

The Snick model and the Hunault model were available both with and without a post-coital test (PCT).

**Materials and methods:** From January 2002 until February 2004, we included consecutive subfertile couples who had not been evaluated previously for subfertility. The couples underwent a basic fertility work-up, consisting of a fertility history, semen analysis, PCT, ovulation detection and assessment of the Fallopian tubes. Couples with anovulation, tubal pathology or severe male factor were excluded. Couples were counselled for either expectant management or treatment (IUI or IVF), depending on a predicted probability of spontaneous ongoing pregnancy within 1 year, calculated from female age, duration of subfertility, previous pregnancy, semen analysis and the PCT.

All couples were followed until the occurrence of ongoing treatment-independent pregnancy, which was the primary endpoint of the study or the start of fertility treatment. Performance of prediction models was assessed using calibration and discriminative capacity. Calibration was assessed by comparing the predicted probability with the observed pregnancy rate at 12 months. Discriminative capacity of the models was assessed with receiver operation characteristic (ROC) analysis and area under the ROC-curves (AUC).

**Results:** As of 1 January 2005, we included 2250 couples, of whom 2005 couples (89%) have complete follow-up. Of the couples, 349 had an ongoing pregnancy within one year. Kaplan-Meier analysis showed an overall ongoing pregnancy rate of 27.5% (SE  $\pm$ 2.8) at the end of 12 months. The mean predicted probabilities and 5th to 95th percentiles at the end of 12 months were: for the model of Eimers 30.0% (SE  $\pm$ 0.8%; 9–53%), for Snick with PCT 25.7% (SE  $\pm$ 0.5%; 8–38%), for Collins 13.1% (SE  $\pm$ 0.3%; 5–28%) and for Hunault with PCT 31.5% (SE  $\pm$ 0.6%; 11–55%). Calibration was good for all models in the range from 0–30%, whereas pregnancy probabilities were overestimated for predictions >30%. The ROC analyses resulted in areas under the curve between 0.58 and 0.63. Both the Snick model and the Hunault model did not improve when the PCT was included. Overall, the synthesis model of Hunault without the PCT performed the best.

**Conclusions:** Our external validation shows that, although calibration and accuracy are not perfect, prediction models can be used to distinguish couples with good fertility prospects from couples with poor fertility prospects.

### O-109 The value of basal serum LH measurement in predicting IVF/ICSI outcome

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**Introduction:** During controlled ovarian hyper-stimulation, age and basal (day 2–3) serum follicle stimulating hormone (FSH) and estradiol (E2) concentrations are considered the main predictors of ovarian response (Muasher et al., 1988; Farhi et al., 1997). Measuring basal serum luteinizing hormone (LH) and FSH were, however, recommended by the National Institute of Clinical Excellence (NICE) in the most recent report on fertility management. The consensus group on PCOS has not included basal serum LH in the diagnosis of the syndrome. In this study, using a large data set, we have tested the hypothesis that basal serum LH levels correlate to ovarian response and overall outcome during IVF/ICSI.

**Materials and methods:** This retrospective study analysed data from 1272 cycles of IVF+/-ICSI performed at our centre between January 2001 and December 2003. All patients had early follicular (day 2–3) measurement of serum FSH, LH, E2 and trans-vaginal ultrasound (TVS) within 6 months of starting treatment. Patients were treated with GnRH agonist (94.2%) or antagonist (5.8%), using a long (91.9%) or short (8.1%) protocol, with either follicular or luteal phase downregulation. The stimulation regimen utilized recombinant FSH or human menopausal gonadotrophin. Basal serum LH and outcome measures of the IVF/ICSI treatment were statistically analysed using SPSS version 11 for Windows.

**Results:** Significant linear regressions of limited predictive value were noted between basal serum LH and number of days of stimulation required ( $R^2=0.018$ ,  $p<0.001$ ), number of oocytes retrieved ( $R^2=0.06$ ,  $p<0.001$ ), number of embryos ( $R^2=0.06$ ,  $p<0.03$ ). The low  $R^2$  values indicate that there is a poor correlation between LH and the above variables, the significant p values indicate that this poor correlation is very unlikely to be due to chance alone.

There was no difference (by ANOVA) between the mean basal serum LH concentration in subjects who achieved pregnancy and those who did not ( $p=0.66$ ) or between subjects who had a live birth and those who did not ( $p=0.56$ ). These results indicate that there is no significant relationship between basal serum LH and pregnancy outcome in IVF/ICSI.

