 μM nicotine were transferred to recipient CF1mothers never exposed to nicotine. Eight to ten blastocysts were transferred in each horn. At day 12.5 of gestation (P12.5), the recipient mothers were sacrificed and abortion rate, embryo development, placenta and fetal weights were assessed.

Results: Exposure to nicotine results in a dose response delay in development: fewer embryos exposed to nicotine reached advanced stage of development ($p < 0.05$; hatched blastocysts: control, 52.1%; nicotine 0.2 μM, 41.9%; nicotine 1.24 μM, 39.8%) and there is an increase of more immature forms in the nicotine group (morula: control, 8.3%; nicotine 0.2 μM, 14.3%; nicotine 1.24 μM, 14.6%). Mice exposed to 1.24 μM nicotine showed a non statistically significant increase in abortive sites (28.7% ± 11.7 3 litters vs. 17% ± 9.7, 5 litters). Results of nicotine exposure on fetal and placental growth were striking: nicotine exposure limited to the preimplantaion stage was sufficient to alter the development of the fetus. Both fetal (n = 56, 47.1 mg ± 5.3 $P < 0.01$) and placenta (n = 56, 52.8 mg ± 6.3 $P < 0.01$) weight were reduced compared to control fetal (n = 56, 78.7 mg ± 16.2) and placenta weight (n = 56, 69.7 mg ± 12.8 resepctively). However the ratio placenta embryo was increased in embryo exposed to nicotine (control, 0.9: nicotine 1.0 $P < 0.01$). One fetus exposed to nicotine during the preimplantation period (1/31 = 3.2%) had evidence of neural tube defect, a known complication of tobacco exposure.

Conclusions Nicotine exposure limited to the preimplantation period, results in long term effects. These results are important because nicotine exposure was: 1) limited in time -4 days- and 2) limited to the embryo -and not the recipient mother or the gametes- 3) the negative outcome was observed long past the exposure. It appears therefore that nicotine exposure reprograms the developmental programs of the exposed embryos. The demonstration that smoking alters the embryos to such extent that they produce less healthy fetuses could motivate parents to stop smoking even before pregnancy occurs.

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 autologeous 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 paternal age, 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.

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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 paternal 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 relation between preterm labour, the incidence of caesarean section and the presence of a twin pregnancy.

Matching could not be performed for 152 live births resulting from 92 singleton pregnancies and 30 twin OD-pregnancies. These oocyte recipients without an available AO-match in our ICSI-population were significantly ($p < 0.001$) older and had a higher parity in both singletons (36.27 vs. 46.28 and 0.23 vs. 0.48) as in twin (36.59 vs. 45.47 and 0.22 vs. 0.77) OD-pregnancies. A higher incidence of preterm births were observed in singletons (15.3% vs. 37.5%; $p = 0.01$) but not in the twin (82.5% vs 89.5%; $p = 0.46$) OD-pregnancies. Singleton pregnancies in the older OD-population had a lower average gestational age (272.35 vs 262.81 days ($p = 0.043$)) and a lower average birth weight (3211.86 vs. 3027.35 g ($p = 0.008$)) and length (49.69 vs. 47.71 cm ($p = 0.003$). These differences were not observed in twin pregnancies. Neither was there any difference in the incidence of low (< 2500g) and very low (>1500g) birth weight babies between the older and younger OD-population in singletons and twin pregnancies.

Conclusion: 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 or age of the recipient, donor or partner. However, 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 medium 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 two 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 medium 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.

Conclusion: 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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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 UI/L, and normal karyotype. The selection criteria for recipients were: the need of oocyte donation for in vitro fertilization (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-lutheal 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. Lutheal 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 (x^2), 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 statistic 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.

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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 requring 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 transfussion one and laws need to reflect these differences based on current evidence as reported here.

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

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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-lutheal 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. Lutheal 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 ultrasound) 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 (KSOM medium with amino acids and 5% Oxygen). Resulting blastocysts were transferred to CF1 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 weekly. 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 difference 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 ($p = 0.051$) 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, but increased fat content starting at 21 weeks of life (IVF: 46.7 ± 5% n = 8.; FB: 40.5 ± 3% n = 8).

Importantly, GTT did not show difference in mice conceived in vivo or in vitro. However, there was a trend for higher fasting insulin in IVF females

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(p = 0.08). In addition, in vitro beta cell insulin secretion after a glucose challenge was reduced in IVF mice (N = 3 mice per group < 0.05).

Conclusion: Adult animals conceived in vitro maintain a memory of the stress experienced during the preimplantation stage. Mice conceived in vitro in optimized culture conditions have a similar but not equal phenotype to mice conceived in vivo. In particular, culture of embryo in optimized culture conditions result in a less severe adult phenotype compared to culture in suboptimal culture conditions. Female mice show differences in fat content, insulin secretion and possibly insulin resistance compared to in vivo conceived mice. These results, for the first time, confirm an IVF effect on growth parameter in adult mice. Importantly, litter size and maternal environment have been controlled and accounted for. Future translational studies to assess metabolic health of IVF children are strongly indicated.

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P-318 Thrombophilia screening in women with thromboembolism (TE) following ovarian stimulation (OS)

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Introduction: TE complicates 1 in 128 patients with severe thromboembolism (Delvigne 19993). Controversy exists regarding the true incidence of thrombophilia in women with moderate to severe ovarian hyperstimulation (OHSS). It ranges from no increase when compared to general population (Fábregues 2004) to as high as 85% (Dulitzky 2002).

Material and Methods: We reviewed the literature for TE following OS to identify the incidence of thrombophilia in these women. We searched MEDLINE, PUBMED, OVID databases and handsearched the relevant journal articles for the reported cases in published literature. Total of 88 cases were identified and we added two unpublished cases from our institution. All cases were analysed by the site of TE and positive thrombophilia screen.

Results: Total of 90 cases were identified and of these there were three reported fatalities. 68 cases (75.6%) were reported to have been screened for the presence of thrombophilia and of these 24 (35.3%) tested positive for one or more thrombophilia marker. 51 cases (56.7%) had TE involving upper limb and neck, of whom 40 (78.4%) were screened for thrombophilia and 18 (45%) screened positive. 19 cases (21.1%) had cerebrovascular TE, of which 13 (68.4%) were screened and 2 (15.4%) screened positive. Lower limb and pelvic TE was reported in 10 cases (11%), 5 (50%) were screened and 2 (40%) screened positive. 8 cases (8.8%) had cardiac TE, all of these were screened and 2 (25%) tested positive. The risk of upper limb and neck TE with positive thrombophilia screen was significantly higher (p = 0.03, one way ANOVA test) than the TE in the other sites.

Data regarding OHSS was reported in 86 cases and 62 (72.1%) were reported to have moderate to severe OHSS. Of these 62 cases, 46 were screened for thrombophilia and 16 (34.8%) screened positive.

