O-119 Oral The value of follicular fluid G-CSF as a biomarker of embryo implantation potential in monofollicular IVF cycle

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Introduction: Evaluating the implantation potential of an embryo is one of the major issues in the assisted reproduction technique. New non invasive methods are recently available. Granulocyte colony-stimulating factor (G-CSF) – a cytokine, belonging to the family of growth factors, detected in follicular fluid (FF) is being proposed as a biomarker of oocyte competence.

The purpose of the study was to investigate the predictive value of G-CSF and other cytokines/chemokines found in the FF in regard to implantation of the embryo and pregnancy outcomes in natural modified IVF cycles. This protocol presents a unique monofollicular research model.

Materials and Methods: Retrospective study was performed in Antoine Béclère hospital. Inclusion criteria for natural modified cycle were: previous implantation failure in conventional ovarian hyperstimulated IVF/ICSI cycles or a low ovarian reserve below 38 years old. We obtained FF from 100 cycles, from 83 patients. For this study we selected FF, belonging to the first attempts of the patients. These 83 cycles led to 54 embryo transfers (ET), resulting in 19 deliveries and 6 first trimester miscarriages. According to embryo morphology 36 high-quality and 18 low-quality embryos were observed. In 10 cycles no oocyte was collected, in 19 no embryo was obtained. Each sample of FF was blindly tested for their cytokine contents by multiplexed microsphere-based immunoassays able to simultaneously measure multiple analytes. Flow cytometric resolution of spectrally distinct microspheres coupled with capture molecules and reporter fluorochromes bound to detect antibodies. IL-1α, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-α, TNF-α, G-CSF, GM-CSF, VEGF, PDGF, GGF, IP-10, MCP-1, CCL5, etoxacin, MIP-1a and MIP-1b were analyzed (Bio-Rad Laboratories, Hercules, CA, USA). In this part of the study G-CSF was established as a potential biomarker using the Area under the ROC (AUROC) curve methodology. Thresholds for Anova analysis according to G-CSF and other cytokines/chemokines found in the FF were respectively both at 0.78 and 0.80 (p = 0.0001). AUROC for FF-GCSF as a biomarker of delivery/puncture and delivery/transfer were respectively both at 0.78 and 0.80 (p = 0.0001). AUROC for FF-GCSF as a biomarker of clinical pregnancy/puncture and transfer were respectively at 0.72 and 0.73 (p = 0.0008). FF G-CSF was lower than 8.74 pg/ml in 24 samples (14 transferred), from 8.74 to 12.1 pg/ml in 14 samples (7 transferred), and over 12.1 pg/ml in 45 samples (33 transferred) and defined low-medium and high G-CSF ranged groups. Clinical pregnancy rates/puncture and transfer were respectively 12.5%–28%–40% and 14%–43%–54% in low-medium and high G-CSF ranged groups (p = 0.03 and 0.06). Delivery rates/puncture and transfer were respectively 0%–14%–37% and 0%–28%–51% in low-medium and high G-CSF ranged groups (p = 0.002 and 0.001). There was no correlation between FF G-CSF levels and pregnancy outcomes, so the selection of the embryo with the best implantation potential is important. New parameters to predict embryo viability, like non-invasive metabolic profiling, have been studied. Metabolomics is the study of small-molecule metabolite byproducts left behind from cellular processes. By measuring byproducts of the embryonic metabolism you get a snapshot of the physiology of an embryo which translates to viability. Recently, several studies showed that metabolomic profiling of biomarkers of metabolism by Near Infrared (NIR) spectroscopy correlated with ongoing pregnancy in fresh IVF/ICSI cycles, when the transferred embryos were selected by conventional selection criteria. In this study, we investigated if metabolomic profiling of biomarkers of metabolism by NIR spectroscopy correlated with ongoing pregnancy after SET of frozen-thawed embryos.

