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 hysteroscopy 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 Ultrasoft 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.

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 imbalances 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 (General Electrics, 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 constitutinal 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 aneuploides, the microarray 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 cytogenetic 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 microarrays can be a new alternative to the aneuploidy testing by FISH and multiple genes analysis in preimplantation genetic diagnosis.

**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 diagnosis (PBD) by polar body biopsy and subsequent fluorescence in situ hybridization (FISH) analysis detects maternally derived aneuploides 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 patterns of signals and 42 (21.7%) polar bodies showed signals indicating a balanced predominition 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 aneuploides.

**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.
Abstracts of the 22nd Annual Meeting of the ESHRE, Prague, Czech Republic, 18–21 June 2006

**O-219**  
**FISH analysis of the whole 4-cell human embryos—relationship to their mono/multinuclear status**  
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**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 (aneu)polidy 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 status 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. For fluorescent *in situ* hybridization (FISH), whole embryos were fixed and hybridized for chromosomes 13, 15, 16, 18, 21, 22, X and Y (Vysis®). According to FISH results, the embryos were divided into three basic groups. Euploid embryos: completely euploid for detected chromosomes. Mosaic embryos: consisting of euploid and aneuploid cells. This group included the embryos with all aneuploid 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 only. In the Group B, neither one embryo of 32 was fully euploid, 56% of them were mosaic and the remaining were completely abnormal. Moreover, FISH results indicate that mosaic embryos differ between these groups. While in Group A we found that 86% of mosaic embryos had 2 or 3 normal cells, controversially in Group B the percentage of such embryos decreased to 38%. In the remaining (18) embryos from Group C we were not able to detect any euploid cell.

<table>
<thead>
<tr>
<th></th>
<th>Euploid</th>
<th>Mosaic</th>
<th>Aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (%)</td>
<td>45</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>Only mononuclear cells: sgr (%):</td>
<td>a vs. b</td>
<td>86 vs. 14</td>
<td></td>
</tr>
<tr>
<td>Group B (%)</td>
<td>0</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>Mono/multinuclear cells: sgr (%):</td>
<td>a vs. b</td>
<td>38 vs. 62</td>
<td></td>
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<tr>
<td>Group C (%)</td>
<td>0</td>
<td>0</td>
<td>100</td>
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</table>

**Conclusions:** According to the results obtained by our study it would be reasonable to perform PGS exclusively in embryos from the group having only mononucleated blastomeres on D2. However, even in this group we can expect high proportion (42%) of mosaic embryos but with equal number or majority of euploid cells. Such embryos, if determined by FISH as aneuploid, may paradoxically be ‘cured’ by the process of biopsy. Nevertheless, they will be rejected from transfer. However, if allowed by legislation and after informed assent of patients, these embryos may represent an ethically more acceptable source of embryonic stem cells. On the other side, the embryos with mono/multinuclear blastomeres should not be used for PGS. With respect to the results we can frequently expect mosaic embryos with minority of normal cells. After recognizing the biosed cell(s) as normal, the remaining cells in analyzed embryo will be most probably abnormal. Finally, in the group of embryos with all multinuclear cells we cannot expect any normal development to healthy embryo and, therefore, they should be rejected from any use in assisted conception. In addition, in the cycles without PGS and mainly in cases of elective single embryo transfers the evaluation of D2 embryos for nuclear status should be one of the most important steps.

**O-220**  
**Comparison of chromosomal mosaicism in the human pre and early post-implantation concepts by analysis of blastomeres and coelomic cells at 6–9 weeks**  
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**Introduction:** A possible cause of early embryonic arrest, failed implantation and miscarriage is the high incidence of nuclear and chromosomal abnormalities. Preimplantation genetic screening (PGS) is therefore increasingly being used to select normal embryos for transfer. Recently, embryonic cells have been sampled from the coelomic cavity at 6–9 weeks gestation for the identification of single gene defects in ongoing pregnancies and for aneuploidy screening. The aim of this study was to compare the incidence of chromosomal abnormalities and mosaicism during preimplantation and early post-implantation development, by studying human embryos, coelomic cells and chorionic villi to molecular cytogenetic evaluation.

**Materials and methods:** Cleavage to blastocyst stage human embryos (n = 227) were spread on poly-L-lysine slides using 0.01 N HCl, 0.1% Tween-20 and processed for sequential FISH in up to four successive hybridizations using probes for chromosomes 1, 4, 6, 3, 7, 9, 17, 13, 16, 18, 21, 22, X and Y. Paired coelomic and chorionic villus samples (n = 13) obtained at 6–9 weeks gestation were fixed uncultured in 3:1 methanol/acetic acid and processed for FISH using probes for chromosomes 3, 7, 9, 17, 13, 16, 18, 21, 22, X and Y. 

**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 multivision 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 coelicentesis for aneuploidy screening in clinical cases and follow-up analysis of pregnancies established after PGS will be discussed.

**O-221**  
**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±28.3 vs. 5.29±24.7 nmol/l for 0 min] and 17OH-progesterone (AUC 4.62±3.1 vs. 3.47±2 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.