Data regarding pregnancy was reported in 83 cases, of which 56 (67.4%) were screened and 18 (32.1%) screened positive.

Conclusion: The incidence of positive thrombophilia screen is higher in women with TE following OS as compared to general population. Those with upper limb and neck TE have significantly higher risk of positive thrombophilia screen than TE at other sites. One-third of women with moderate to severe OHSS and were screened had positive thrombophilia screen and one-third of the women who fell pregnant following OS and had TE, had positive screen. Therefore, there is need for consensus on screening women with moderate to severe OHSS for evidence of thrombophilia and duration of thromboprophylaxis in those with positive screen and moderate to severe OHSS.

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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.

Here we investigate whether such undesired outcomes are due to preemptive placental adaption or to an epigenetic insult.

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. were a derived singleton, or a derived twin). Neonatal measures such as birth weight and gestational age were also recorded.

Results: A total 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) was 39.2% (n = 6.818) with 16.7% pregnancy loss. The overall occurrence of multiple gestations (twins, triplets, quadruplets) was 38.3% (2,608) and of those, 701 (10.3%) underwent spontaneous embryo reduction. Approximately 18.6% of twin gestations, 54.2% of triplets, and 76.3% of quadruplets spontaneously reduced ($P < 0.01$). This reduction was not impacted by maternal age or gross embryo morphology. Interestingly, over 75% of the triplets and quadruplets spontaneously reduced to twins.

sET and mET resulting in a single embryo implantation as judged by ultrasound at 6-7 weeks generated offspring with comparable body weights and gestational ages (3222.3 ± 567 vs 3297.0 ± 576g; 38.6 ± 2 vs 39.0 ± 2 wks, respectively).

The decreasing body weight of derived singletons correlated inversely with the increasing number of implantation sites (3153.1 ± 642g vs 2927.9 ± 765 vs 2126.8 ± 662; $P < 0.01$). The derived singletons (38.6 ± 3wks) had gestational ages comparable to true singletons, while those coming from higher order gestations (triplets, quadruplets) were shorter (36.7 ± 5, 37.4 ± 4wks; $P < 0.05$).

The birth weights of derived twins and triplets did not differ from those of true twin (2413.2 ± 582g) or true triplet (1945.5 ± 283g) counterparts nor from weights found in the spontaneously conceived population.

Conclusions: Maternal and placental cues induce constraints to prenatal development to ensure the survival of both mother and fetuses, particularly in monotocous 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

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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 23.5 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 fail 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-321 Obstetric outcome for IVF children born after single or double embryo transfer

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Introduction: IVF children are known to have a poorer obstetric outcome compared to children born after spontaneous conception, mainly due to a higher rate of multiple pregnancies. However, also singletons after IVF have showed increased rates of preterm birth and low birth weight (LBW), about double the rates compared to spontaneous conception. With a broad introduction of single embryo transfer (SET) this scenario might change.

The aim of this study was to compare the obstetric outcome of children born after SET, elective SET (eSET), non elective SET (non eSET) and double embryo transfer (DET) with children in the general population.

Material and Methods: All IVF children born in Sweden after IVF treatment during the years 2002-2006 were included (13 583 children) and compared with all children born during the same time period (583 079 children). Data were collected from all 16 Swedish IVF clinics and cross-linked with the Medical Birth Registry.

Main outcome measures were preterm birth (< 28 w, < 32 w, < 37 w), very low birth weight (VLBW) (< 1500 g) and LBW (< 2500 g). Other outcome measures were small for gestational age and perinatal mortality. Adjustment was performed for year of delivery, mother's age, parity, smoking, BMI and years of infertility. Mantel-Haenzsel statistics was used.

Results: In total, and including fresh and frozen transfers 7777 children were born after SET and 5745 children were born after DET. The multiple birth rates were 1.2% for SET and 21.2% for DET.

Comparing all IVF children (SET, DET, fresh and frozen transfers, singletons and multiples) with all children from the general population significantly higher rates were found in the IVF children for all main outcome measures (adjusted odds ratio (AOR) between 1.7 and 1.9).

Comparing only IVF singletons (SET, DET, fresh and frozen transfers) with singletons from the general population significantly higher rates were found for < 28 w, < 37 w, LBW and VLBW (AOR between 1.1-1.7).

The rates of LBW and VLBW for singletons after all SET (fresh + frozen) were 5.0% and 1.2%, and for singletons after all DET (fresh and frozen) 5.1% and 1.2%. Separated into eSET, non eSET and DET singletons (only fresh) these rates were 5.3% and 1.1% vs 5.8% and 1.3% vs 5.3% and 1.3%. For preterm birth (< 37 w) and very preterm birth (< 32 w) the rates for all SET and DET singletons (fresh + frozen) were 7.2% and 1.3% vs 7.5% and 1.4%. Separated for eSET, non eSET and DET (only fresh) the rates were 7.5% and 1.2 % vs 7.3 % and 1.6% vs 7.6% and 1.4%.

No significant differences were found for children born after SET (only fresh) vs general population (AOR 0.95-1.4) or for children born after DET (singletons, only fresh) vs general population (AOR 1.0-1.2).

For eSET singletons (only fresh) vs singletons in the general population no significant differences were found except for < 37 w (AOR 1.15, 95% CI 1.02-1.30).

When comparing non eSET singletons (only fresh) vs singletons in the general population significantly higher rates of < 28 w, < 32 w and < 2500 g were found for the non eSET children (AOR 2.63, 95% CI 1.56-4.42, AOR 1.48, 95% CI 1.01-2.16, AOR 1.31 95% CI 1.07-1.61).

Conclusion: Children born after SET as well as DET singletons have comparable preterm birth and low birth weight rates as children in the general population. For eSET singletons no significant differences was found except for a higher rate of preterm birth < 37 w. Non eSET singletons had significantly higher rates of very preterm birth (< 28w and < 32 w) and LBW as compared with singletons in the general population.

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

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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 an gonadotropin-releasing hormone (GnRH) antagonist for IVF or ICSI.

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-

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tients). This analysis was performed separately for stimulation day 8 and the day of human chorionic gonadotropin (hCG) administration. Ongoing pregnancy rates for patients stratified according to percentiles of serum LH levels (P75) were assessed and odds ratios (ORs) with 95% confidence intervals (CI) were calculated to determine the association of endogenous LH levels with the likelihood of ongoing pregnancy.

Results: In the stepwise logistic regression model including serum hormone values measured on day 8 of stimulation (among other factors), pregnancy was predicted by serum progesterone levels and by the number of oocytes retrieved. In the stepwise regression model including serum hormone values measured on day of hCG administration (among other factors), the only predictor of ongoing pregnancy was female age. In the combined analysis of 6 trials, the OR of the LH analysis on stimulation day 8 was therefore adjusted for trial, female age, serum progesterone values, and oocytes and at the day of hCG for trial and female age. Comparing ongoing pregnancy rates according to P75 LH levels provided an OR of 0.88 (95% CI, 0.69–1.12) on stimulation day 8 and of 0.97 (95% CI, 0.77–1.22) on the day of hCG. Thus, neither low nor high endogenous LH levels on day 8 of stimulation or on the day of hCG administration have an association with ongoing pregnancy likelihood.