**Conclusions:** Basal serum LH levels have no predictive value for IVF/ICSI outcome and hence add little to patient management. We suggest that LH measurement can be dropped from routine testing prior to IVF/ICSI cycle. The measurement of FSH, E2 and TVS are solely required for the identification of low and high responders.

## FREE COMMUNICATION

### Session 30 – Preimplantation genetic diagnosis

Tuesday 21 June 2005

10:00–11:30

#### O-110 Comparison of one- and two-cell biopsy for PGD

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**Introduction:** To date, no controlled data are available comparing the possible damage inflicted to cleavage-stage embryos after the biopsy of one or two blastomeres.

The aim of this study is to evaluate the diagnostic accuracy and the embryonic implantation potential after the removal of one or two cells.

**Materials and methods:** The patients included in this study underwent pre-implantation genetic diagnosis (PGD) for monogenic diseases (if duplex-PCR was available) and X-linked disorders using FISH. Patients undergoing pre-implantation genetic screening (PGS) were included in a later phase. If PGS was performed, chromosomes X, Y, 13, 16, 18, 21 and 22 were tested.

A pilot study was carried out in which a randomization for one- or two-cell biopsy was performed per cycle going to biopsy. Different end-points were measured: efficiency and accuracy of the diagnosis, in vitro embryo development, implantation rates and the course of ongoing pregnancies.

**Results:** The results of 186 cycles are presented.

In the PCR group ( $n=91$ ), one cell was biopsied in 46 cycles and two cells were removed in 45 cycles. In the FISH cycles ( $n=95$ ), one cell was removed in 47 cycles and two cells were biopsied in 48 cycles. Of the 288 embryos, 46 (16.0%) remained without diagnosis for the PCR cycles in the one-cell biopsy group compared with 11/274 (4.0%) in the two-cell biopsy group. This is statistically different ( $p<0.01$ ). Of the 562 embryos (two-cell group), two were misdiagnosed, both with an acceptable misdiagnosis. For the FISH cycles, 2/209 and 3/228 embryos remained without diagnosis (0.96% and 1.32%) in the one- and two-cell groups, respectively. This is not statistically different ( $p>0.05$ ). In the PCR as well as in the FISH group, there was no statistical difference for the further in vitro development between the one- and the two-cell biopsy groups: 46% of the embryos developed to blastocyst in the one-cell biopsy group, whereas 49% of the embryos developed to blastocyst in the two-cell group. In the PCR group, 39 of the 46 (85%) of the one-cell biopsy cycles ended in a transfer, whereas 35 of the 45 (78%) cycles in the two-cell group ended in a transfer. For the FISH cycles, these numbers are 32/47 (68%) and 33/48 (69%), respectively. In neither of the two groups there is a statistical difference in transfer rate between the one- and two-cell biopsy.

For the implantation and pregnancy rates, numbers are still too small to draw conclusions. In the second part of the study, one cell will be removed in all PCR cycles, where a multiplex is available, whereas two cells will be removed when only a simplex PCR is available.

**Conclusion:** More embryos remain without diagnosis when only one cell is removed in a PCR-based diagnosis. PCR-technology is more susceptible to technical problems than FISH. However, this did not lead to a lower transfer rate. The *in vitro* developmental potential of embryos after one-cell and

two-cell biopsy also did not show a statistical difference. Whether this will be reflected in the implantation and pregnancy rates remains to be shown as soon as the study is completed. Only then will it be possible to make an informed choice between one- and two-cell biopsy.

### O-111 The absence of embryo compaction on day 4 of PGD conception cycles is associated with an increased risk of miscarriage

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**Introduction:** A greater number of pregnancies end in miscarriage following PGD than in IVF/ICSI cycles. The purpose of this study was to evaluate the factors in a PGD conception cycle that may contribute to the cycle resulting in an ongoing pregnancy.

**Materials and methods:** Of the 310 cycles that commenced between July 1997 and December 2004, 85 cycles resulted in a positive pregnancy test result. Of these, 29 cycles were for autosomal conditions, 20 for X-linked chromosome conditions and 36 for chromosomal rearrangements. The 85 cycles were divided into two groups: those that resulted in an ongoing pregnancy and those in which the pregnancy failed. An ongoing pregnancy was defined as one reaching beyond eight weeks gestation.