Material & Methods: Between January and April 2008, embryos of 52 patients scheduled for a frozen-thawed SET were included. Day 4 embryos were thawed using a standard slow protocol and then cultured for 20–24 hours prior to transfer. The embryos were cultured individually overnight in 25 μl pre-equilibrated medium drops. Alongside, embryo-free media drops were incubated as controls. Embryos were selected for transfer by routine morphological criteria. After transfer, the medium drop in which the transferred embryo was cultured and a control medium drop were immediately frozen (−196°C). Individual metabolomic profiles were obtained from 10 μl media samples using NIR spectroscopy (Molecular Biometrics Inc.). Cryopreserved embryo viability scores were calculated from a logistic regression of genetic algorithm selected NIR spectral regions, and leave-one-out cross validation. The metabolomics data were compared to pregnancy outcomes.

Results: Of the 52 cryopreserved SET 9 (17.3%) ongoing pregnancies were established as detected by fetal cardiac activity (FCA) 12 weeks post embryo transfer. Viability scores calculated from four distinct NIR spectral regions significantly discriminated (P = 0.0007) between cryopreserved embryos that established ongoing pregnancies (FCA positive, 0.33 ± 0.12) compared to those that failed to implant (FCA negative, 0.16 ± 0.27). A partial least squares discriminant analysis model was also developed to discriminate between the FCA positive and negative samples and was able to successfully distinguish 11 of 19 (58%) positive and 37 of 44 (84.1%) negative at 90% confidence limits for each group.

Conclusion: The results indicate that NIR spectral analysis of post-thaw samples may allow discrimination of viable and non-viable cryopreserved embryos. Coupled with a pre-freeze analysis this may allow stronger predictability for frozen SETs. The data awaits confirmation in a blinded study.

SELECTED ORAL COMMUNICATION SESSION

Session 32: Ethics and law

Tuesday 30 June 2009 10:00–11:30

O-120 Oral Viability assessment of cryopreserved embryos by near infrared spectroscopy: preliminary results

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Introduction: Cryopreservation of supernumerous, good quality embryos is routinely offered in IVF/ICSI programs. The number of frozen-thawed cycles and its contribution to overall pregnancy results has increased over the last few years, mainly because of an increase in applying Single Embryo Transfer (SET) in the fresh cycle. SET is even often performed when cryo-thawed embryos are transferred. The quality of an embryo has a great influence on pregnancy outcomes, so the selection of the embryo with the best implantation potential is important. New parameters to predict embryo viability, like non-invasive metabolic profiling, have been studied. Metabolomics is the study of small-molecule metabolite byproducts left behind from cellular processes. By measuring byproducts of the embryonic metabolism you get a snapshot of the physiology of an embryo which translates to viability. Recently, several studies showed that metabolomic profiling of biomarkers of metabolism by Near Infrared (NIR) spectroscopy correlated with ongoing pregnancy in fresh IVF/ICSI cycles, when the transferred embryos were selected by conventional selection criteria. In this study, we investigated if metabolomic profiling of biomarkers of metabolism by NIR spectroscopy correlated with ongoing pregnancy after SET of frozen-thawed embryos.

Material & Methods: Retrospective study of U.S. egg donors: risks, informed consent, anonymity, satisfaction, & need for long-term follow-up: results & recommendations

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Introduction: Success in U.S. IVF clinics is measured by the likelihood of live births. Less attention is paid to the outcomes and health of egg donors or the
future concerns of donor-conceived children. After egg retrieval, donors are discharged from the IVF clinic but are rarely contacted afterwards. Long-term medical risks to egg donors have never been systematically studied. Only a few published studies have considered the emotional and psychological effects of egg donation on donors. Potential egg donors sign informed-consent forms without actually receiving information on long-term risks, because such risks are not known.

Methods: This study presents findings from a large sample of egg donors, up to 22 years after egg donation; 155 of them completed a survey on the website of Donor Sibling Registry (DSR), a US-based registry that helps donor-conceived people make mutual-consent contact with their half siblings and/or donors. An online survey asked about medical complications and subsequent health problems, contact with IVF clinic, donors’ satisfaction with the donation process, and current feelings.