Conclusion: This is the largest study to date on the association of LH levels with pregnancy likelihood in a GnRH antagonist protocol employing multivariate analyses that control for potential confounders. It provides robust evidence from prospective trials with central laboratory assessment that endogenous LH concentrations have no association with pregnancy likelihood in normogonadotropic women undergoing COS with rFSH. Thus, endogenous LH levels cannot serve as a rationale for adding LH activity to the ovarian stimulation protocol.

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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 (150 µg corifollitropin alfa, first cycle only). Inclusion/exclusion criteria were very similar in all 3 trials, with the exception of age which had an upper limit of 39 years in the Trust trial and of 36 years in the Engage and Ensure trials. In all 3 trials a single dose of corifollitropin alfa (Elonva, N.V. Organon) was administered on menstrual cycle day 2 or 3 and, after 7 days, treatment was continued with daily FSH until the criteria for human chorionic gonadotropin (hCG) trigger were met. To prevent premature luteinizing hormone surges, daily treatment with a GnRH antagonist was started on stimulation day 5 or 6. To trigger final oocyte maturation patients received either 10,000 IU (78.9%) or 5000 IU (14.7%) hCG or 250 µg recombinant hCG (6.3%). For each case of OHSS, all signs and symptoms and their severity were collected. Cases of OHSS were graded into 3 classes of mild, moderate, or severe OHSS in line with the guidelines provided by the World Health Organization (1973).

Results: In total there were 95 out of 1705 patients (5.6%) in the corifollitropin alfa group with signs or symptoms of either mild, moderate, or severe OHSS, while in the rFSH reference group there were 53 out of 880 patients (6.0%) with signs or symptoms of OHSS. The incidence of early versus late OHSS onset 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 vs 30.3 years) and body mass index (23.7 vs 24.2 kg/m^2), and had a similar antral follicle count at stimulation day 1 (13.0 vs 13.9) and FSH levels at stimulation day 1 (5.8 vs 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 1.4% in the corifollitropin alfa treated patients and 3.5%, 1.3%, and 1.3% 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 multifollicular 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/m^2) 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 ((S)AEs); 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 (ganirelix or cetrorelix acetate) up to 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/m^2, 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.

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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 corifollitropin alfa 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.

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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 IVF. Although increased BMI had a negative impact, the effect was much less strong than female age even for women under 40.

Conclusions: We did not find evidence for an increased risk of overweight and BMI on the complication rates from ART. The impact of female age on ART success rates was much stronger than that of BMI. Although overweight and obese women in ART programs should be informed about their increased risk of pregnancy complications, an increased BMI in itself is not a reason to withhold IVF. As society allows overweight and obese women to aim for spontaneous conception, there is no reason to withhold ART to these women.

P-326 Perinatal outcome of 120 children born from oocyte in vitro maturation treatment

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Introduction: Oocyte in vitro maturation (IVM) is a promising approach for fertility treatment of specific categories of women. Because it requires no or minimal gonadotropin stimulation, IVM can offer the advantage to reduce costs, patient discomfort, and ovarian hyperstimulation syndrome (OHSS) risk in in-vitro fertilization (IVF) procedure. On the other hand, questions concerning the efficacy and safety of IVM remain open. In particular, because meiotic and cytoplasmic oocyte maturation occurs in vitro, it has been hypothesized that IVM treatment might involve specific epigenetic and congenital abnormalities risks to the health of the conceptus. In this study, we assessed the perinatal outcome of babies born from IVM treatment.

Material and Methods: Normo-ovulatory women or presenting with polycystic ovary (PCO) or polycystic ovary syndrome (PCOS) were selected for IVM treatment, after informed consent was obtained. IVM was performed in natural 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.

Results: 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 pielectasy, 1 of labiopalatoschisis associated with hypospadias, 1 of sinus pilonidalis, and 1 of plagiocephaly in a twin pregnancy. No major abnormalities were found.

Conclusions: IVM is gaining increasing interest as a fertility treatment and preservation strategy. One of the factors currently limiting its adoption as a routine procedure is the concern that maturation per se may entail an increased risk of congenital abnormalities. Data on children born from IVM cycles are still scarce. In 120 babies born from IVM treatment, to our knowledge the largest set of data reported by an individual centre, we did not detect major congenital abnormalities, confirming previous evidence. Although larger and more systematic studies are needed, our preliminary experience does seem to raise specific concerns on the safety of IVM.

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. Antimüllerian 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-

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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.

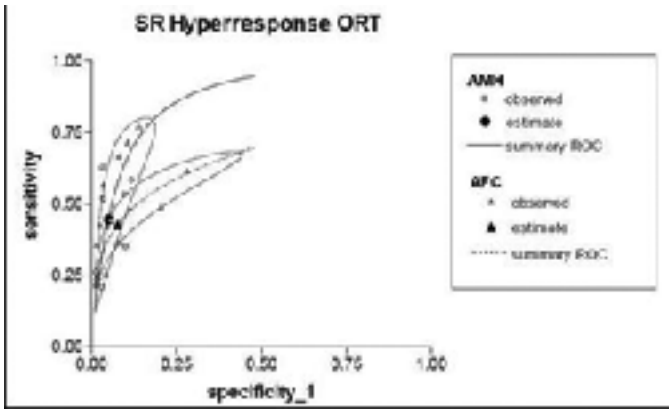

Figure 1: Summary curve for AMH and AFC performance in excessive response prediction

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 cyle 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 uhMG.

In agonist 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 (13-15.8) with uFSH, and 17 (15.5-19.2) with hMG; in antagonist, mean number of oocytes were 15.5(14.4-16.6), 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 retrival

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Introduction: Since we introduced ultrasound guided oocyte retriveal for IVF local analgesi using a para-cervical block and sedation has been the main procedure for oocyte retrieval. We have further improved the method by testing a local analgesy in the vaginal vault in the position for the puncture, in which the local analgesy were placed ultrasonically guided just prior to the puncture.
Material and Methods: In a prospective observational study where one doctor continued the paracervical block method for analgesy and sedation with Rapifen, was compared to 3 other doctors using the ultrasound guided placement of the local analgesy and Rapifen sedation. A VAS 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 paracervical 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 analgesy 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 20th 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; 31-35; 36-40; > 40); BMI (< 25; 25-29.9; ≥ 30); infertility cause (Male,Tubal, Endometriosis, and PCO); ovarian response in terms of the 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).

