2 (2%) twin deliveries. Three hundred and thirty-eight (72% of cycles) 2 embryo transfers resulted in 199 deliveries, 139 (41%) single and 60 (18%) twin deliveries.

Conclusion: The results indicate that in routine IVF, embryo scoring focused on a few well defined scoring parameters can help to choose optimal pre-embryos for transfer, especially for single transfers. However, pre-embryos with 100% implantation potential did not always have top embryo scores using the current scoring parameters, indicating that there is only partial overlap between maximal implantation potential and morphologically scored top embryos.

O-170   Predicting in vitro oocyte fertilization in assisted reproductive techniques: intra-follicular leptin concentration

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Introduction: Granulosa cells are able to produce and store leptin, suggesting that this hormone is locally involved in the regulation of follicular growth. In Italy, a recent law established that a maximum of three oocytes for each patient can be selected and utilized for IVF/ICSI. Thus new strategies for selecting mature oocytes would be developed. In this study, the role of follicular fluid (FF) leptin concentration in predicting oocyte fertilization and embryo quality was preliminary evaluated in normogonadotrophic women undergoing controlled ovarian stimulation (COS) for assisted reproductive techniques.

Materials and methods: Leptin concentration was measured in 34 consecutively collected FF from 26 women. In order to avoid contaminations from other follicles and inappropriate matching between oocytes and follicles, only the first follicle for each ovary was considered. In addition, only FF in which a mature oocyte had been found during the ovum pick up were analysed. Thus, single oocytes were matched with their FF and followed during the technique until the embryo transfer. Embryos deriving from fertilized oocytes were submitted to quality scoring systems.

Results: Mean leptin concentration was significantly higher in FF whose oocytes showed two pronuclei ($n=19$) when compared with those with no evidence of fertilization ($n=15$) at the 16–18 hours check (25.9 ± 6.6 versus 16.1 ± 11.3 ng/ml, respectively, $p<0.05$). Follicular mean diameters were similar in the two groups (21.3 ± 3.2 and 20.5 ± 5.2 mm, respectively). Logistic regression analysis identified FF leptin levels as the best predictive parameter for oocyte fertilization ($p<0.001$). When receiving operating characteristics curve was employed, a FF leptin concentration of 20.25 ng/ml was the most reliable cut-off in predicting fertilization of oocytes. FF with leptin concentrations higher than this value ($n=18$) had an oocyte fertilization rate of 85.7%. In contrast, FF levels of 20.25 ng/ml ($n=16$) were associated with a rate of 16.7% ($p<0.05$). No correlation emerged between FF leptin and the score attributed to 15 valuable embryos at the stadium of zygote ($r=-0.01$) and at 48 h after insemination ($r=0.1$).

Conclusions: Our preliminary results suggest FF leptin levels as possible predictor of oocyte competence and fertilization success rates. These results underline the relevance of FF variables in developing methods for oocyte selection. Following confirmation on our data, this variable may be adopted in a multivariate scoring system aimed at a real-time oocyte selection.

O-171   Mannose-binding lectin and tubal factor infertility

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Introduction: Tubal factor infertility is mainly caused by obliteration of the fallopian tubes. The most common cause of tubal obliteration is a past salpingitis, e.g. infection with *Chlamydia trachomatis*. The serum protein mannose-binding lectin (MBL) is an important component of the innate immune system.

By binding to carbohydrate residues on microbes, MBL enables activation of the complement system. Common polymorphisms in the gene encoding for MBL, which cause low levels of functional MBL are associated with various types of infectious and inflammatory diseases. Thus we speculated whether the presence of MBL variant alleles could be related to tubal infertility and whether these alleles could influence the clinical pregnancy rate after IVF/ICSI.

Materials and methods: One hundred and seven women with tubal infertility referred to IVF treatment and 113 women with no history of tubal disease referred to ICSI or IVF with donor semen due to male factor infertility were prospectively included. All were of Danish Caucasian descent. MBL polymorphisms were investigated by established PCR techniques using peripheral blood DNA from the women. The frequencies of the normal wild-type allele A coding for normal MBL levels and the mutant alleles, jointly called O alleles, coding for low MBL levels were calculated.

Results: No significant difference in MBL genotypes between the women with and without tubal disease was found. The MBL genotypes A/A, A/O and O/O were present in 59.8, 36.4 and 3.7% of the patients with tubal factor and in 61.1, 33.6 and 5.7% in women with no tubal disease, respectively. The pregnancy rate (a living pregnancy in week 7) in the next IVF/ICSI cycle was 29% both in the tubal factor and male factor infertility groups. There was no relationship between the MBL genotype and the likelihood to achieve a living pregnancy at week 7 in either group.

