Results: In Group 1, a statistically significant increase in the incidence of difficult ET, was evidenced by the frequency of tenaculum use (19.8% vs. 5.9%), the requirement of hysterometer to negotiate the cervix (5.0% vs. 1.2%) and the presence of blood at the post-transfer inspection of the ET catheter (9.9% vs. 3.8%). Conversely, the implantation rate was significantly higher (16.4% vs. 13.0%) in the Ultrasound catheter group.

Conclusion: The use of a softer catheter improved the ET procedure regarding its ease and efficacy.

O-214 Removal of cervical mucus prior to embryo transfer does not improve pregnancy rates in IVF/ICSI

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Introduction: There are experimental indications that a film of cervical mucus may cover the embryo transfer catheter during passage of the cervical canal. Hypothetically, this ‘condom’ of cervical mucus might interfere with the correct placement of the embryo(s) into the uterine cavity. (1) Consequently, it seems conceivable that meticulous removal of cervical mucus prior to embryo transfer could result in higher pregnancy rates. The current study was undertaken to evaluate the effect on pregnancy rates of removal of cervical mucus prior to embryo transfer in IVF and ICSI treatments.

Materials and methods: The study was set up as a double-blind randomized controlled trial. Couples undergoing IVF or ICSI treatment were invited to participate. The experimental group underwent meticulous removal of cervical mucus prior to embryo transfer by means of a cervical brush. The control group underwent a mock procedure during which the ectocervix was touched but no endocervical removal of mucus occurred. Subjects were randomized by computer during which they were stratified for age, cycle number and method of treatment (IVF or ICSI). Patients were blinded with regard to the procedure used. Doctors were blinded with regard to the outcome of treatment. The primary outcome parameter was clinical pregnancy.

Results: Four hundred and twenty-four patients were included after written consent, two were lost to follow-up. No significant differences were found between the two groups with regard to age, cycle number and method of treatment (IVF or ICSI). Also, the two groups did not differ statistically with respect to parity, gravidity, duration of infertility, cause of infertility, use of fresh or frozen embryos, number of oocytes retrieved and number and quality of embryos transferred. Clinical pregnancy occurred in 64 out of 217 (29%) in the treatment group and in 64 out of 205 (31%) in the control group (OR 1.0 (0.9–1.2)).

Conclusion: The results of this randomized controlled trial suggest that removal of cervical mucus prior to embryo transfer does not improve the pregnancy rates.

FREE COMMUNICATION

Session 56 – Genetics

Wednesday 21 June 2006 10:00–11:45

O-215 IVF with PGD for sex selection: characteristics of couples, outcomes and evidence-based ethics

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Introduction: IVF and PGD for determination of blastomere sex chromosome number is the most effective method known for choosing the sex of a future baby. With such certainty comes ethical controversy. In Australia it has been argued (1) that in a liberal society there is no prevailing reason for prohibition, especially if there is no compromise to recipients of IVF/PGD for medical indications and no community subsidy. With enablement of such a social novelty, however, there is a duty to determine the characteristics of, and outcomes for, couples electing sex selection, to assist in ascertaining resulting benefits or harm (2).

Materials and methods: Summary data are reviewed for 239 couples. A specific questionnaire was introduced in April 2002 to more specifically query motivation; this was completed by couples prior to commencing on a cycle and was brought into the process for obtaining informed consent. Focussed implications counselling was compulsory in addition to conventional preparatory counselling. In December 2005, an additional questionnaire was provided to all couples who had commenced a cycle and who agreed to participate in a follow-up study. All steps were approved by a properly constituted ethics committee, the members of which are from outside the institution.

Results: Of the total cohort of 239 couples, the preferred sex was 59% for females (x²=4.9, compared with population sex ratio). Overall the desire for a sex-selected child was rated as 3% more father-led, more mother-led in 27% (majority for a girl) and 68% equal by both partners. The most common reason cited for sex selection was a desire to balance their family (>65%) followed by the desire for a parent child relationship by the mother (26%) and family pressure felt by the father (12%). For couples whose treatment was successful, the predominant feeling by the mother was that they felt complete (95%) and by the father was that treatment success had confirmed the decisions that the couple had made (67%). For couples whose treatment was not successful, 47% of mothers stated that it was ‘difficult’ to deal with treatment failure, while 16% stated it was ‘extremely difficult’ (a ‘life crisis’). Following IVF–PGD treatment, 21% discarded the embryos of the non-desired sex, 40% donated them to research, 6% transferred them and 20% of couples there were no such embryos. Only 6% of couples would not recommend IVF–PGD for sex selection to others.