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Results: Patients with a serum P level > 1.5 showed a significantly lower OPR with an Odds Ratio (OR) = 0.53 (95% Confidence Interval: 0.38-0.72); p = 0.00006.

According to age intervals, the OR (95% CI) for OPR were: 0.58 (0.22-1.56); 0.50 (0.31-0.80); 0.49 (0.27-0.89) and 0.0. (0.0-0.0).

According to BMI, OR (95% CI) were: 0.64 (0.42-1.01); 0.22 (0.05-0.94) and 0.76 (0.09-6.89).

Outcome related to ovarian response in terms of the number of oocytes collected was: 0.58 (0.13-2.55); 0.52 (0.25-1.08); 0.43 (0.24-0.79); 0.27 (0.14-0.51) and 0.44 (0.23-0.84). In terms of serum E2 on day of hCG was: 0.28 (0.07-1.17); 0.56 (0.32-1.01); 0.35 (0.20-0.63) and 0.45 (0.26-0.79).

According to the total gonadotrophin consumption, OR (95% CI) were: 0.83 (0.38-3.61); 0.54 (0.32-0.89) and 0.51 (0.33-0.79).

The outcome related to infertility cause were: Male: 0.51 (0.32-0.82); Tubal: 0.77 (0.27-2.21); Endometriosis: 0.30 (0.89-1.03) and PCO: 1.76 (0.59-5.20).

Conclusions: A serum P level above 1.5 ng/mL the day of hCG administration in COS for IVF is detrimental for cycle outcome in terms of OPR in all patients subpopulations, according to age, BMI, ovarian response, gonadotrophin consumption and infertility cause. The only exception to this rule was observed in PCO patients. This latest finding needs further research to be confirmed.

P-331 Varying the ovulatory trigger minimises ovarian hyperstimulation without compromising pregnancy rates

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Introduction: Ovarian hyperstimulation syndrome (OHSS) is a major complication of assisted reproductive treatment (ART) that can result in hospitilisation 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 Cetrotide 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:

1 Kolibianakis et al (2005) Hum Reprod 20, 1213-1220.

P-332 The necessity of attentive cardiac evaluation in women with Turner Syndrome requesting oocyte donation to become mothers

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Introduction: Turner syndrome (TS) is defined by a total or partial absence of X chromosome associated to phenotypical traits. Most women with TS willing to achieve pregnancy need oocyte donation (OD) because of their ovarian dysgenesis. However, TS is associated to a high mortality risk due to frequently associated cardiac malformations as coarctation, bicuspid aortic valve (BAV) and also aortic dilation. A high prevalence of aortic dissection is observed in TS majored by hyper blood pressure. Indeed pregnancy is at risk of aortic dilation and dissection in TS women. Pregnancy should be contraindicated if a cardiac malformation is also present. We present our experience of the cardiac evaluation of 25 women who requested OD and the results of their managements.

Material and Methods: Between 2001 and 2008, a total of 25 women with Turner syndrome asked for OD in our centre. They were 29 ± 5 years old. Seven women had a karyotype 45 XO and 18 were mosaic. Eighteen women had a systematic cardiac evaluation before OD by the referent cardiologist of our centre including clinical examination, blood pressure measurement, chest bi-dimensional echocardiography and cardiac MRI. There was a systematic measure of the aortic diameter (AD) which was normalised to the body surface area (aortic size index ASI) so that the shorter height of TS women was taken into account contrarily to classical AD measurement (Matura et al. 2007). During that period 7 women benefited of an OD.

Results: A previous cardiac examination including echocardiography had been done in 11 women but none had discovered a cardiac malformation. However, a BAV was revealed at echocardiography in 5 of them when re-examined by our referent cardiologist. Two others, never explored before, also had a BAV, resulting in a total percentage of BAV of 38.8% (7/18). No other cardiac malformation was found and only one woman had systemic hypertension. Seven women had an ASI ≤ 15 mm/m^2 (no dilatation), nine between 16 and 19 mm/m^2 (mild dilatation) and one had an ASI > 20mm/m^2 (significant dilatation). Three BAV were associated to a moderate aortic dilation. Among the eighteen women explored, 7 women with no cardiac risks and ASI < 19 mm/m^2 beneficiated from OD. The clinical pregnancy rate per transfer (38.5%) was similar to other indications. The baby take home rate per couple was 57%, with normal pregnancy term (39 ± 1.7 WG) and delivery weight (2728 ± 376.9 g).

Conclusion: Cardiac malformations are frequent in women with TS. Cardiac evaluation of those women has to be realised by a referent cardiologist trained to search for the specific malformations associated to the disease since non specialised cardiologist could miss them, as our past experience highlights it. Indeed, a woman died in our centre of aortic dissection because her initial BAV was not diagnosed before pregnancy (Boissonnas CC et al. 2009). Echocardiography has to be associated to MRI to precisely screen the anomaly. Aortic diameter measured before pregnancy must be related to women's height so ASI assessment should be the reference before including women for oocyte donation even in the absence of cardiac malformation. BAV and any cardiac malformation and/or an ASI ≥ 20 mm/m2 should contraindicate a pregnancy and should be a cause of exclusion of the OD program. During pregnancy, ASI should stay < 20 mm/m2 to limit the risk of aortic dissection, even if this risk can never be totally excluded. Our cohort of women with strict cardiac screening that beneficiated of OD had no complication during pregnancy. However, TS women must be informed that pregnancy is at higher risk even in absence of initial cardiovascular risk factor detected.

P-333 Is there any benefit of blastocyst transfer in frozen-thawed cycles?

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Introduction: Many investigators suggested that fresh blastocysts transfer cycles result in better pregnancy outcomes with fewer number of transferred embryos compared with cleavage stage embryo transfer. However, in terms of frozen cycle outcomes using blastocysts, only few studies are available.

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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 vs. 33.4 ± 3.8 yrs), BMI (22.3 ± 3.5 vs. 21.5 ± 3.2 kg/m^2), and basal FSH (10.6 ± 5.5 vs. 10.9 ± 6.5mIU/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 shows 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.

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 it s role GnRH antagonists regime is insufficient.
Objective:s: 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 E_2.
Methodology: An observational study was conducted at Medically Assisted Centre, HUKM and IIUM from 1st of Jan 2009 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 E_2 valerate orally (n = 170, progesterone/E_2 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/E_2 group (40.0 %) (p < 0.005).
Conclusion: It appears that the addition of E_2 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.