**Results:** Of the 85 cycles, 61 (72%) resulted in an ongoing pregnancy and 24 pregnancies failed. There was no difference in the chance of an ongoing pregnancy between the types of condition being tested for (autosomal 76%, rearrangement 72% and X-linked 65%,  $p=0.71$ ). The two groups were comparable with regard to age at the time of treatment ( $33.1\pm 4.2$  versus  $32.7\pm 3.8$  years,  $p=0.67$ ), history of previous pregnancy (75.9% versus 71.8%,  $p=0.22$ ), pre-treatment basal (day 2–4) FSH level ( $5.7\pm 1.9$  versus  $5.9\pm 1.8$  IU/l,  $p=0.59$ ), daily dose of 75 IU r-FSH ampoules ( $3.75\pm 1.26$  versus  $3.57\pm 1.12$  ampoules,  $p=0.53$ ) and duration of ovarian stimulation ( $10.9\pm 1.43$  versus  $10.5\pm 1.65$  days,  $p=0.21$ ). Nor were there any significant differences observed between the groups with regard to response to ovarian stimulation (mean number of follicles of 14 mm or more in diameter on the day of hCG administration) ( $11.3\pm 6.2$  versus  $12.4\pm 5.5$ ,  $p=0.45$ ), endometrial thickness ( $11.7\pm 2.20$  versus  $10.8\pm 2.6$  mm,  $p=0.18$ ) or mean number of retrieved oocytes ( $13.7\pm 6.7$  versus  $14.7\pm 6.6$ ,  $p=0.70$ ). An ongoing pregnancy was more likely if the mean number of embryos replaced was greater ( $1.97\pm 0.48$  versus  $1.63\pm 0.50$ ,  $p=0.04$ ) bearing in mind that generally no more than two embryos were replaced. Couples who had one or more compacting/cavitating embryos replaced post biopsy were more than twice as likely to have an ongoing pregnancy compared with those that had only cleavage stage embryos to replace (79.5% versus 33.4%,  $p=0.001$ ).

**Conclusion:** A pregnancy resulting from a PGD cycle is more likely to continue as an ongoing pregnancy if at least one of the biopsied embryos replaced on day 4 is compacting.

### O-112 PGD: relief of pain or antagonistic to the values of society?

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**Introduction:** Preimplantation genetic diagnosis (PGD) is a modern technique in reproductive medicine which can be used to diagnose genetic disorders of an embryo. PGD is currently not legal in Germany, still there are debates being held regarding the issue of legalization. Both the internationally 'accepted' indications and the new or alternative approaches (such as HLA typing) can lead to complex ethical dilemmas. Several international studies show that, in countries where PGD is allowed and practiced, the general opinion is ambivalent and can not be divided into two opposite positions: there is a general perception of positive and negative implications of the PGD subject. International research in similar fields, such as prenatal diagnosis and genetic testing, has pointed at the importance of religiousness and personal values in attitude-formation. The analysis of public perceptions of science has also gained in importance as input to the design of public policies. Especially developments that could collide with societal values, such as PGD, should be opened up to

some kind of public participation (Pardo, 2003; Enquete Kommission, 2002). Therefore, an analysis of the perceptions of the general population should be taken into account for the regulation of PGD in Germany.

**Materials and methods:** In our study, 2110 persons aged 18–50 years were interviewed about their attitudes towards PGD and related topics in a written survey in December 2003. Data was analysed with descriptive and inferential statistical procedures (ANOVAs and binomial logistic regressions) to prove our hypothesis that religion, world views and the evaluation of PGD would influence the attitude a person has towards this technique.

**Results:** In our survey, the majority of respondents (76%) would agree to a restricted legalization of PGD in Germany. No differences regarding sex, age or education of respondents were observed, while the religious affiliation did have an effect: persons affiliated to a religious group agreed more to the prohibition of PGD in Germany. The attitudes, however, were found to be rather ambiguous and complex: the perception of both risks and benefits could coexist and ambivalence was present in a large number of respondents. The most important argument pro-PGD was, the relief of pain and suffering for the affected individuals. An important argument contra-PGD was the concern regarding societal values. Our results confirmed some of our assumptions regarding the factors that influence attitude-formation, but religiousness and religious affiliation did not have the expected influence, although the majority of respondents reported to be somewhat religious. Findings regarding the influence of certain personal values on the attitudes were similar, in contrast, the evaluation of costs and benefits of PGD explained most of the final attitude whether PGD should be legalized in Germany or not.

**Conclusions:** Our results indicate that the guidelines provided by the most influential churches are not sufficient for decision-making in the context of ethically problematic medical developments. Nonetheless, the self-consciousness of a religious person and underlying convictions do have an influence on the way costs and benefits of a technique, such as PGD, are evaluated by an individual. Still, the agreement to a restricted legalization may also be connected to an overall rejection of prohibitions made by the State, concerning the individual reproductive autonomy, and a tendency to trust in the self-regulation of the medical practice in issues of human reproduction.