Conclusions: Presence of MBL variant alleles was not associated with tubal infertility or decreased IVF outcome. Thus MBL does not seem to play a direct overall role in protection against tubal obliteration. Furthermore, the presence of MBL variant alleles does not seem to affect the clinical pregnancy rate after IVF/ICSI.

FREE COMMUNICATION

Session 45 – ART/Ovarian stimulation 2

Tuesday 21 June 2005   **17:00–18:00**

O-172   The effect of recombinant HCG on oocyte maturation in assisted reproduction

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Introduction: Recombinant HCG (rHCG) was recently introduced as an alternative to urinary HCG for the final maturation of oocytes in women treated with IVF and ICSI. However, little information is available on the nuclear and cytoplasmic maturation of the oocytes in women receiving this new preparation. The aim of this work was to conduct a prospective randomized study in order to investigate the effect of rHCG on oocyte maturity compared with urinary HCG (uHCG), in women treated with ICSI.

Materials and methods: A total of 89 infertile couples due to male factor who attended our centers for ICSI treatment from January to December 2003 were included in the study. None of the patients showed FSH >10 IU/ml and oestradiol >60 ng/ml on cycle day 3. All women were treated with a long protocol in which GnRH analogue (Buserelin subcutaneous, 0.4 mg daily) was given as a pre-treatment and rFSH administration (Gonal-F, 225 IU daily) was started when pituitary desensitization was established. From the 7th day of stimulation in both groups, daily monitoring of follicle size by ultrasound was performed and plasma levels of oestradiol were measured. The dose of rFSH was adjusted according to the individual response of each patient. Ovulation was triggered with HCG when plasma oestradiol levels reached between 1000 and 4500 pg/ml and at least four follicles >16 mm diameter were visualized on ultrasonography. Patients were randomly assigned to one of the two study groups. Group A consisted of 42 women who received 250 μg of rHCG, while group B consisted of 47 couples who received 10,000 IU of uHCG. Oocyte retrieval was performed 34–37 h after HCG administration. The number of oocytes retrieved was recorded. The rate of metaphase II oocytes was calculated and the oocytes were assessed for cytoplasmic maturity. The fertilization rate, pregnancy rate and implantation rate were also calculated.

Results: There were no statistically significant differences between the two study groups regarding the patient's age, body mass index, basal levels of FSH or estradiol levels on the third day of cycle. The two groups showed comparable data for total units of FSH administered, estradiol levels at HCG day, days of stimulation, number of follicles and number of oocytes harvested. The rate of metaphase II oocytes in women treated with rHCG was significantly higher (88.1%) than in patients treated with uHCG (80.8%) (P<0.01). The rate of metaphase II oocytes with cytoplasmic maturity was significantly higher in women treated with rHCG (89.1%) than in women treated with uHCG (81.5%) (P<0.01). There were no significant differences between the two groups in the fertilization rate, cleavage rate, top quality embryo rate, pregnancy rate, implantation rate or miscarriage rate.

Conclusion: In women treated for ICSI, rHCG increases the number of metaphase II oocytes as well as their cytoplasmic maturity compared with uHCG. However, our study showed no significant difference between both preparations in terms of clinical outcome and a larger study may be needed to clarify this point.

O-173 Characteristics of follicular fluid from women receiving either Buserelin or hCG to induce ovulation

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Introduction: The use of GnRH antagonists during ovarian hyperstimulation has allowed final follicle maturation and ovulation to be induced with either a single bolus of a GnRH agonist (GnRHa) or with the traditionally used hCG. The GnRHa induces an endogenous LH as well as FSH flare-up with ovulatory levels of gonadotropins comparable with those of the natural cycle and may theoretically be more physiological. However, a recent prospective randomized study from our group showed a very poor reproductive outcome when Buserelin was used to trigger ovulation despite a luteal phase support (i.e. progesterone and oestradiol) similar to that normally administered. Although an insufficient luteal phase may cause the poor outcome, it is currently unknown whether this also reflects an improper follicular maturation caused by the GnRH agonist. By analysing follicular fluid samples from the above mentioned prospective randomized study the specific effects of the GnRHa on the hormone milieu of preovulatory follicles was compared with that of hCG.