P-335 Altered gene expression in neuroactive ligand-receptor interaction pathway in the brain of the mouse conceived by IVM

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Introduction: As an intellectual, scientific and clinical challenge with a number of potential applications, in-vitro maturation (IVM) of oocyte plays great role in avoiding ovarian hyperstimulation syndromes (OHSS) in patients with polycystic ovaries (PCO) during in-vitro fertilization (IVF). IVM could increase the supply of high-quality oocytes of more uniform characteristic, and would provide new opportunities for restoring female fertility after chemotherapy. However, some studies showed the process of IVM might induce abnormality in gene expression and organelle structures in oocytes. Up to date, whether IVM is a safety or effective technique for the assisted reproductive technology (ART) has been still under debate. The long-term health of offspring from IVM is still unclear. The goal of this project was to evaluate the long-term safety of IVM on the brain development from newborn to adult using a mouse model, assessed by gene chip microarrays.
Material and Methods: Metaphase II oocytes were obtained by maturation of GV-stage oocyte in vitro or administration of gonadotrophins in vivo (the control group) from C57BL/6J mice. All oocytes were fertilized by intracytoplasmic sperm injection (ICSI), and 2-cell stage embryos were transferred to the oviducts of pseudo-pregnant foster mothers for production of IVM and the control mice. The gene chip array (mouse genome 430 2.0) was used for the analysis of the gene expression in the brains of newborn mice from each group. The differentially expressed genes were verified by real-time RT-PCR. Meanwhile, the states of these genes during the development from newborn to adult were identified.
Results: Gene chip microarray analysis showed that there were 231 differentially expressed probes between the brain tissues of IVM and the control mice, representing 196 differentially expressed genes with 158 up-regulation and 38 down-regulation. For the pathway analysis, in general, differential gene expression followed specific molecular pathways, and the most significantly altered one was the pathway named neuroactive ligand-receptor interaction (P value was 2.0E-6 and the Q value was 4.7E-5). There were eight genes found to be up-regulated expression in this pathway, that is, sphingosine-1-phosphate receptor 5 (S1pr5),glycine receptor, alpha 1 subunit (Glra1),glutamate receptor, ionotropic, delta 2 (Grid2),galanin receptor 1 (Ga1r1), glutamate receptor, ionotropic, AMPA4 (alpha 4) (Gria4), gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 6 (Gabra6), angiotensin II receptor, type 2 (Agtr2) and glutamate receptor, metabotropic 4 (Grm4). All those changes were confirmed by real-time RT-PCR. Interestingly, we found that the different expression of those genes between the two groups disappeared, except Glra1 and Agtr2 which showed down-regulated in IVM group.
Conclusions: Evidence presented here using a mouse model suggests that IVM could affect the gene expression in the brain of newborn mice, especially in the neuroactive ligand-receptor interaction pathway. However, along with the growth and development, parts abnormal expressions could be rectified while others retained alterative. The exact functions of these changed genes contributing to the development of the brain need further investigation

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 59 IVM collections at our centre between January 1st and Dec 31st, 2009. The results of all cycles in terms of total

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number of mature oocytes (MII) collected, maturation rate of immature oocytes, 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) oocyte 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, IVM 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 extraction 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 coagulation tests and a randomized control group (1:3) were asked to complete a bleeding 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 coagulation 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 implemented in daily clinical practice [1]. To ensure proper implementation, the aim of our study was to identify existing barriers and facilitators for guideline adherence according to professionals and patients.

Methods: Qualitative focus group interviews were performed with 17 gynaecologists (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: I) the guideline, II) professionals, III) patients, IV) organisation.

Results: 96 potential barriers, at all four domains, were identified among professionals. 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 patients 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 information is not up to date with guideline for professionals, II) professionals having 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 effective.

The potential facilitators identified were immediate availability of the guideline or local protocol derived from the guideline, an electronic decision tool to advise on diagnostic work up and let patients report their family history prior to their first visit. All participants agreed that complete adherence to the guideline was theoretically achievable.

Conclusion: Both professionals and patients experienced barriers and facilitators for guideline adherence in recurrent miscarriage. These barriers should be addressed in guideline implementation strategies to be able to improve care.

References:

1 Franssen MTM, Korevaar JC, *et al.* Management of recurrent miscarriage: evaluating the impact of a guideline. Human Reproduction 2007;22(5):1298-1303.

P-339 Long term outcomes in women with pcos randomised to receive laparoscopic electrocautery of the ovaries or ovulation induction with gonadotrophins

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Background: Although laparoscopic electrocautery of the ovaries is a well accepted treatment for women with clomiphene citrate resistant polycystic ovary syndrome (PCOS), its long term benefits and harm are unknown. We followed a cohort of women allocated to electrocautery or gonadotrophins to study long term effects on fertility and chronic illness.

Methods: Between February 1998 and October 2001 168 women with clomiphene citrate resistant PCOS were included in a randomised controlled trial comparing an electrocautery strategy and rFSH treatment. In the year 2009

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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 gravidity, multiple pregnancy and intrauterine 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 electrocautery 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 electrocautery 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 electrocautery group (RR 1.7; 95% CI: 1.1 – 1.6). For women not using anticonception and a pregnancy wish, no differences were observed in the birth of a second live born child, 90% after LEO and 85% after rFSH (RR 1.1: 95% CI: 0.9 tot 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 tot 1.4) between the treatment groups.

Conclusion: In women with clomiphene citrate resistant PCOS, electrocautery 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 asses 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 878 (22.2 %) had 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 with an ROC-AUC of 0.52 and 0.50, respectively. In the univariate analysis of the ovarian reserve tests AMH was the best predictor of an excessive response with an AUC of 0.80, compared to the AFC and FSH (ROC-AUC 0.71 and 0.66, respectively). The added predictive value of AMH with age was highest with an AUC of 0.80 compared to the added predictive value of AFC and FSH (ROC-AUC 0.72 and 0.69, respectively). However, there was no significant improvement from the prediction of the ovarian reserve test alone.

Conclusion: In this individual patient data meta-analysis it was illustrated that the baseline characteristics BMI and duration of subfertility do 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* DNA 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. DNMT1o lacks the N-terminus of 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: Spare 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 trophectoderm and inner cell mass.

DNMT1o and DNMT1s could not be detected in the majority of human oocytes (n = 37) while a weak DMNT1o expression could be detected in most zygotes (n = 7). DNMT1s was mainly found in the cytoplasm from the 2-cell stage on whereas DNMT1o was constantly present in the nucleus from the 8-cell stage onwards. This pattern seems opposite to mice, where Dnmt1s shows a nuclear localization during all preimplantation embryonic stages and Dnmt1o remains cytoplasmic with only a transient nuclear switch at the 8-cell stage.

Conclusion: In human preimplantation embryos there is a gradual increase of DNMT3L in the cytoplasm from the 8-cell stage onwards, which may correspond with the activation of the embryonic genome. After 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 where 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 DNMT1 expression is only associated with maturation competency. Alternatively, maternally stored *DNMT1* 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.

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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 after underwent 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/aGnRH 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) of each group as well Levels of VEGF, sVE-Cadherin an Angiopietin 2 were determined by ELISA in serum and in follicular fluid. VEGF, Ang-2 and VE-cadherin gene expression were determinated by RT-PCR.