Conclusions: Experience with clinically effective sex selection reveals a mild but significant excess of couples attempting a daughter. Women were more likely than men to regard themselves as the driving partner seeking sex selection, usually for a daughter after a series of sons. Couples varied in their opinion on the most difficult part of the IVF–PGD process, but 94% of those who completed a treatment would recommend sex selection by PGD to other couples in a similar situation. These data will assist other couples to appreciate the hazards and to make an informed choice. In circumstances where there is no call for societal subsidy and no disadvantage for couples with disabilities competing for services, we conclude that there is a sound evidence base for enabling access to effective sex selection for family balancing as part of a couple’s right to reproduce responsibly.

References

O-216 Genome profiling of blocked embryos using whole genome amplification and genomic microarray and potential application for PGDAS

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Introduction: Recently, genomic and/or expression microarray technology has become a common tool for genome and gene investigation in research and development and clinical diagnostics. For genome profiling and high resolution molecular karyotyping, array comparative genome hybridization (array CGH) methods appear to be far better than classical CGH as they do not suffer from dependence on having metaphase preparations and have much higher sensitivity and specificity for subtle genomic changes. This primary study was
focused on microarray application after whole genome amplification to assess the possible unbalances of the 24 chromosomes in blocked embryos regenerated by ART.

Materials and methods: From 22 patients undergoing ICSI programme, 37 blocked embryos on day 4 were treated for zona pellucida digestion and submitted to whole genome amplification (WGA). Parallely, aliquots of few somatic cells were also submitted to WGA to be used as reference DNA. Embryos and somatic cells were collected in 0.5 ml PCR tubes containing 5 μl of lysing buffer and incubated at 45°C for 15 min, followed by proteinase K inactivation at 96°C for 20 min. Lysates were used directly for whole genome amplification using Phi 29 kit (Genentech, USA) by adding 45 μl of the master mix in a total volume of 50 μl. The mix was then incubated at 30°C for 6 h followed by heat inactivation at 65°C for 3 min. WGA products were labelled by random priming (test-Cy3 and control-Cy5, and the converse, as well as Cot-1 blocking DNA) are mixed and precipitated together. The labelled probes DNA solutions were hybridized to a constitutional genomic microarrays for 16 h. A post-hybridization wash of the arrays was performed and then the arrays were scanned on a two-colour fluorescent scanner and the images were analysed using a commercial software.

Results and discussion: The data showed that the use of WGA to amplify a minimum of 8–10 blastomeres is producing an acceptable quantitative and qualitative DNA without significant preferential amplification. The microarrays gave a useful data for 31 embryos (31/37). For the other six embryos we could not conclude because of poor hybridization quality and noisy profile, possibly by WGA efficiency. Among 31 embryos we detected 14 embryos (nearly 45%) with chromosomes abnormalities. Compared to aneuploidy testing by FISH, more than simple and complex aneuploies, the microarrays showed unbalanced and cryptic disorders also, but this observation should be confirmed by quantitative PCR using WGA products. In practice, array CGH use is not without limitations, these include the inability to detect polyplody or balanced chromosome abnormalities. For most other clinical cytogenetics studies, array CGH is likely to become the method of choice because of the ability to apply it to non-dividing cells or even few cells.

Conclusions: If we resolve the problem of WGA from single or double blastomere caused by preferential amplification and allele drop out. Genomic microarray CGH can be a new alternative to the aneuploidy testing by FISH and multiple genes analysis in preimplantation genetic diagnosis.

O-217 Results of polar body analysis in 215 unfertilized oocytes

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Introduction: Aneuploidies of human oocytes are presumed to be a major cause of fertilization failure in assisted reproductive treatment (ART) cycles. Aneuploid oocytes result from meiotic segregation errors during oocyte maturation. As underlying mechanisms non-disjunction of bivalent chromosomes as well as unbalanced premature division of chromatids are discussed. Polar body analysis (PBD) by polar body biopsy and subsequent fluorescence in situ hybridization (FISH) analysis detects maternally derived aneuploies in both fertilized and unfertilized human oocytes in ART. The diagnosis of aneuploidies in oocytes failing to fertilize in ART cycles would allow direct estimation of aneuploidy rates in female gametes showing fertilization failure. Therefore, the purpose of this study was to evaluate the frequency of aneuploidies in unfertilized oocytes and to identify the underlying mechanism of formation by means of PBD.