Materials and methods: Patients were stimulated with recombinant FSH and once the leading follicle had reached a size of 15 mm, a GnRH antagonist (Orgalutran) 0.25 mg administration was initiated and continued until the day of ovulation induction. Patients were randomized (sealed envelopes) to ovulation induction with either a single bolus of 0.5 mg Buserelin s.c. or 10.000 IU of hCG s.c. Oocyte retrieval was performed 34–36 h later followed by fertilization by either IVF or ICSI. Luteal support was given in the form of micronized vaginal progesterone, 90 mg a day as well as estradiol 4 mg a day. Two follicular fluid samples from each of 69 women (i.e. 32 receiving Buserelin and 37 receiving hCG) were monitored for the following hormones: LH, FSH, oestradiol, progesterone and inhibin-B.

Results: The implantation rate (34% versus 3.4%; p<0.001) and clinical pregnancy rate (36% versus 6%; p<0.005) and rate of early pregnancy loss (4% versus 79%; p<0.001) were all in favour of hCG. Follicular fluid levels of LH (IU/l) in the agonist group as compared to the hCG group was [11.1±0.5 versus 3.6±0.3 (mean±SEM); p<0.001], FSH (IU/l) (6.3±0.6 and 3.3±0.2; p<0.001), oestradiol (μmol/l) (1.9±0.2 versus 1.8±0.2; p>0.10), progesterone (μmol/l) (70±4 versus 90±6; p<0.001) and inhibin-B (ng/ml) (35.6±2.8 versus 40.1±3.1; p>0.10). In addition, serum levels of inhibin-B were comparable on the day of ovulation induction and on the day of OPU, despite a highly significant difference in circulating levels of progesterone.

Conclusions: This study demonstrates that the detrimental effect on the clinical outcome in the group of patients subjected to ovulation induction with GnRHa (Buserelin) is most likely not related to a defect in final follicular maturation. Despite significantly reduced levels of progesterone in follicular fluid from women in the GnRH agonist group compared with the hCG group, the levels are still comparable to levels observed with the use of other protocols and levels of inhibin-B and oestradiol remain similar. Intrafollicular concentrations of gonadotropins remain significantly higher at the time of OPU than those

observed in the hCG group suggesting that a proper follicular maturation has occurred. These data enforce that ovulation induction with a GnRHa cause a defective corpus luteum and results in a luteal phase that cannot be rescued by progesterone and oestradiol administration.

O-174 IVF/ICSI cycles with the addition of rhCG microdose

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Introduction: Many publications propose the necessity of adding LH in IVF cycles to increase the production of viable oocytes. Most physicians use hMG as the source of LH add back, but due to lack of purity and high variability of this product, we decided to start using microdoses of rhCG (Ovidrel, Serono). The objective of this pilot study was to compare the outcome of IVF/ICSI patients treated with antagonists and pure rFSH or rFSH with the addition of microdoses of rhCG during the last phase of follicular development.

Materials and methods: Fifty-four IVF patients between 25 and 35 years of age, with BMI 18–29 kg/m^2, basal FSH <10 mIU/ml and regular cycles were included in the study. Patients in control group (n=16) were treated from day 3 to 10 of the cycle, with 225 IU of rFSH. When the leading follicle reached 14 mm, 0.25 mg of Cetrotide was administered daily, until the day of ovulation triggering. The study group (n=38) started with 225 IU of rFSH but 1 day after starting Cetrotide, rFSH dose was decreased to 75 IU and 7.7 μg of rhCG (0.1 ml of a solution containing 250 μg of rhCG), equivalent to 200 IU of LH activity per day. Microdoses of rhCG were continued until day of 250 μg of Ovidrel SC. The day after starting rhCG, rFSH was stopped. Follicular aspiration was performed 36 h after rhCG injection. All oocytes collected were evaluated and fertilized using ICSI. The embryos produced were transferred at day 3 using standard procedures. Laboratory and clinical data were analyzed. Statistical analysis was performed using Mann–Whitney, t-test and chi-square as required, with p<0.05 considered statistically significant.