Data are expressed as mean ± SEM). Metric variables were analyzed by the independent t-test, and nominal variables by X^2 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 when 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 GnRHa 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 folicular 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

No significant differences in serum samples of VE-Cadherin were found but we observed a trend of higher levels in the group with hCG.
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: In 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 PGD/PGS conceptions. A control group of children born after intracytoplasmic sperm injection (ICSI) with embryo transfer on day 5, similarly to the procedure after PGD/PGS 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 PGD/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 PGD/PGS ($p < 0.001$). Less multiples had a low birthweight (< 2500g) after PGD/PGS ($p = 0.005$). Perinatal death was more frequent in multiples born after PGD/PGS ($p = 0.003$).
Conclusion: Embryo biopsy is not adding risks to the health of singleton newborn PGD/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 PGD/PGS could be related to the absence of infertility in many couples undergoing PGD. The higher perinatal death rate in PGD/PGS multiples in comparison to ICSI multiples, needs to be confirmed in other follow-up series en further explored. Data on long-term health all PGD/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.

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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 (Väestöliitto 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 of his/her conception. There were 146 children aged 3-14 years and 38 had already been informed (26.0%). The majority of the children (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 ($P < 0.05$, X^2). 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.

Conclusions: Approximately 60% of OD parents plan to inform their child of their donor oocyte origin and at least 26% of children up to 14 years of age have already been informed. Parents with young OD children are clearly more inclined to disclosure compared to parents with older children.

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 wich 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, inseminated 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.

Materials 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 importance 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 Luteolisis 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 oocyte recruited are considered the two main predictors of the hCG-dependent ovarian hyperestimulation syndrome (OHSS). Lately, GnRH agonist has been introduced as an alternative to hCG for final oocyte maturation (in GnRH antagonist cycles) as a mean to avoid the OHSS. Nevertheless, the absolute estradiol serum levels to prevent the development of OHSS have not been evaluated. Thus, the objective of this communication is, to determine the incidence of OHSS in IVF cycles in which ovulation was trigger with a GnRH agonist in the presence of very high circulating levels of estradiol (≥ 6,000 pg/mL).

Material and Methods: 51 egg donor cycles in which estradiol levels were ≥ 6000 pg/ML (study group), were compare with a 124 egg donor cycles in which estradiol levels were < 6000 pg/ML (control group); determined by the day ovulation was triggered with a GnRH agonist. All the cycles started with a fixed dose of 200 UI of recombinant FSH followed by a step-down protocol. The antagonist was introduced when the leading follicle was 14 mm, and 75 UI of LH was added at the same time. This study is a restrospective data analysis. Data were collected from our medical record database. For descriptive statistics, we used means ± SD. Student's t-test, Fisher's exact test and chi-square test were used where appropriate. $P < 0.05$ was considered significant.

Results: Mean estradiol levels were 9575.2 ± 4329.6 pg/ml in the study group and 3867 ± 2004.62 in the control ($p < 0.05$). Clinical pregnancy rates in the recipients who received eggs from both groups were similar(51.2% vs 49.6%, $p > 0.05$). The number of mature follicles was significantly higher in the study group (17 ± 7.6 vs 12.2 ± 6.1, $p < 0.05$). No significant differences in the amount of FSH (1760 ± 53 vs 1652.74 ± 388.9) or days of stimulation (11.7 ± 1 vs 10.9 ± 0.8) was seen. None of the donor developed symptoms of OHSS.

Conclusions: Estradiol serum levels from patients in which ovulation is triggered with a GnRH agonist seems to be of relative importance since the luteolysis will prevent the development of OHSS, independently of the hormone concentration.

P-349 Assessment of the genotoxicity of cryoprotectants commonly used in ART for vitrification with an in vitro biological model using CHO cells

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Introduction: : Cryopreservation of embryos or oocytes by vitrification is widely performed in ART. Vitrification is based on a rapid cooling that converts aqueous solutions into an amorphous solid phase, without ice crystals. The advantage of vitrification is that physical damages associated with ice formation are avoided. However, vitrification requires high concentrations of cryoprotectants, which may be cytotoxic and mutagenic. With a genetic toxicology approach, the aim 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-based 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 2,989,613 euros.
Conclusions: Even without full implantation, 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 menotrophin (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/m^2; underweight (BMI < 18.5 kg/m^2) and obese (BMI ≥ 30.0 kg/m^2) women were not included. The analysis population was distributed according to BMI as follows: N = 1,140 with BMI 18.5-24.9 kg/m^2 (normal weight) and N = 316 with BMI 25.0-29.9 kg/m^2 (overweight), with the mean being 21.6 ± 1.7 and 26.9 ± 1.3 kg/m^2, 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 regressing 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/m^2 was significantly ($p < 0.05$) lower compared to those with BMI 18.5-24.9 kg/m^2: 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/m^2) 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/m^2). 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/m^2.

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-

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periences with care. This questionnaire is subdivided in 7 dimensions, namely: accessibility; information; communication; respect for patients' values; continuity; autonomy; and competence. The FertiQoL-questionnaire consisted of 24 items, assessing the impact of fertility problems in the emotional, mind-body, relational and social domains. Lastly, the HADS comprised 14 items, subdivided into two scales measuring Anxiety and Depression. Using multivariate multilevel regression analysis ($p < 0.05$), we examined if adjustment for quality of life components, anxiety or depression was necessary when measuring patient experiences with fertility care. 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 items 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. The mean HADS-anxiety -and HADS-depression scores were respectively 5.6 (SD 3.8) and 3.5 (SD 3.3) on a scale from 0 to 21. Anxiety was related to the aspects 'communication', 'continuity', 'autonomy' and 'competence', whereas depression was associated with 'accessibility' and 'respect for patients' values'. Neither anxiety or depression was related to the aspect 'information'.

Conclusions: Overall, 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 placenta is important for mammalian fetal survival and growth by controlling of metabolism and fetal nutrition, gas and metabolite exchange, and so on. So we hypothesized that the environment of GDM could affect the potential of placenta. Furthermore, since epigenetic reprogramming begin at the time that migratory primordial germ cells (PGCs) enter the embryonic genital ridge through gametogenesis and epigenetic abnormality happening during this phase maybe involve the transgenerational transmission, intrauterine hyperglycemia may also have effects on the second filial generation (F2 offspring). Therefore, the aim of our study is to use gene chip-based approach to examine differences in gene profile in placenta, investigating whether the intrauterine environment of GDM has potential influence on both first and second filial generations.