Materials and methods: The study concerned 95 women (average age of 36.6±4.8 years with a range of 20.9–44.7 years) who underwent ART at the Fertility Center at Kaiser Wilhelm Memorial Church in Berlin from June to December, 2005. Indications for aneuploidy screening by PBD were previous unsuccessful ART cycles and/or advanced maternal age. First, polar bodies from a total of 215 oocytes remaining unfertilized after ART were biopsied. Each polar body was hybridized with a MultiVysion™ PB multicolor probe panel (Abbott) specific for chromosomes 13, 16, 18, 21 and 22. After overnight hybridization slides were washed and observed with a Nicon Eclipse 80i fluorescent microscope. Polar bodies were analysed and judged according to the number of signals for each given chromosome.

Results: PBD provided analysable patterns of FISH signals in 194 out of 215 (90.2%) analysed polar bodies. Forty-two (21.7%) polar bodies showed normal pattern of signals and 42 (21.7%) polar bodies showed signals indicating a balanced predivision of chromatids. Abnormal patterns of FISH signals were detected in 110 (56.6%) polar bodies; among them, gain or loss of single signals (chromatids) were detected in 63 (57.3%) polar bodies and additional or missing double signals (chromosomes) were detected in 14 (12.7%) polar bodies. Thirty-three (30.0%) polar bodies showed aberrant signal patterns for more than one chromosome indicating complex aneuploidies.

Conclusions: Oocytes showing fertilization failure were diagnosed to be aneuploid in a high frequency of 56.6%. This supports the hypothesis that aneuploidy may be a cause for high rates of fertilization failure during ART. Moreover, using the technique of polar body biopsy followed by FISH analysis the underlying mechanism of aneuploidy formation can be diagnosed. Our results suggest that unbalanced premature division of chromatids is the main mechanism of aneuploidy formation in female gametes failing to fertilize. The information of aneuploidy rate of unfertilized oocytes may be an important prognostic factor for patients having no or only poor fertilization of their oocytes and who consider continuation of ART.

O-218 Paternal contribution to aneuploidy in preimplantation embryos

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Introduction: The increased incidence of de novo chromosomal abnormalities in the children born after ICSI and its widespread use for the treatment of male factor infertility gave impulse to investigate on the safety of the procedure. The aim of this study was to evaluate the paternal contribution to aneuploidy in selected groups of patients. The effect of sperm indices on the chromosomal constitution of preimplantation embryos was assessed in couples with a female partner younger than 36 years.

Materials and methods: A group of 210 patients underwent 270 cycles of PGD for aneuploidy. The results were analyzed according to the sperm parameters: normospermia treated by conventional IVF insemination (N-IVF; 65 cycles), normospermia requiring ICSI (N-ICSI) on the basis of the couple’s reproductive history (26 cycles), moderate oligoasthenoteratospermia (M-OAT; 56 cycles), severe OAT (S-OAT; 71 cycles) and azoospermia with sperm retrieved by microsurgical epididymal aspiration (MESA; 20 cycles) or testicular extraction (TESE) in obstructive (OA; 7 cycles) or non-obstructive azoospermia (NOA; 25 cycles). One blastomere was biopsied from day 3 embryos and analyzed for the chromosomes XY, 13, 15, 16, 17, 18, 21 and 22 by FISH.

Results: A total of 1379 embryos were FISH diagnosed. When compared with N-IVF patients, the proportion of chromosomally abnormal embryos was significantly higher in NOA (70% vs. 55% in N-IVF, p<0.01) and in S-OAT (63% vs. 55%, p=0.05), while the other groups had comparable figures (60% for N-ICSI and M-OAT; 64% for MESA). The frequency of aneuploidy for gonosomes increased proportionally with the severity of the male factor condition being significantly higher in NOA (11.4%) compared to N-IVF (3.1%), N-ICSI (2.3%) and M-ICSI (3.1%). In MESA patients, aneuploidy for gonosomes was detected in 7% of embryos. In addition, embryos from NOA had the highest incidence of complex abnormalities (69%) compared to the other groups. From the reanalysis of all blastomeres in 377 non-transferable embryos, 95% of NOA embryos were chaotic mosaics; this figure was significantly higher compared to the other studied groups.