Results were based on 155 women < 1 to 22 years (mean, 9.4 ± 5.2 years) past their first donation, which occurred at a mean age of 26.4. Reported medical complications included 32.6% with some degree of OHSS and 4.9% with subsequent infertility. Only 2.6% had been contacted by the IVF clinic for medical updates; 34.2% reported medical changes they thought would be of interest to donor children and half had attempted to report these to the clinic, with variable results. Many of those who did not report didn’t realize they could or should. Almost all were open to contact with recipient families (but this finding of course reflects selection bias in the sample). A common theme was desire to know the outcome of the egg donation. Donors frequently had not sought information because they were confused about the definition of “anonymity” or “confidentiality,” believing that anonymity meant they were not to contact the clinic and/or that the clinic could not contact the recipients to provide them information.

Conclusions and recommendations: IVF clinics need to give anonymous egg donors clearer guidelines re asking for outcome information or giving the clinic medical updates to benefit their biological children. Counselling should also inform donors that in later years they might feel differently about the egg donation than at the time of donation. Additional long-term studies are needed to ascertain egg donors’ risks of infertility or cancer. We recommend that IVF clinics maintain donor records indefinitely (This will also permit systematic follow-up of egg donors to finally determine the potential health risks); develop protocols to contact donors regularly to update medical information on the donor’s health and information of interest to recipients; educate egg donors about the importance of contacting the IVF clinic, even years later, to provide such information; contact recipient families with relevant information provided by the egg donor; notify donors if any IVF-conceived children are born with genetic abnormalities; educate egg donors of the possible curiosity of the child to be born and make egg donors and recipient families aware of resources for updating and sharing medical information, such as the Donor Sibling Registry.

O-122 Oral On consent rules for storing embryos

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Introduction: Couples have embryos frozen in order to gain the option of transferring them in future, with the aim of creating a pregnancy and ultimately a live birth. Problems can arise when couples separate. Under UK law, embryos cannot be transferred without the current consent of both genetic parents. The issue came to public prominence in the case of Natalie Evans, who lost the opportunity to have her own genetic children after her relationship ended and her partner withdrew consent for embryo storage. This study examines the case for a change in consent laws to give couples a choice of consent agreements.

Materials & methods: A possible change in the law, to give couples a choice of two types of consent agreement, is considered. One type of agreement would be in line with current consent procedures, allowing either genetic parent to withdraw consent up to embryo transfer. The other type of agreement would involve one member of the couple signing away the right to withdraw consent, so that their partner would have control over the embryo. The couple would then decide between themselves which type of consent agreement to sign. The benefits of and possible objections to this change in the law are analysed according to philosophical and legal principles.

Results: The proposed change in the law may directly benefit both members of a couple in a situation where they would not create frozen embryos together under the current law but would do so under the proposed new law. For example, a woman in the position of losing her fertility to urgent medical treatment may be unwilling to have embryos created with her partner and frozen if the man retains the right to withdraw consent at a later date. It is then in the man’s interests to be able to sign away the right to later withdraw consent. More generally, the change gives couples more autonomy by allowing them a choice, and does not remove any option currently open to them. Possible objections to this change in the law take three main forms: (i) concern about the welfare of a child born to parents who have separated; (ii) concern about any broader costs to society from the birth of such children; and (iii) concern that a genetic parent who signed away his or her right to withdraw consent would be the victim of an unfair contract. It is shown that the first objection would imply that a baby born to parents not together has a life not worth living; this seems absurd and also conflicts with the general principle under English law that ‘wrongful life’ is not recognised. Both (i) and (ii) conflict with the legality and practice of offering fertility treatment to single women, and the removal of the ‘need for a father’ in the UK Human Fertilisation and Embryology Act 2008. For (iii), it is generally accepted that a necessary condition for a contract to be unfair contract is that the wronged person is either likely to be in an unfit state of mind or has insufficient information at the time of signing the contract. Thorough counselling, with mandatory individual counselling for both partners, may answer possible concerns about unfair contracts.