Materials and Methods: We established a mouse model of GDM which induced by a single intraperitoneal injection of streptozotocin on the first day of pregnancy. On day 3 (D3) of pregnancy, diabetes was confirmed by measurement of blood glucose concentrations via tail vein and defined as a blood glucose level above 16.7mmol/L (300mg/dl). Maternal blood glucose was also examined on D7 and D18 of pregnancy to confirm the diabetic condition. At D18 of pregnancy, we collected the placenta of GDM offspring (F1-GDM) by uterine-incision. In addition, some GDM mice were allowed to deliver spontaneously and the pups were fostered by normal female mice and remained with their foster mothers until they were weaned. We intercrossed male and female adult F1-GDM mice and collected the placenta of F2 offspring (F2-GDM). Differences in gene expression of placenta were examined by Mouse Genome 430 2.0 Array of Affymetrix GeneChip.

Results: (1) Compared with control group, totally, there were 109 differential genes in the placenta of F1-GDM group and 71 differential genes in the placenta of F2-GDM group ($|Score(d)| \geq 2$, Fold Change ≥ 2 or Fold Change ≤ 0.5, number of samples per group ≥ 3); Notably, some genes detected to be differentially expressed in both F1-GDM and F2-GDM groups were identified as Gt-pbp4, Embigin, H2-D1, Ang2 and so on. (2) The differential genes are involved in many pathways including: cell adhesion molecules, B cell receptor signaling pathway, antigen processing and presentation, type I diabetes mellitus, TGF-beta signaling pathway, cytokine-cytokine receptor interaction and so on; (3) Using GO analysis, the differential genes participate in molecular function, biological process and cellular component such as cellular process, physiological process, biological regulation, development, metabolism and catalytic activity; (4) The results also included some relatively differential imprinting genes in the placenta of F1-GDM and F2-GDM groups, such as Igf2 and Meg3, which 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 complied (PR = 40.6%, multiple PR = 0%). 391 patients did not comply with the MPS and 2 embryos were transferred (PR = 57.8%, $p < 0.0001$; twin PR = 28.3%, $p < 0.0001$; triplets PR = 1.3%, $p < 0.001$). MPS transfer 2 embryos = 1850 patients: 1641 complied (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%, $p < 0.0001$; 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 recommended, 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.

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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 (NVOG). 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 to12 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 monozygotic 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 expressional 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

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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 *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 placentas 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 placentas. 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 placentas 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 γ-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 + /GSTT1 + , GSTM1-/GSTT1 + , GSTM1 + /GSTT1- and GSTM1-/GSTT1-. The odds ratio for development of IUGR was significantly higher in GSTM1-/GSTT1- group (OR = 2.47, 95%CI = 1.111-5.495, $\chi^2 = 5.085$, $p = 0.024$*,). Moreover, GSTM1-/GSTT1- genotypes showed a statistically significant increased risk of IUGR in combination with high maternal and cord blood levels of γ-HCH.
Conclusions: The results of this study suggest an association between high blood levels of γ- 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.[1-3] 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 cycles[1]. 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 ± 0.80 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 ± 8.6 (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.
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P-360 64-row multidetector computed tomography virtual hysterosalpingography. Three years of experience

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Introduction: 64-row multidetector computed tomography virtual hysterosalpingography (CT-VHSG) is an innovative, minimally invasive imaging test that combines the benefits of high temporal and spatial resolutions of the multidetector CT technology with the traditional X-ray hysterosalpingography technique. CT-VHSG provides an integral evaluation of the the female reproductive system. The purpose of this presentation is to illustrate the spectrum of findings and the differential diagnosis with other pathologies.
Material and Methods: We retrospectively evaluated 1500 CT-VHSG studies performed in our institution for the evaluation of infertility. The mean age of the studied population was 37.2 ± 3.9 years old.

CT-VHSG exams were performed using a 64-row CT scanner (Brilliance 64, Philips Medical Systems). Scans parameters were: 0.9 mm slice thickness, 0.45 mm reconstruction interval, 120 kV and 50-200 mAs. A total volume of 20 ml iodine contrast dilution (3ml of iodine contrast and 17ml of saline solution) were administered into the uterine cavity using a semi-rigid plastic cannula.

CT images were evaluated on a workstation using multiple post-processing techniques. The scanning time, the radiation exposure and the grade of discomfort were also documented.
Results: In the cervical region CT-VHSG demonstrated: wall irregularities (23%), folds thickening (10%), cervical polyps (9%), diverticulaes (6%), cervical stenosis (8%), cervical synechiaes (1%).

CT-VHSG findings in the uterus were divided according to the location. Uterine cavity findings were: polyps (40%), submucous myomas (9%), synechiaes (11%). Uterine wall abnormalities included: intramural and subserous myomas (9%), uterine malformations (8%), adenomyosis (5%) y C-section scar (3%). Fallopian tubes findings included: unilateral hydrosalpinx (8%) and bilateral hydrosalpinx (2%), tubal obstruction (4%). The 7% of the fallopian tubes were partially visualized in the CT-VHSG studies.

The scan time was 3.2 ± 1.1 sec. The effective radiation dose was 2.01 ± 0.4 mSv. The 81.3% of patients referred only mild or no disconfort.
Conclusion: CT-VHSG allowed an adequate and accurate evaluation of the female internal genital organs, providing a complete diagnostic information in patients with infertility. This is minimally invasive low radiation dose technique, well tolerated for the vast majority of the patients. This modality appears as a valid alternative diagnostic test in the algorithm of patients with infertility.

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 remaining 180/632 (28.4%) showed a secondary infertility. All patients have been valued with HyCoSy and have been checked in the 25 minutes after the examination. The patients have been examined in the lithotomic position, during the first phase of the menstrual cycle. A preliminary transvaginal ultrasound was performed with Logiq 5 Expert GE by two operators (A.A.M.; I.M.)to assess whether there where contraindications to perform the examination. The sonographic probe was removed, and a speculum was inserted to visualize the cervix. A stylet catheter (5F), was inserted into the cervix and a distal balloon was slowly filled with 1.5 ml of sterile saline solution, to fix the catheter and prevent saline backflow. The speculum was then removed, and the probe was inserted, to confirm the correct position of the catheter. Patency has been investigated by injecting air and saline solution, in a small amounts of 1-2 ml each. We distributed a questionnaire to patients to verify the onset of the late collateral effects and following complication to the diagnostic procedure. Tolerability to the procedure has been evaluated by means of a 11-points numeric rating scale of the pain experienced (0 to 10: 0-4 mild pelvic pain; 5-7 moderate pelvic pain; 8-10 severe pelvic pain), besides pelvic infections, haemorrhage and post procedural fever were recorded. The questionnaire has been returned in our structure within 30 days from the execution of the ultrasound examination.
Results: Of 700 patients, 632 (90%) returned the questionnaire. The mean numeric rating scale was 4,1 (range 0-9). 15 (2.37%) 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 out 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); endometriosis 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 afterloaded 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).
Matherial 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 embryologist 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 $\alpha < 0.05$.