Results:

Table I. Mean values for every variable+standard deviations and p values

Variables	Study group	Control group	p-Value
Number of patients	38	16	
Age and cycle start	30.3±3.5	31.3±3.4	0.33
BMI (kg/m^2)	22.9±3.2	21.6±2.7	0.18
Total amount of FSH used (IU)	2167±648	1597±254	0.002
FSH basal (IU)	4.32±1.46	4.52±1.18	0.61
E2 basal (pg/ml)	53.0±21.5	47.3±14.1	0.34
Total oocytes	14.9±10.7	18.5±9.7	0.22
Mature oocytes (MII)	11.9±8.8	14.4±6.9	0.17
MII/follicles (%)	56.7±14.2	57.2±17.4	0.99
Viable embryos	5.45±3.29	8.38±5.26	0.04
Embryos transferred	3.5±0.92	3.62±1.09	0.74
Embryos frozen	1.95±3.76	4.75±5.29	0.02
Implantation rate	11.0±20.9	14.1±11.3	0.26
Pregnancy rate (%)	26.3% (10/38)	12.5% (2/16)	0.47

Conclusions: The preliminary results of this study showed same results in terms of the number of oocytes retrieved, MII and embryos transferred. Implantation and pregnancy rates were obtained using a smaller dose of rFSH along with microdoses of rhCG as a source of LH. This procedure is an alternative for good prognosis patients, mainly those younger than 35 years of age. More studies using microdoses of rhCG in these patients are necessary to confirm our findings.

O-175 Prospective, randomized, dose finding study of low-dose human chorionic gonadotropin (hCG) administration in controlled ovarian stimulation (COS)

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Introduction: Follicle-stimulating hormone (FSH) is critical to induce follicle recruitment and growth; nevertheless, we showed that the combination of FSH and low-dose hCG monotherapy across the COS cycle, as currently achieved

with the use of highly purified human menopausal gonadotropin (HP hMG), stimulates steroidogenesis and folliculogenesis more effectively than FSH alone. Furthermore, FSH can be replaced at least in part by the administration of luteinizing hormone (LH) activity once the LH/hCG receptor is expressed by granulosa cell. We previously demonstrated that low-dose hCG administration can be used instead of FSH in the last 3–4 days of gonadotropin treatment to effectively complete COS and achieve clinical success and pregnancy. However, HP hCG at these low concentrations is not currently available and the most suitable hCG dose for these novel COS regimens has not yet been established. Thus, we elected to assess the effect of different amounts of low-dose hCG on folliculogenesis, steroidogenesis and ICSI outcome.

Materials and methods: A total of 80 ICSI patients (20/group) were randomized to be treated with four different gonadotropin regimens in a long GnRH agonist protocol. Group A received only recombinant (r) FSH (250 IU/day) throughout treatment. The other patients received rFSH (250 IU/day) until estradiol (E_2) was 600 pg/ml and three follicles >12 mm were detected; thereafter, rFSH was lowered to 50 IU/day and hCG 100 (group B), 200 (group C) or 400 IU/day (group D) was added. Treatment was monitored with daily serum samples, pelvic ultrasound at 2-day intervals and follicular fluid (FF) hormone measurements.

Results: Low-dose hCG was administered for an average of 4 days. The per cycle rFSH dose was significantly reduced, fewer small (<10 mm) and a greater number of large preovulatory follicles (>14 mm), and more oocytes were obtained in groups B, C and D. In preovulatory serum and FF, E_2 and testosterone (T) levels were lowest in group A, while progesterone (P) levels were highest in group D. E_2/T, E_2/androstenedione and E_2/P ratios in FF were higher and a significantly greater number of oocytes were obtained and fertilized in groups B and C. Although there was a trend towards higher pregnancy rates in groups B and C, it did not achieve statistical significance.

Conclusions: Combining low-dose hCG and FSH positively impacts on outcome. However, an hCG dose of 400 IU/day in the late COS stages may be excessive and potentially associated with lower oocyte yield and premature luteinization. Conversely, hCG at a dose of 100 or 200 IU/day combined with rFSH 50 IU/day versus rFSH alone: (1) reduced rFSH consumption; (2) resulted in the development of a greater number of mature follicles and a higher oocyte yield; (3) induced a more estrogenic preovulatory and intrafollicular environment; (4) tended to improve clinical outcome of ICSI; (5) is a suitable clinical COS regimen.

Session 46 – ART/Risks and complications

Tuesday 21 June 2005   **17:00–18:00**

O-176   Ovarian hyperstimulation syndrome (OHSS) incidence in GnRH antagonist IVF cycles. Can estradiol levels or the number of follicles on the day of hCG administration predict the patients at risk of developing OHSS?

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Introduction: Cochrane meta-analysis of the five prospective randomized studies with a GnRH antagonist suggested that the incidence of severe OHSS was not dependent on the type of analogue used. Moreover, the role of variables like estradiol or the number of follicles, on the day of hCG, in identifying patients at risk for OHSS is still debatable. This might be explained by the lack of distinction between early and late OHSS type, and the inclusion of iatrogenic OHSS cases after the luteal supplementation with hCG. The aim of this prospective cohort study was to assess the incidence of OHSS in GnRH antagonist stimulated cycles. In addition, to estimate the predictive value of estradiol levels and/or the number of follicles present on the day of hCG, taking into consideration the type of OHSS.