Some practical considerations remain, such as the time periods over which embryos can be used under different consent agreements.

Conclusion: There is a strong case for revising the law to allow couples a choice of consent agreements when they opt to have embryos frozen. Robust consent-advisory procedures will be needed, helping couples to think through their consent decisions more thoroughly than at present.

O-123 Oral Avoiding transgenerational health risks: a morally acceptable reason for sex selection?

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Introduction: Mitochondrial DNA (mtDNA) mutations are an important cause of severe diseases. As there is no curative treatment, helping carriers to have healthy children has been a central focus of attention. New techniques aimed at achieving this are preimplantation genetic diagnosis (PGD) and possibly in the future nuclear transfer (NT). However, when applying PGD for mtDNA disorders, it is conceivable that only affected embryos are available for transfer. Moreover, in a clinical application of NT it will be difficult to avoid small amounts of affected mitochondria to come along with the oocyte, pronuclei or nucleus of the recipient woman.

Although neither PGD nor NT can ‘guarantee’ a mutant free child, the mutant load of the resulting child is expected to be (far) below the threshold to disease expression. Nevertheless, due to the existence of a genetic bottleneck, the mutant load may rise again in the third generation - the couple’s grandchildren. As mitochondria are transferred maternally, male offspring will not pass on their mutant DNA to the next generation. This leads to the question whether the avoidance of transgenerational risks provides a morally acceptable reason for sex selection.

Material & methods: Literature review; ethical analysis.

Results: We do not consider the possible occurrence of transgenerational health risks to be a contra-indication for PGD and NT. After all, it is far from certain that the genetic bottleneck will lead to a higher mutant load in a possible third generation. Nor is it certain that female offspring will reproduce and if she does, she may resort to PGD or NT herself. However, both these risks and the burden of difficult reproductive decisions are important enough to be prevented if reasonably possible. Theoretically, this can be achieved by creating or transferring only male embryos.

Whereas sex selection is highly controversial for nonmedical use, it is generally accepted to avoid the birth of a child with a severe X-linked genetic disorder. In our case, sex selection would be used to avoid health effects further along the line of generations. Would that be acceptable? A similar question has arisen in PGD for X-linked diseases, where sex selection against healthy
carrier embryos has the same purpose. Because these applications of sex selection are still done for reasons of health, they should not give rise to the moral concerns associated with sex selection for nonmedical reasons.

Ideally, sex selection would be an integrated part of PGD for mtDNA mutations, which may perhaps also be done as a possible confirmatory step after NT. Information about the sex would then be obtained as a by-product of PGD performed for other reasons, or sex identification is added to PGD. Given the limited nature of the risk to be avoided, the proportionality of this extra element must be carefully observed. This allows to preferentially transfer male embryos, but only as a secondary criterion and not as a reason to conduct a new cycle. PGD solely to avoid a transgenerational risk would not be acceptable either from this point of view. Preconceptional sex selection (sperm separation) could be considered to increase the number of male embryos available for transfer. However, since these methods are not fail-safe, sexing of the embryos afterwards would still be needed if one wants certainty. Unfortunately, the most promising technique (flow cytometry) is not completely established yet. High costs would also affect the proportionality, given the limited nature of risks to be avoided.

Conclusion: Notwithstanding the theoretical acceptability of sex selection to avoid transgenerational health risks, the proportionality of this application in the context of mtDNA mutations depends on various factors and needs further scrutiny.

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Introduction: Traditionally, gamete donation has been practiced so that donors and recipients are unknown to each other and individuals conceived following a donor procedure receive little or no information about their donor. However, a key topic of recent debate, policy formulation and regulation has been the extent to which donor-conceived people should be allowed to ascertain information about their genetic and biographical history. Worldwide, ten jurisdictions currently enable donor-conceived individuals to learn the identity of their donor and an eleventh has passed legislation enabling them to do so that is yet to be implemented.

