Results: Out of 105 tested heterozygous single leukocytes, 103 (98.1%) showed a positive amplification signal, while five cells (4.9%) showed ADO. Amplification in single blastomeres was shown in 13 out of 14 (92.9%) and ADO was observed in 2 out of 13 single blastomeres (15.4%). PGD of SCA3 was performed for a couple in which the male showed a normal 26 CAG-repeats allele and an expanded allele of 67 CAG-repeats and his wife demonstrated two normal alleles of 21 and 22 CAG-repeats, respectively. After the first superovulation cycle seven embryos were available for biopsy. All blastomeres showed amplification and no ADO occurred. One embryo was diagnosed as affected. Six embryos were diagnosed as unaffected and two of them were transferred and resulted in a singleton pregnancy without complications.

Conclusions: As was shown by the single leukocytes test the single cell SCA3 CAG-repeats PCR protocol appeared to be an efficient and accurate method for application in PGD of SCA3. The first PGD for SCA3 resulted in the birth of a healthy girl.

11:15–11:30

O-107. Single cell co-amplification of polymorphic markers on Xq28 for the preimplantation genetic diagnosis by linkage analysis of hemophilia A, X-linked adrenoleukodystrophy, X-linked hydrocephalus and incontinentia pigmenti

Gigarel N.[1], Burlet P.[1], Frydman N.[2], Kerbrat V.[2], Vekemans M.[1], Frydman R.[2], Munnich A.[1] and Ray P.F.[1]

[1]Hôpital Necker, Genetics, Paris and [2]Hôpital Antoine Béclère, Obstetrics and Gynaecology, Clamart, France

Preimplantation genetic diagnosis (PGD) first consisted in the selection of female embryos for patients at risk of transmitting X-linked recessive diseases. Advances in molecular biology now allow the specific diagnosis of almost any pathology. We feel it becomes almost unethical to realise such a 'reverse sex discrimination' especially since the unnecessary disposal of healthy male embryos reduces the procedure success rate by diminishing the pool of embryos eligible for transfer. The most telomeric part of the X-chromosome long arm is a highly gene-rich region encompassing disease genes, such as haemophilia A, X-linked adrenoleukodystrophy, X-linked hydrocephalus and incontinentia pigmenti (IP). Most haemophilia A cases are caused by a large inversion within Factor VIII gene while the predominant IP mutation is a 11.7 kb deletion. The direct detection of such mutations is not possible from single cells and a specific diagnosis can only be carried out by identifying the mutant allele using polymorphic markers. We set out to develop a panel of fluorescent primers that can be used to follow the segregation of these disease genes. Such markers would also allow the indirect diagnosis of adrenoleukodystrophy and X-linked hydrocephalus whose genes are located less than 150 kb away. For these last two pathologies this will avoid the lengthy task of developing new single cell PCR assays for each familial mutation. To circumvent the risk of misdiagnosis due to a meiotic recombination between the gene of interest and the marker it is important to analyse two markers located on each side of the gene. The co-amplification of several polymorphic sites also reduces the chances of amplification failure and increases the assay accuracy by allowing the detection of potential allele drop-out. We set up one duplex single cell co-amplification assay with dinucleotide repeats DXS1073 and BGN for the diagnosis of adrenoleukodystrophy, X-linked hydrocephalus and one triplex test with DXS1073, G6PD, DXS1108 for the diagnosis of hemophilia A and IP. Amplification rate on single lymphocytes ranged from 90% to 98% with an ADO rate comprised between 2% and 10%. Familial linkage analysis was carried out for eight couples. At least two markers were informative for two couples at risk of transmitting adrenoleukodystrophy and X-linked hydrocephalus and for five of six couples with hemophilia A. We are currently developing a F8C intragenic marker (F8C intron 13CA) to reduce the number of couples for which this test is not applicable. All of these couples are now scheduled for PGD and will be treated in the months to come. If the F8C intron 13CA is not informative for the last haemophilia family we will for them, have to return to the 'good old sex selection'.