### O-113 Assessment of embryonic competence for the two-cell biopsy technique in preimplantation genetic diagnosis for beta thalassaemia in combination with HLA matching

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**Introduction:** The aim of the study was to evaluate the effect of harvesting two blastomeres from day 3 embryos on embryo viability and developmental potential in couples undergoing PGD for  $\beta$ -thalassaemia combined with HLA matching to obtain an HLA matched donor for allogeneic haematopoietic stem cell transplantation to an affected sibling with thalassaemia.

**Materials and methods:** A total of 34 PGD cycles were performed in 19 couples for  $\beta$ -thalassaemia combined with HLA matching. On day 3 after ICSI, two blastomeres were harvested by laser biopsy from embryos with  $\geq 6$  blastomeres and sent for analysis. Total number of embryos biopsied was 264, with a mean number of 7.8 embryos/cycle. Single embryo culturing was utilized with sequential culture media. On day 5, embryo morphology assessment was carried out and only HLA matched unaffected embryos (normal or heterozygous for  $\beta$ -thalassaemia mutations) were transferred. Progression to the blastocyst stage on day 5 and implantation rates were taken as markers of embryonic competence.

**Results:** The mean age of women was 32 and the 2PN fertilization rate with ICSI was 82%. Blastomere biopsy was performed only in embryos with  $\geq 6$  blastomeres. Twenty seven embryos were found to be HLA matched and unaffected (either normal or heterozygous for  $\beta$ -globin gene mutation). Though only 22 of these embryos developed appropriately to be eligible for transfer in 16 cycles (1.4 embryo/transfer). One ectopic, 1 biochemical and 3 clinical pregnancies (1 twin, 2 singleton) were achieved with the transfer of these embryos yielding an overall implantation rate of 18.2% (4/22). In one of the women who became pregnant, the transferred embryo originated from the biopsy of a six-cell embryo, while the other three implantations were of seven- and eight-cell embryo origin. The blastocyst formation rate on day 5 was 43.4%

(46/106) for the eight-cell embryos biopsied, 24.5% (24/98) for the seven-cell embryos biopsied and 5% (3/60) for the six-cell embryos biopsied.

**Conclusions:** The incidence of no or misdiagnosis by allelic dropout, contamination or amplification failure can be lowered by taking two blastomeres from the day 3 embryos in PGD cycles. Two-cell biopsy technique yields acceptable overall blastocyst formation and implantation rates in a PGD programme for  $\beta$ -thalassaemia combined with HLA matching. Although the blastocyst formation rate following the two-cell harvest from six-cell embryos is very low, there is still the chance of such an embryo to result in viable pregnancy.

#### **O-114 Experience on preimplantation genetic diagnosis combined with HLA matching**

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**Introduction:** Recently, preimplantation genetic diagnosis (PGD) has been considered for several indications beyond its original purpose, not only to test embryos for a genetic disease, but also to select embryos for a non-disease trait, such as specific human leukocyte antigen (HLA) haplotypes, related to immune compatibility with an existing affected child in need of an hematopoietic stem cell transplant.

We report our experience on preimplantation HLA matching, describing strategies and overall outcome data of 60 cycles (54 for  $\beta$ -thalassaemia, 1 for Wiscott-Aldrich syndrome, 2 for Diamond-Blackfan anemia and 3 for acute lymphoid leukaemia) from 45 couples.

**Materials and methods:** An indirect single-cell HLA typing protocol based on a multiplex fluorescent polymerase chain reaction (PCR) of short tandem repeat (STR) markers scattered throughout the HLA complex was used. By selecting a consistent number of STR markers an accurate mapping of the whole region can be achieved. The HLA region can be indirectly typed by segregation analysis of the STR alleles and the HLA identity of the embryos with the affected sibling can be ascertained evaluating the inheritance of the matching haplotypes. A nested multiplex PCR assay was used to co-amplify all the selected loci. The first round PCR contained the external primers for the amplification of the informative HLA STR markers selected during the pre-clinical work-up of each PGD case, the gene regions involved by mutations, STR markers linked to these regions for ADO detection and STR markers used for detection of aneuploidies in patients of advanced reproductive age. The first round multiplex PCR was followed by separate second round PCR reactions for each locus. Mutation analysis was performed using the minisequencing method.