Material and Methods: The presentation is based primarily on a review and analysis of legislation and policy documentation in the eleven jurisdictions that have either already implemented legislation enabling donor-conceived individuals to learn the identity of their donor (10) or have yet to implement such legislation that has been approved by the relevant legislature (1). Where such information is not readily available in print, this has been obtained directly from relevant authorities in each jurisdiction.

Results: The analysis provides details of the legislation that has been passed and presents data on the following areas: (1) safeguards to the interests of donors and the promotion of donors’ rights; (2) limits placed on the number of offspring or families per donor; (3) arrangements for managing a formal register of donor procedures; (4) the age at which a donor-conceived person can obtain information and any provisions for earlier access to information and/or access to information by the parent of a donor-conceived child acting on behalf of their son or daughter; (5) access to donor information by the descendant of a donor-conceived person; (6) restriction on the provision of information; (7) provisions for access to information in respect of a donor procedure undertaken before the implementation of legislation permitting disclosure of donor identity, and (8) provisions enabling a donor-conceived person to ascertain information about any other individual who shares the same donor.

Conclusions: The presentation will conclude by identifying a range of measures that may be taken to promote the ability of donor-conceived people to learn about their genetic and biographical history.

O-125 Oral Who should pay for assisted reproductive techniques? - answers from patients, professionals, and the general public in Germany
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Introduction: In 2004, new legal regulations have been introduced in Germany, restricting the coverage of statutory health insurances to 50% of the costs for ART procedures and medication. As a result, the amount of performed treatment cycles as well as the amount of children born after IVF/ICSI declined by about one third. Against this background, we conducted a survey among patients, professionals, and the general public in Germany on their opinions regarding financing ART.

Material and Methods: The views of German patients (n = 1598, response rate RR = n.d.), reproductive physicians (n = 230, RR = 36%), psychosocial counselors (n = 66, RR = 67%), medical ethicists (n = 135, RR = 55%), social lawyers (n = 140, RR = 45%), health politicians (n = 78, RR = 27%), and the general public (n = 1005, RR = n.d.) regarding costs, cost-efficiency, savings potential, funding, co-payment, and special financing plans (egg-sharing, money-back-guarantee) of ART have been surveyed using standard questionnaire techniques (Paper and Pencil Interviewing PAPI, Computer Aided Web Interviewing CAWI, Computer Aided Telephone Interviewing CATT).

Results: In all groups, the vast majority supported coverage of ART by statutory health insurances and/or tax money. At the same time, about 1/3 of the patients, 2/3 of the physicians and 3/4 of all other groups considered co-payments of patients as appropriate. The median amount of co-payment considered appropriate ranged between 15–25% of the costs, considerably less than what patients in Germany actually have to pay (50%). Regression analyses showed that the support for public coverage of ART was strongly correlated with the views (i) of infertility as an illness, (ii) that there is a need for assisted reproduction for infertile couples with an unfulfilled desire for children, and (iii) that every human should have the opportunity to have children.

To increase the health insurance premium by 1.50 Euros per month in order to cover ART was rejected by a relative majority of psychosocial counselors, medical ethicists, and health politicians, but supported by most respondents of the other groups. To reduce services in other areas of health care in favour for reproductive medicine was supported by the group of reproductive physicians only. They saw some potential for savings with regard to the medication, but not with regard to diagnostics, laboratory techniques, and anaesthesia. Among the patients and physicians, about the same number of respondents was in favour for and opposed to the possibility for women to receive a discount on infertility treatment by sharing their eggs with other couples. All other groups rejected such a commercial egg-donation with a majority of two thirds. A money-back-guarantee in case of unsuccessful treatments was unanimously objected by all groups, including patients.

Conclusions: Policy makers should take notice of the opinions of patients, professionals and the general public in Germany regarding financing ART. The data show a significant tendency in favour of reduced co-payments and increased health insurance coverage. Potential for significant savings were neither seen in the field of assisted reproduction nor in other areas of health care. Introducing elements of commodification and market mechanisms in reproductive medicine was rejected.