ART/Ovarian stimulation 2

Tuesday 1 July 2003
Madrid

10:00–10:15

O-108. A prospective, multicentre study assessing the tolerability and convenience of the use of a new formulation of follitropin α (Gonal-F multidose) in ovulation induction, intrauterine insemination and IVF/ICSI

Avril C., The r-hFSH multidose Study Group

Clinique Saint Antoine, Assisted Reproductive Technique, Bois Guillaume, France

Introduction: A prospective, multicentre study was carried out to assess the tolerability and convenience of the use of a new formulation of follitropin α (Gonal-F multidose) in ovulation induction (OI), intrauterine insemination (IUI) and IVF/ICSI. Recombinant-hFSH is largely used for the treatment of infertility. This study aims to assess the tolerability and convenience of use of Gonal-F 600 IU/ml preparation. Patient's and clinician's perception were also studied.

Materials and methods: Patients included were treated in OI, IUI or IVF/ICSI. Eligibility criteria were kept simple to reflect normal clinical practice. In OI, the chronic low dose protocol was recommanded with a starting dose of 75 IU. In IVF/ICSI, patients received a starting dose of 225 IU combined with GnRH agonists or antagonists. Recombinant-hFSH was supplied in a multidose vial containing 1050 IU follitropin alfa. During FSH treatment, information including the dose of FSH, local tolerance and adverse events was recorded. After treatment completion, a questionnaire was given; one was completed by the patient and one by the physician.

Results: Two hundred and one patients were included and 189 were analysed (143 in IVF/ICSI and 46 in OI/IUI). Characteristics of patients were as follows: age 32.5 ± 4.4 years; BMI 22.3; primary infertility in 61.4%; and duration of infertility 4.1 years. Self-administration of Gonal-F 600 IU/ml was performed in 72% of patients. Patients with experience of auto-administration found this new preparation more convenient to use (67%) and easier to inject (75%). Patients who never made any auto-injection (52.6%) considered the treatment easier to use (90.1%) and simple to administer (86%). Most physicians found this new preparation appropriate to their needs (96.3%) and better than the conventional one (86.2%). Patients reported no (85.9%) or mild (14.3%) local reaction. In IVF/ICSI, the total dose of rhFSH used was 2204 IU ± 870, corresponding to two or three vials of multidose in 91% of patients.The number of oocytes was 9.3 ± 4.7, the number of embryos was 5.5 ± 3.5, 2.2 embryos were transferred, and the on going pregnancy rate was 32.8% per cycle. In OI/IUI, the starting dose was 78.3 IU and the total dose of rhFSH was 903 IU, the number of mature follicles was 1.9, and 15.2% of patients were pregnant.

Conclusions: Gonal-F 600 IU/ml is perceived by most patients and physicians as a medication simple to use and well tolerated with a satisfying pregnancy rate.

10:15–10:30

O-109. A new highly purified urinary FSH (Fostimon IBSA). Meta-analysis results of four multinational clinical studies comparing urinary FSHs in IVF

de Mouzon J.[1], Anserini P.[2], Casper R.[3], Yuzpe A.[4], Grudzinskas J.G.[5], Zivn J.[6] and Forgács V.[7]

[1]INSERM, U569, Le Kremlin-Bicetre, France, [2]University of Genoa, Department of Obstetrics and Gynecology, Genoa, Italy, [3]Toronto General Hospital, Toronto, Canada, [4]Genesis Fertility Centre, Vancouver, Canada, [5]St Bartholomew's Hospital, The Fertility Centre, London, UK, [6]Prenosta gynekolicko-porodnická klinika, Prague, Czech Republic and [7]Jahn Ferenc Memorial Hospital, Department of Obstetrics and Gynecology, Budapest, Hungary

Introduction: Highly purified urinary FSH has been satisfactorily and widely used for controlled ovarian hyperstimulation. Over the last few years, recombinant products have appeared in order to improve, theoretically, safety and efficacy. However, their high cost is a limitation to their wide use and urinary products still have some future. Thus, a new urinary FSH (Fostimon; IBSA, Switzerland), obtained by a new and very potent purification method, has been developed and compared to existing HPuFSH.

Materials and methods: Four randomized controlled comparative studies were carried out in four countries (Canada, Hungary, Italy, UK). In total 350 patients, enrolled in an IVF programme, were randomized into two groups, receiving Fostimon (group I) or the comparative HPuFSH (group II), Fertinorm (Canada) or Metrodin-HP (Europe). The four studies were pooled and the two groups were compared for their characteristics and clinical outcomes. The meta-analysis was performed using a study factor. Statistical methods included chi-square, ANOVA, CO-ANOVA and logistic model.