**Results:** A total of 486 embryos were tested for HLA typing in combination with a genetic disease and 44 embryos for HLA typing only. A total of 922 blastomeres was analysed, in 848 (92.0%) a successful amplification was obtained. A reliable HLA haplotype was obtained in 848/848 (100%) of the blastomeres with positive PCR results. Testing for chromosome 6 copy number revealed 47 (8.9%) embryos with aneuploidies, including a total of 4 (0.8%) trisomies, 43 (8.1%) monosomies, which affected the HLA matching diagnosis for these embryos, leading to a conclusive diagnosis in only 483/530 embryos (91.1%). Recombination was found in 23 (4.8%) embryos, 5 (1.0%) of which were still unaffected and HLA compatible, but were not considered for transfer. In total, 74 (15.3%) embryos revealed an HLA match with the affected siblings, 55 (11.4%) of which resulted unaffected and 46 (9.5%) have been transferred back to patients in 30 of the 60 cycles performed (1.5 on the average). Nine pregnancies were achieved (30.0% per transfer); only two pregnancies resulted biochemically, one spontaneously miscarried, and one resulted ectopically and was then terminated. Five healthy HLA matched children have been already delivered and cord blood stem cells were transplanted to three affected siblings, resulting in a successful hematopoietic reconstruction.

**Conclusion:** These results represent one of the most extensive experience in the field and, complemented by other similar experiences, demonstrate that preimplantation HLA matching is a reliable alternative for the achievement of a successful treatment in children affected by severe congenital or acquired bone marrow disorder, in the absence of a compatible related donor.

#### **O-115 Sperm fluorescence in situ hybridization analysis and preimplantation genetic diagnosis results of 13 male translocation carriers undergoing 17 cycles**

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**Introduction:** Sperm FISH analysis is important in estimating abnormal gamete cell and thus abnormal embryo rates for male translocation carriers undergoing ART. This study makes a comparison between results in sperm FISH analysis and PGD outcomes.

**Materials and methods:** During 2000–2004, sperm FISH analysis was performed for eight reciprocal translocation carriers [46,XY,rcpt(7;13)(q22;q12); 46,XY,rcpt(9;18)(p11;q11) 46,XY,rcpt(2;5); 46,XY,rcpt(9;18)(p13.3;q21.3); 46,XY,rcpt(2;9)(q33;q34); 46,XY,rcpt(1;7)(q32;q34); 46,XY,rcpt(15;17)(q24;q21.3) and 46,XY,rcpt(1;16)(q11;q11) for which both male and female partner have the same translocation] and five robertsonian translocation carriers [three 45,XY,robt(13;14), one 45,XY,robt(14;15)(p11;p11) and one complex translocation carrier 45,XY,robt(13;14)(q10;q10),rcpt(11;22)(q23.3;q12)]. Semen samples were fixed in methanol/acetic acid (2:1). For each reciprocal translocation case at least three specific probes and for each robertsonian translocation case, at least two specific probes were used to analyse abnormal segregation patterns in more than 1000 sperm cells. Eleven of 13 translocation carrier males underwent a total of 17 PGD cycles. One cell per embryo at the 6–8-cell stage was biopsied. Blastomeres were fixed and FISH analysis was performed with specific probes.

**Results:** The percentage of average unbalanced sperm in carriers of reciprocal translocations was 77.1 and 22.55 for robertsonian translocation carriers. Abnormal embryo rates were 75% and 44.5% in reciprocal and robertsonian translocations carriers, respectively. Four couples achieved clinical pregnancy (36%) in 11 ET cycles.

**Conclusion:** FISH analysis in sperm cells of translocation carriers gives a good estimation of abnormality rates in the embryos. There was a strong correlation between sperm FISH results and abnormal embryo rates in PGD cycles of reciprocal carriers, while the rate of chromosomal abnormality in embryos was found to be higher than sperm samples of robertsonian translocation carriers. Sperm FISH can give good approximations of success in a PGD cycle but besides abnormal sperm rates, type of rearrangement; especially positions of breakpoints on the chromosomes, maternal age and number of embryos available for biopsy are other important factors predicting the outcome of ART for infertile males carrying structural rearrangements.

#### FREE COMMUNICATION

### Session 31 – Clinical endocrinology

Tuesday 21 June 2005

10:00–11:30

#### **O-116 Simvastatin decreases levels of endothelial adhesion molecules VCAM-1 and ICAM-1 in women with PCOS: results of prospective randomized trial**

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**Introduction:** Polycystic ovary syndrome (PCOS) is associated with an increase of cardiovascular risk factors including systemic inflammation and endothelial dysfunction. Statins inhibit HMG-Co-A reductase, a rate-limiting step of the mevalonate pathway. Statin use improves lipid profile and alleviates systemic inflammation. Recently, we have demonstrated that use of simvastatin in women with PCOS results in a decrease of serum testosterone, LH, LH:FSH ratio, as well as improvement of lipid profile. In this report, we analyzed the effect of simvastatin on markers of endothelial function