Afterloaded 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

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(*35.91* ± 3.87 in AT vs. 36.44 ± 3.68 years in DT), hormonal levels (*basal FSH 7.13* ± 2.72 in AT vs. 7.18 ± 2.43 mIU/L in DT), body mass index (*22.63* ± 3.51 in AT versus 22.32 ± 3.9 in DT), oocytes retrieved (*10.29* ± 5.74 in AT versus 11.28 ± 6.77 in DT) and number of embryos transferred (*1.95* ± 0.63 embryos in AT versus 2.07 ± 0.58 in DT).

Clinical pregnancy rate was similar in both groups (AT: 55/152, 36.2% vs DT: 54/150, 36%; difference 0.18%; 95% CI, -10.6% to 11%).

According to duration of embryo transfer AT was shorter than DT (131.5 seconds vs 152.6 seconds, respectively; 95% CI, 8 to 33; P < 0.05).

There was no difference according to difficulty of embryo transfer (AT: 9.54 vs DT: 9.52) (ns).

Conclusions: Therefore to our knowledge this would be the first prospective randomized study performed to compare AT vs DT. Although there is a statistically significant difference according to embryo duration (shorter in AT compared to DT), this does not reflect on reducing embryo transfer difficulty or improving clinical pregnancy rate.

Clinical Trials NCT 00824629

P-363 The role of acupuncture in patients at unfavourable reproductive prognosis in IVF: a prospective randomised study

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Introduction: 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 according 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 according 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-364 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: Vitrification has become a reliable routine procedure in the program of assisted reproductive medicine. However, changes of physical condition during cryopreservation can impact on the implantation potential of early cleavage-stage embryos. From historic examination and experiences of IVF, it is suggested that the inactivated blastomere can inhibit the differentiation of embryo. Indeed, partially damaged vitrified-warmed embryos can affect the development and viability of cryopreserved embryos. Based on them this study evaluated the effect of laser-assisted removal of lysed blastomere of thawed embryos on implantation and pregnancy rates in human cleavage-stage cryo–embryo transfer (ET) cycles.

Material and Methods: This was a prospective randomized study conducted in Mirae and Heemang OB/GYN clinic. From July 2009 to December 2009, a total of 515 embryos were thawed in 117 cryo-ET cycles. All embryos were vitrified on day 3. Eight cell embryos were equilibrated in 7.5% ethylene glycol (EG) + 7.5% DMSO for 15 min and then vitrified in 15% EG + 15% DMSO + 0.5M sucrose within 1 min. The vitrification solution containing embryos was placed onto the pull and cut (PNC) straw, which was designed and made by pulling and cutting of 0.25ml plastic straw, and immediately plunged into the liquid nitrogen. Vitrified embryos were warmed in 1M sucrose at 37°C for 1min, 0.5M sucrose for 3min, and washed twice in PBS. After then the embryos were evaluated: the intact and lysed cell number was counted. All examined embryos were performed laser-assisted hatching. Fully intact 244 embryos (Group 1, n = 72) did not be treated another approach. Half of the 155 embryos with partially damaged blastomeres were not performed laser-assisted removal of lysed blastomere (Group 2, n = 23) and the others were performed laser-assisted removal of lysed blastomere (Group 3, n = 22).

Results: A total of 515 embryos were thawed and the survival rate was 77.5% (399/515). There were no statistical differences in the mean number of thawed embryos, survived embryos and transferred embryos between groups. The number of intact blastomeres after thawing was significantly lower in Group 3 (5.8 ± 0.4) as compared between Group 1 (7.2 ± 1.4) and Group 2 (6.2 ± 1.2). The mean number of blastomeres per transferred embryo after culture in Group 3 (7.3 ± 1.4) was significantly lower than Group 1 (8.8 ± 2.7), but it was similar to Group 2 (7.6 ± 1.7). Clinical pregnancy rate (CPR) and implantation rates (IR) in Group 1 [38.9% (28/72) and 18.4% (45/244)] were higher than Group 2 [26.1% (6/23) and 11.5% (9/78)] and Group 3 [31.8% (7/22) and 10.4% (8/77)], but it was not statistically significant. When fully intact embryos were less than half of all thawed embryo, CPR and IR were higher in Group 3 [25.0% (3/12) and 9.3% (4/43)] than Group 2 [0% (0/5 and 0/19)].

Conclusions: Laser-assisted removal of lysed blastomere improved the developmental potency of the partially damaged vitrified-warmed embryos by post-thaw resumption of mitosis and CPR and IR were equivalent to fully intact embryos. These results show that laser-assisted removal of lysed blastomeres may improve the clinical outcome, especially, in case of fully intact embryos less than half of all thawed embryos, in human cleavage-stage embryo cryo-ET cycles.

P-365 The diameter of leading follicle on the day of oocyte retrieval affects clinical outcome in IVM 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

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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 I.U. or GnRH agonist (GnRHa; buserelin acetate 300 μg). Follicular monitorings 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 intracytoplasmic 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.

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 by means of a report was tested versus a maximal multi-faceted strategy that was both professional as well as patient-oriented. Elements of the latter strategy comprised the feedback report and discussion of it, information checklists, leaflets on shared decision making, suggested tools for implementation of the guidelines on local level and patient leaflets which explained the professional guidelines in lay language. Sixteen Dutch fertility clinics participated in the trial. Primary outcome measures were the extent of adherence to 25 guideline-recommendations, reflected in quality indicator scores. To get insight into unwanted side-effects, patient anxiety and depression scores were gathered as secondary outcomes by means of the State Trait Anxiety Index and Beck's Depression Index. Data collection encompassed medical record search, patient and professional questionnaires.

Results: 1499 couples were included at baseline and 1396 at after-measurement. After an implementation period of six months, no overall significant improvement in indicator scores was found for either strategy nor for any specific guideline. Secondary outcomes did not differ for both groups, selected anxiety 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-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 ($P <$ or $= 0.05$ for any outcome measure) or non-significant ($P > 0.05$ or not reported) and in favor of experimental arm ($P <$ or $= 0.05$ 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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rank = 0.013), whereas laboratory studies were published significantly later compared to all other studies (log-rank test 0.034).
Conclusion: Almost half of the randomized trials presented in ESHRE annual meeting are not published even 7 years after presentation. Trials presented orally and trials which provided significantly higher results in favor of the experimental arm are more likely to be published and are published sooner compared to poster presentation and studies that do not favor the experimental arm. Publication bias in reproductive medicine is a fact.

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 IUI 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% *(*$P \leq 0.05$). 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%), *(*$P \leq 0.05$).
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 (Frydman® 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 environnemental toxic compounds in IFV 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

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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 tot 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.

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 recovery 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 fully 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% + collagene 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% CO_2 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% + collagene 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 momolayer 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 × 1mm × 1mm)and cryopreserved by vitrification method. Decidua biopsy was performed during induced abortion surgery. Decidae 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% CO_2 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 χ^2 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.

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