Results: The two groups of patients were very similar concerning women's age, tubal status, endometriosis, ovulation, basal FSH, LH, estradiol and prolactin. The cancellation rate was equivalent (11.0% in group I, 10.2% in group II). There was no statistical difference in the total dose administered (1955 ± 843 versus 1902 ± 781 IU, $P = 0.38$), in estradiol peak, in the numbers of follicles and recovered oocytes (10.4 ± 4.4 versus 11.0 ± 5.3, $P = 0.58$), respectively in groups I and II. The pregnancy rate was not statistically different, per initiated cycle (20.8% versus 16.9%, $P = 0.36$), per oocyte recovery (27.4% versus 20.8%, $P = 0.24$) or per embryo transfer (28.7% in group I versus 22.5% in group II, $P = 0.29$), the number of transferred embryos being the same (2.64 ± 0.88 in both groups). When a logistic model was applied, taking into account the main prognosis factors and a study factor, the pregnancy odds ratio was higher, but not significantly, for group I (OR=1.42, 95% CI 0.80–2.50).

Conclusions: In this meta-analysis study the new uFSH (Fostimon) was associated with satisfactory results compared with the competitor HPuFSH (Fertinorm/Metrodin HP) and it can be used in IVF with similar chances of success than other gonadotropins.

10:30–10:45

O-110. Combining cycle day 7 follicle count with the basal antral follicle count improves predicting ovarian response

Elter K., Durmusoglu F., Erenus M. and Yoruk P.

Obstetrics and Gynecology, Marmara University, Istanbul, Turkey

Introduction: At present, various tests have been developed to assess ovarian reserve, and to predict the reponse to ovarian stimulation. Even so, the chance of success with IVF treatment is not predictable for some women. The result is a cancellation or a decreased success rate due to a poor ovarian response. In this retrospective study, we analysed the predictive value of cycle day 7 follicle count (CD7FC) for poor ovarian response with a long protocol.

Materials and methods: Ninety-one consecutive IVF cycles with a long protocol between January 2002 and December 2002 were analysed in this study. Women were divided into two groups according to the number of mature oocytes retrieved: group A (poor responders) $\leqslant 3$ and group B > 3. Basal serum FSH and estradiol (E_2) levels were determined in the previous spontaneous cycle. Basal antral follicles were counted in the IVF cycle. Groups were compared by using student's t and chi-squared tests, where appropriate. ROC analysis was performed to determine the optimum cut-off values. SPSS 10.0 was used for the statistical analysis, and a P value of <0.05 was considered significant.

Results: In the group of poor responders ($n = 24$), age (mean ± SD; 34.54 ± 4.84 versus 30.15 ± 5.52 years), duration of infertility (8.63 ± 5.62 versus 5.82 ± 4.83 years) and total number of rFSH ampules used from day 3 to day 6 (11.21 ± 3.30 versus 8.58 ± 5.12) were significantly higher. Basal antral follicle count (4.55 ± 3.00 versus 8.55 ± 3.22), CD7FC (4.58 ± 2.90 versus 10.64 ± 3.73), and CD7 serum E_2 levels (165.28 ± 145.28 versus 448.87 ± 430.66 pg/ml) were significantly lower than the normal responders ($n = 67$). BMI and basal serum FSH and E_2 levels in the previous spontaneous cycle were comparable between groups. Ovum retrieval cancellation and pregnancy rates (/cycle) were 33.3% and 0%, and 4.2% and 34.3% for the poor and normal responders, respectively ($P < 0.05$). On ROC analysis, an optimum cut-off value of 7.5, with a sensitivity of 83% and specificity of 79%, was observed for the CD7FC. The cut-off value for the antral follicle count was 6.5 with a sensitivity of 85% and specificity of 74%. When both antral follicle count and CD7FC values were lower than their optimum cut-off values, both sensitivity and specificity improved to 85% and 90%, respectively. The positive and negative predictive values for this combination were 74% and 94%.

Conclusions: Cycle day 7 follicle count during a long IVF protocol is helpful in predicting ovarian response in combination with the antral follicle count. This combination has high positive and negative predictive values. This may help clinicians and women to make earlier cancellations, and decrease the psychological, financial and medical burden of a later cancellation.

10:45–11:00

O-111. Expected poor responders in IVF do not benefit from a higher starting dose

Klinkert E.R., Broekmans F.J.M. and Te Velde E.R.

Department of Reproductive Medicine, University Medical Center, Utrecht, the Netherlands

Introduction: To optimize ovarian stimulation in the first IVF cycle it is important to predict the response. The antral follicle count (AFC) correlates well with the number of recovered oocytes at ovum retrieval. In this study we used the AFC to identify expected poor responders. The aim of the study was to evaluate the effect of doubling the starting dose of gonadotrophins in this group.