Materials and methods: Between January 2002 and December 2003, 2526 consecutive IVF/ICSI cycles with a GnRH-Antagonist/recFSH ovarian stimulation protocol were analysed. Exclusion criteria included (1) the use of hCG for luteal supplementation (n=121) and (2) oocyte donation cycles. According to the day of oocyte retrieval, OHSS cases occurring within 9 days after OPU were addressed as early OHSS and those occurring after 10 days were classified as late OHSS. Only patients with moderate or severe OHSS were hospitalized and were considered as OHSS cases.

Results: Fifty-five patients out of 2526 cycles were hospitalized due to OHSS (2.1%; 95% CI: 1.6–2.8). Early OHSS presented in 33 patients (1.3%; 95% CI: 0.9–1.8) whereas the late type complicated 22 patients (0.8% 95% CI: 0.5–1.3). Patients with hyperstimulation syndrome were significantly younger than the controls (30.3 days versus 33.1 days, p<0.01). Late OHSS compared with early type had significantly higher probability to be severe (72.7% versus 39.4%, p=0.02). Several cut-off values of estradiol and number of follicles were tested for sensitivity and specificity in an attempt to predict high risk patients in developing OHSS. None of the estradiol thresholds could predict severe, late or even early OHSS. The higher sensitivity (67%) and specificity (81%) for estradiol was given for a threshold of 2500 ng/l and only for early OHSS cases. On the contrary, a threshold of 13 follicles with a diameter above 11 mm could predict all the early OHSS cases (100% sensitivity, 70% specificity) and additionally 87% of the severe cases, which represent the most clinically significant (70% specificity). Moreover this cut-off level of 13 follicles had a highly significant negative likelihood ratio of <0.01.

Conclusions: This study is the largest series ever reported on the incidence of OHSS in GnRH antagonist stimulated cycles. The best estimate of the reported incidence of 2.1% indicates that OHSS does not appear to be different between GnRH antagonist stimulated cycles and those reported in IVF cycles treated with a GnRH agonist. On the other hand, it becomes evident from the current analysis, that even low levels of serum estradiol above which one could be considered at high risk for OHSS have no predictive validity. However, the number (≥13) of follicles present on the day of hCG administration could be an excellent way of differentiating the patients at high risk of developing severe OHSS and especially the early cases. Therefore, future preventive strategies for OHSS can be based on that threshold to replace the ovulatory dose of hCG with either administration of a single dose GnRH agonist, or rec-hCG or rec-LH in antagonist cycles.

O-177   Results of IVF/ICSI cycles after the new Italian law

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Introduction: The Italian law regulating assisted reproduction treatments (ART) stated that no more than three oocytes can be fertilized and that all the embryos have to be replaced in a single transfer. Embryo cryopreservation is allowed only in cases of unexpected risk to the mother. The effect of these limitations are of great concern for the couples. Data on treatments performed were collected to identify the consequences of this law on ART in our study.

Materials and methods: Between March and December 2004, 765 cycles of ovarian stimulation for ART have been performed in our centre: 217 were IVF cycles and 548 were ICSI cycles. In 62 cases frozen spermatozoa rescued after testicular sperm extraction was used. A total of 690 oocyte retrievals was performed and 75 cycles (9.8%) were cancelled because of poor ovarian response to the therapy. The three best quality oocytes in each patient were inseminated or injected according to the characteristics of the semen sample. The remaining metaphase II oocytes were cryopreserved. Oocytes were observed 18–20 h after insemination or injection in order to asses pronuclear formation. Every embryo and every fertilized oocyte, even if no cleavage had occurred, have to be transferred without selecting for embryo quality. Embryo replacements were performed on day 2 or 3 after retrieval. According to the law, when abnormal fertilization was observed, couples were informed and decided to accept or refuse the transfer of the abnormal embryos.

Results: 7488 oocytes were retrieved (mean±SD 10.79±6.64). In 11 cases no oocytes were retrieved or there were no suitable oocytes for insemination or injection. A total of 528 oocytes were inseminated and 1526 were injected, 1554 (75.7%) oocytes were fertilized and 1493 (96.1%) embryos were transferred. In 32 cases (4.6%) no transfer was performed, 3 (0.4%) because no