The majoritarian approval of covering ART was founded on the beliefs that couples suffering from involuntary childlessness are in need for treatment because infertility is a condition of ill health and having children is a basic opportunity everyone should have. A rational and normative assessment of the respondents’ opinions on financing ART should deal with these foundational beliefs.

O-126 Oral Cross-border fertility care: a survey of Canadian and American clinics, assessing scope of practice and communication between patients and caregivers
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Introduction: Patients seek cross-border fertility care for many reasons. This practice challenges the continuity, quality and ethics of care. In order to better understand these issues, a survey of Canadian and American fertility clinics was undertaken. Its objectives were to: 1) identify the scope and volume of cross-border services in North America and 2) evaluate communication between patients and their caregivers.

Materials and Methods: Each survey was developed with input from clinicians, nurses and patients. Surveys were pre-tested in four different centers
for time and ease of use, ambiguity and comprehension. Between October 1st and November 30th 2008, the survey was mailed twice to 26 Canadian fertility clinics and to 8 individual Canadian MDs, satellite with these clinics. A modified survey was distributed online, to 392 American SART-member clinics. Results were tabulated and summarized using SPSS.

**Results:** 28 Canadian and 125 American surveys were completed (78% and 32% response rates). For Canada, the largest proportion of surveys (50%) was from Ontario. Respondents reported offering a total of 6927 stimulated IVF cycles per year, equivalent to 77% of the total cycles provided in Canada for 2007 (n = 9019). The most common out-of-country treatment sought by Canadians was IVF with anonymous donor-eggs: 363 of 452 patients treated (80%). Canadian respondents provided satellite monitoring for 431 women undergoing out-of-country IVF. For patients entering Canada in order to receive fertility treatment (n = 146), the largest demand was for IVF (73% of patients treated). 52% of respondents recommended specific destination countries to their patients, but not specific providers. Confidence in safety, effectiveness and ethics were considered very important by 71–80% of respondents. Respondents felt that patients were most concerned with effectiveness (88%) and safety (80%). 88% of Canadian respondents always provide the information requested by the destination clinic. Canadian clinics were most interested in receiving information about complications of treatment, number of embryos transferred and frozen.

For the United States survey, the largest proportion of responses came from the Southern US (31%). Respondents reported offering 35,387 stimulated IVF cycles per year, equivalent to 41% of the total 85,326 stimulated cycles reported to SART for 2006. Responding US clinics reported treating 927 out-of-country patients, 51% of them with standard IVF. 36% of incoming patients were from Latin America and 23% from Europe. The largest proportion of the 220 patients leaving the US in order to receive IVF or donor egg IVF, traveled to India / Asia: 41% and 52% respectively. Respondents indicated that confidence in treatment effectiveness, and as well as information from other patients, were very important factors in patients’ decisions to come to their clinics. The majority of respondents felt that recent laboratory results and track sheets from previous cycles should always be sent with out-of-country patients. Good concurrence was seen between Canadian and American clinics’ ratings of key data that should be provided along with returning patients.

**Conclusions:** The number of Canadians traveling to the United States for ART is equivalent to approximately 5% of the total cycles performed in Canada. Eighty percent of these women seek anonymous donor egg IVF. Less than 1% of US patients leave the country for fertility care and for them, the most popular destination is India / Asia, for standard or donor egg IVF. In the USA, approximately 3% of the total ART volume is made up of women coming into the country for care. US clinicians stress the need for recent lab data and previous stimulation track sheets. All parties surveyed rated effectiveness and safety of care as paramount in patient choice of destination.

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**SELECTED ORAL COMMUNICATION SESSION**

**Session 33: Preimplantation genetic diagnosis**

**Tuesday 30 June 2009 10:00 – 11:30**

**O-127 Oral**  PCR-based detection of chromosomal unbalances on embryos: a possible future (r)evolution of PGD for chromosomal translocations

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**Introduction:** Preimplantation genetic diagnosis (PGD) has been offered to carriers of balanced translocations as an alternative to prenatal diagnosis. Fluorescence in-situ hybridisation (FISH) is the method of choice for detecting chromosome rearrangements. The FISH strategy involves the simultaneous use of telomeric probes in combination with centromeric probes (reciprocal translocations), or alpha-satellite/locus-specific enumerator probes (Robertsonian translocations).