Materials and methods: Fifty-two patients between 31 and 46 years of age with a regular menstrual cycle participated in this randomized trial. Expected poor response was defined as an AFC of less than five follicles of 2–5 mm measured just before starting the gonadotrophins. There were no restrictions regarding the basal FSH level. All patients were treated with a long suppression protocol receiving 150 IU (group I, $n = 26$) or 300 IU (group II, $n = 26$) of recombinant FSH as a starting dose. In group I, the dose was doubled after 7 days of stimulation if the estradiol level was <200 pmol/l. The main outcome measures of the study were number of poor responders (<4 oocytes at retrieval or cancellation due to insufficient follicle growth), number of oocytes and clinical pregnancy rate (7 weeks of gestation). The treatment groups were compared using the Mann–Whitney U-test and the chi-squared test.

Results: The groups were comparable regarding patient characteristics. In 35% of the patients in group I, the dose had to be increased. The number of cancelled cycles, duration of stimulation, maximum estradiol level and number of embryos transferred did not differ between the two groups. The median number of oocytes collected was three for both groups. The total dose of rFSH differed significantly (2100 IU in group I versus 3600 IU in group II, $P < 0.01$). The clinical pregnancy rate was 12% in group I

and 4% in group II ($P = 0.30$). Sixty-five percent of the patients in group I experienced a low response and 62% in group II ($P = 0.77$)

Conclusions: Patients with an antral follicle count below 5 do not benefit from a higher starting dose of gonadotrophins. The outcome of an IVF treatment in this group of patients is poor. The antral follicle count performs satisfactory as a test to predict poor response.

11:00–11:15

O-112. Effects of low-dose aspirin on oocyte quality, fertilization rate, implantation and pregnancy rates in unselected patients undergoing IVF

Lentini G.M., Falcone P., Guidetti R. and Mencaglia L.

Florence Center of Ambulatory Surgery, Firenze, Italy

Introduction: Many studies have analysed the possible role of low-dose aspirin in IVF programmes. Some authors affirmed the positive role of low-dose aspirin in improving the pregnancy rate in patients with seropositivity for anti-phospholipid or autoimmune antibodies. Further studies have also found a positive role in increasing the ovarian responsiveness, the implantation and pregnancy rates in unselected patiens. On the other hand other studies have not found any benefical effect. The aim of this study was to evaluate the effect of low-dose aspirin treatment on oocyte quality, fertilization, implantation and pregnancy rate in a group of unselected patients undergoing IVF.

Materials and methods: We analysed 84 patients undergoing IVF from January to December 2002. The patients were randomly divided into treatment (A) and control groups (B). Group A (42 patients, mean age 34.6 ± 3.5 years) received a daily oral dose of 100 mg of aspirin starting a month before the first dose of gonadotropin. Group B (42 patients, mean age 34.2 ± 4.6 years) did not receive any treatment. The variables analysed for the evaluation of ovarian responsiveness and IVF outcome included number of oocytes retrieved, oocytes grade, fertilization rate, implantation and pregnancy rates.

Results: The mean number of oocytes retrieved/subject was 10.29 ± 5.45 in group A and 11.24 ± 5.49 in group B. The distribution of oocyte quality was G1 19 %, G2 53.4 %, G3 27.5 % in group A and G1 18.9 %, G2 54.5 %, G3 26.5 % in group B. The fertilization rate was 63.43 % in group A and 70.57 % in group B ($P > 0.05$). The implantation and pregnancy rate were 9.5 % and 30.9 % in group A and 6.5 % and 23.8 % in group B ($P > 0.05$).

Conclusions: Low-dose aspirin therapy does not improve ovarian responsiveness, oocyte quality, implantation and pregnancy rates in un unselected group of patients undergoing IVF.

11:15–11:30

O-113. Coasting period and embryo development with outcome

Aygun M.[1], Karlikaya G.[1], Vanlioglu F.[1], Karagozoglu H.[1], Melil S.[1], Kumtepe Y.[2] and Kahraman S.[1]

[1]Reproductive Endocrinology and Genetics, Istanbul Memorial Hospital, Istanbul and [2]Department of Obstetrics and Gynaecology, Ataturk University, Erzurum, Turkey

Introduction: Gonadotropin withholding (coasting) has been offered to patients as a promising method for the prevention of cycle cancellations, as well as complications associated with severe ovarian hyperstimulation syndrome (OHSS). However, the data on the duration of coasting period regarding oocyte morphology and embryo development is still limited and controversial. In the present study we aim to evaluate oocyte quality, fertilization, further embryo development and clinical outcomes after coasting with reference to its duration.