Conclusions: There is growing evidence that a severe male infertility condition could contribute to the generation of chromosomal abnormalities in the resulting embryos. This is especially evident in cases of NOA in which the high incidence of chromosomal abnormalities is mainly due to mosaicism and gonosomal aneuploidy. For these patients, the possibility of performing aneuploidy screening on the generated embryos could represent the prevailing approach in order to decrease this novel form of reproductive risk.
Introduction: Owing to different limitations, preimplantation genetic screening (PGS) is not realistic choice for all couples. Therefore, there is an effort to assess the genetic status of an early embryo by other methods. One of them is assessment of multinucleation in cleaving embryos. The aim of the study was to determine (an)euploidy in all blastomeres of day 2 (D2) 4-cell human embryos with respect to their nuclear status.

Materials and methods: After ethical approval a total of 106 human embryos donated for research were evaluated under microscope for presence of multinucleation. Using hatching pipette the embryos were rotated until the status of all cells was clarified. Only embryos with clear state of all blastomeres were included in this study. According to this assessment the embryos were divided into three groups. Group A: embryos with four mononucleated cells. Group B: the embryos with at least one multinucleated blastomere and in Group C there were the embryos with all multinuclear cells.

Results: After FISH, 45% of 56 fully mononucleated embryos in Group A contained all euploid blastomeres, 42% were mosaic and 13% had aneuploid cells. This group included two subgroups(sgr) as follows: sgr/a: two or three blastomeres were euploid; and sgr/b: if only one cell was euploid. Aneuploid group included the embryos with all aneuploid cells.

Results: The majority of embryos examined were diploid/aneuploid mosaic (45/227; 19.8%), followed by the uniformly aneuploid (38/227; 16.7%), the diploid/chaotic (33/227; 14.5%), the diploid/tetraploid/aneuploid (30/227; 13.2%), the complex polyploid (28/227; 12.3%), the uniformly diploid (28/227; 12.3%), the diploid/tetraploid (24/227; 10.6%) and the diploid/haploid (1/227; 0.4%). The results from the coelomic fluid samples were concordant with those from the analysis of chorionic tissue. One sample was uniformly aneuploid (Trisomy 13), whereas 12/13 samples were normal diploid, 6 being female and 6 male, with moderate levels of mosaicism (<10%). The percentage of normal diploid cells was 92–97% for the chromosomes identified by the multiversion probe (13, 16, 18, 21, 22) and 91–96% for the urovision probe (3, 7, 9, 17). Tetraploid and aneuploid cells that were observed at low levels could reflect genuine mosaicism, owing to cytokinetic failure, non-disjunction or chromosome loss.

Conclusions: Chromosomal mosaicism was present in both preimplantation and post-implantation samples but the incidence was higher before implantation. In coelomic cells, derived from the embryonic lineage, low-level mosaicism (<10%), including aneuploid, tetraploid and chaotic cells, was observed. Tetraploid and chaotic cells were, however, more frequently observed in chorionic villus cells derived from the extraembryonic lineage. Aneuploid cells were present in both preimplantation and prenatal samples, but were more frequent before implantation. The potential to use coelocentesis for aneuploidy screening in clinical cases and follow-up analysis of pregnancies established after PGS will be discussed.

O-220 High rate of biological loss in ART according to eggs retrieved, fertilization, cleavage and preimplantation genetic screening results

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Introduction: A recent report indicated that only ~15% of embryos transferred result in live birth, underscoring the enormous biological wastage during IVF cycles. In addition, it was speculated that if the number of live birth was calculated per oocyte retrieved or inseminated, the rate of loss would have been even greater. In this study we calculated the overall biological wastage, from oocytes inseminated to ongoing pregnancies, in a group of patients undergoing preimplantation genetic screening (PGS) because of advanced age, recurrent pregnancy losses or multiple failed IVF cycles.
Materials and methods: A total of 26 patients (31 cycles) underwent IVF/ICSI procedures for PGS at the Yale University Fertility Center from June 2004 to December 2005. To assess the overall biological wastage, the following variables were analyzed: number of oocytes retrieved, inseminated and fertilized; number of embryos cleaved and biopsied on day 3; number of chromosomally normal embryos and rate of blastocyst development; and implantation rate and pregnancy outcome. One or two blastomeres were biopsied from each embryo and fluorescence in situ hybridization (FISH) was performed in-house using a 5-chromosome followed by a 4-chromosome probe set (13, 15, 16, 17, 18, 21, 22, X, Y).