Here we present the development of a polymerase chain reaction (PCR)-based PGD approach for detection of chromosomal imbalances on embryos derived from both reciprocal and Robertsonian translocation carriers. The procedure involves testing of single blastomeres by fluorescent multiplex PCR analysis of polymorphic short tandem repeat (STR) markers located along the chromosomes involved by translocation.

**Material & methods:** STR markers were selected to be located at either side of each breakpoint (reciprocal translocations) or at any point of the chromosomes involved (Robertsonian translocation). STR markers were also included to determine the copy number of chromosomes 13, 14, 15, 16, 18, 21, 22, X, Y in patients of advanced maternal age. Informativity testing of STR markers was performed for both partners of each couple. Only fully informative markers presenting alleles not shared by the partners were selected. In order to avoid misdiagnosis due to possible allele drop-out (ADO) occurrences, at least three STR for each chromosome were included in the protocol. Embryos were diagnosed as “normal-balanced” if PCR results indicated two signals (peaks) for each chromosome tested. Embryos were diagnosed as “unbalanced” if the PCR results showed a deviation from the “normal-balanced” signal pattern, such as trisomies (three peaks), monosomies (one peak) and nullisomies (no PCR signals).

**Results:** Twelve PGD cycles were carried out for 12 couples carrying six different reciprocal translocations and two Robertsonian translocations. The mean maternal age was 36.4 ± 4.6 years. A total of 204 oocytes were collected, 152 (74.7%) were MI, 126 (79.7%) fertilized and 110 embryos were biopsied on day 3. PCR was successful in 102/110 (92.7%) blastomeres, accounting a positive amplification on a total of 1048/1128 (92.9%) loci. Overall, 102 (92.7%) embryos were successfully diagnosed, 52 of which resulted normal/ balanced, 44 were unbalanced and 6 resulted to be haploid. PGS was included in the PGD protocol of five couples, involving testing of 45 embryos, 40 (88.9%) of which were successfully diagnosed and 24 (60.0%) showed aneuploidies. Embryos suitable for transfer where identified in 10 cycles. Following transfer of 23 embryos (mean 1.9 ± 1.1), 7 women had a clinical pregnancy confirmed with fetal sacs and heart beat (70.0% pregnancy rate per embryo transfer). A total of 13 embryos implanted (56.5% implantation rate per embryo transferred), for 10 of which heart beat was also detected. Only 2 couples accepted to undergo to prenatal diagnosis, performed by chorion villus sampling (CVS) or amniocentesis, which confirmed the PGD results. All pregnancies are still ongoing.

**Conclusions:** The above results demonstrate the feasibility and reliability of our PCR-based PGD protocol for detection of chromosomal imbalances. The present technique has the potential to overcome to several inherent limitation of the FISH procedure, such as suboptimal fixation, overlapping signals, split signals, lack of signals, cross-hybridization, polymorphisms, limited availability of the probes, combination of colours, decreasing of the accuracy with re-probing. This approach has the advantage to be rapid, low expensive, amenable to automation, involving an easy procedure and data interpretation. Unlike FISH, with the presented protocol is also possible to distinguish the parental origin of chromosones, allowing detection of uniparental disomies and the achievement of a DNA fingerprint for each embryo, useful for identification of embryos that have implanted. Finally, because cell fixation is not necessary, the PCR-based protocol represents an easier procedure for management of transport PGD. Considering the encouraging preliminary clinical outcome obtained, this approach has the potential to represent a valuable alternative to FISH-based PGD.

**O-128 Oral**  IVF with non-elevated E2 levels and PGD for BRCA1 mutation in a breast cancer patient. First report on mutation-free twin’s pregnancy

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**Introduction:** Young breast cancer patients frequently wish to have biologic children but reproductive decisions are difficult especially when fertility