Materials and methods: In our centre 79 patients undergoing coasting in 83 assisted reproduction treatment cycles were recruited for this study: group A ($n = 49$), with coasting period ranging from 1 to 3 days and group B ($n = 34$), with coasting period ranging from 4 to 7 days. Oocyte morphology, pronuclear (PN) morphology scoring, fertilization rate and embryo quality were compared.

Results: Of 83 cycles, five cycles (two in group A) underwent total embryo freezing because of severe OHSS risk. The most dominant abnormal morphological feature of the oocytes was wide perivitelline space and perivitelline granulation, which was significantly higher in group B ($P < 0.05$). Fertilization rates (73.1% versus 76.3%), PN-scoring (A–D) rates (81.7% versus 86.5%) and rate of the grade I embryos on day 3 (74.7% versus 77.2%) between the two groups were not significantly different. There was a higher cleavage rate (e6 blastomers) on day 3 in group A (76% versus 33.3%, $P < 0.05$). A total of 173 embryos in group A and 117 embryos in group B were transferred. In group A, 26 pregnancies (55.3%) and in group B, nine pregnancies (29%) per embryo transfer were achieved ($P < 0.05$). Implantation rates in group A were higher but were not significant.

Conclusions: Coasting is used as a preventive method against OHSS and cycle cancellation. But the effect of the coasting period on embryo development and pregnancy rates in assisted reproduction treatment cycles has not been clearly defined. Fertilization rates seem similar in coasting periods regardless of duration. In our study we observed that a prolonged coasting period (>3 days) has a significantly negative effect on the cleavage rate on day 3. Prolonged coasting may also negatively contribute to total pregnancy rates.

Female fertility/Clinical

Tuesday 1 July 2003
Paris

10:00–10:15

O-114. Reduced ovarian function in survivors of childhood cancer

Larsen E.C.[1], Müller J.[2], Schmiegelow K.[3], Rechnitzer C.[3] and Nyboe Andersen A.[1]

[1]The Fertility Clinic, Rigshospitalet, Section 4071; [2]Department of Growth and Reproduction, Rigshospitalet, Section 5064; [3]Department of Pediatrics, Rigshospitalet, Section 5002, Copenhagen University Hospital, Denmark

Introduction: Seventy percent of children with cancer survive. Anti-cancer treatment may, however, impair ovarian function. Alkylating agent chemotherapy and radiotherapy below the diaphragm, in particular, have shown to have an excess risk of inducing premature ovarian failure. The aim of this population-based study was to achieve a comprehensive knowledge of the degree of ovarian damage.

Materials and methods: Ovarian function was evaluated in 100 childhood cancer survivors and 21 controls of similar age. Menstrual cycle pattern was recorded and strictly timed ovarian sonography and hormonal assessment was performed.

Results: The median age of the survivors was 5.4 years (range 0.1–15.3) at the time of diagnosis and 25.7 years (18.5–44.4) at study entry. Seventeen survivors with premature ovarian failure had follicle depleted or non-detectable ovaries, elevated gonadotrophins, and immeasurable inhibin B. Thirteen survivors used oral contraception. Survivors with spontaneous menstrual cycles ($n = 70$) had smaller ovarian volume per ovary than controls (median 4.8 versus 6.8 cm^3; $P < 0.001$), and a lower number of antral follicles per ovary (median 7.5 versus 11, $P < 0.001$). Furthermore, they had lower inhibin B levels than controls (median 94 versus 111 pg/ml, $P = 0.03$), and higher estradiol levels (median 0.12 pmol/l versus 0.08 pmol/l, $P = 0.04$). Even survivors, who had not received treatment previously shown to impair ovarian function, had smaller ovarian volume per ovary than controls (median 5.6 versus 6.8 cm^3; $P = 0.03$), and a lower number of antral follicles per ovary (median 9 versus 11, $P = 0.046$). Multiple linear regression analysis was performed to predict the total antral follicle number per ovary, and showed a reduced number with ovarian irradiation (beta = -0.40, $P < 0.001$), alkylating