Results: The mean age (±SD) was 37 (±3.7). A total of 356 oocytes were retrieved and 333 inseminated by either IVF (23 cycles) or ICSI (8 cycles). Of these, 209 fertilized (63%) and 199 cleaved (cleavage rate 95%). On day 2, 166 embryos were grade I or grade II (83%) and 135 were grade I and grade II on day 3 (68%). A total of 182 embryos were biopsied on day 3; of these only 33 (18%) were normal and 25 (14%) developed to blastocyst and were suitable for transfer. Four pregnancies were obtained (5 sacs for an implantation rate of 20%), but only two are ongoing (3 sacs, twins and a singleton). The analysis of the overall biological wastage showed that of the 333 eggs inseminated, only 25 (7.5%) produced normal embryos at PGS, of which 5 (1.5%) implanted and only 3 (1%) produced ongoing pregnancies (1 set of twins at 28 weeks and a singleton at 18 weeks).

Conclusions: The use of PGS in patients with recurrent pregnancy loss, advanced age or multiple failed IVF cycles showed an extremely high rate of oocyte/embryo wastage during ART procedures. The use of PGS allowed an objective assessment of how many inseminated oocytes have the potential to become normal embryos (10%) and live births (1%) in these instances. These data, if confirmed by future studies, may help both physicians and patients in considering oocyte donation as a reproductive option.

FREE COMMUNICATION

Session 57 – Endocrinology—PCOS

Wednesday 21 June 2006 10:00–11:45

O-223 Pioglitazone administration in hyperinsulinemic women with PCOS decreases the adrenal androgen response to corticotrophin-releasing factor

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Introduction: Hyperinsulinemia, frequently affecting women with polycystic ovary syndrome (PCOS), seems to disturb the hypothalamic–pituitary–adrenal (HPA) axis function. The insulin-sensitizing drug pioglitazone was demonstrated to reduce the adrenal response to corticotrophin (ACTH) in PCOS patients.

Materials and methods: To assess the site of action of insulin in the HPA-axis, the pituitary–adrenal response to the corticotrophin-releasing factor (CRF) was evaluated in 7 hyperinsulinemic PCOS patients before and after 4 months of treatment with pioglitazone (30 mg/die). Hormonal and lipid assays and an oral glucose tolerance test were also performed before and after therapy.

Results: We observed a significant reduction in insulin secretion (p<0.05) and an improvement in HDL levels (p<0.01) after therapy. Pioglitazone administration did not modify ACTH and cortisol response to CRF. A significant reduction in the adrenal CRF-induced secretion of androstenedione [area under the curve (AUC) 60.7±2.83 vs. 5.29±2.47 nmol/l for 0 min] and 17OH-progesterone (AUC 4.62±2.31 vs. 3.47±2.26 nmol/l for 0 min) occurred after treatment. A scarce response to CRF was observed for dehydroepiandrosterone sulfate and testosterone both before and after pioglitazone.

Conclusions: This study seems to indicate that, in PCOS hyperinsulinemic subjects, insulin may enhance the adrenal steroidogenesis by acting directly on the gland, with no significant effects on the pituitary ACTH response to CRF stimulation.

O-224 Effects of metformin alone, rosiglitazone alone and in combination on polycystic ovary syndrome: prospective randomized trial

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Introduction: Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, chronic anovulation and is associated with insulin resistance and compensatory hyperinsulinemia. Insulin sensitizing agents were recently demonstrated to effectively treat women with PCOS, acting through divergent cellular mechanisms. However, clinical trials directly comparing the effectiveness of selected insulin sensitizers are limited. The aim of this study was to prospectively compare the effects of metformin (MET) alone, rosiglitazone (RSGN) alone and a combination of MET and RSGN on clinical, endocrine and metabolic parameters in hyperinsulinemic women with PCOS.