cent in situ hybridization was performed using a combination of commercially available probes, which target the whole chromosome, centromere, locus specific sequences and/or sub-telomeric regions specific to the chromosomes involved in the translocation. Clinical pregnancies were determined by the presence of a gestational sac with fetal heartbeat.

RESULTS: Following IVF and PGD, an implantation rate of 61.6% was realized. Follow up of these pregnancies revealed a significantly lower spontaneous abortion rate of (7/45) 15.6% when compared to the previous obstetric history of these same patients in which the spontaneous abortion rate was (115 / 131) 87.8%. After IVF and PGD, 27 deliveries occurred in which 30 healthy infants with balanced or normal karyotypes were born resulting in a take home baby rate of 79.4% with 11 ongoing pregnancies. This is in contrast to the previous obstetric history of these couples in which the take home baby rate was only (15/131) 11.5% and included the birth of 4 children with unbalanced karyotypes and one stillborn with a normal karyotype.

CONCLUSION: These results demonstrate the significantly positive impact of PGD when performed for couples in which one partner carries a chromosomal rearrangement, including a 5.6 fold reduction of spontaneous abortions and a 6.9 fold increase in take home baby rates.

Supported by: Reproductive Genetics Institute

Monday, October 18, 2004
4:45 P.M.

O-74

The peptide nucleic acids (PNAs) as probes for chromosomal analysis of human oocytes and preimplantation embryos. F. Pellestor, P. Paulasova, T. Anahory, B. Andréo, M. Macek, S. Hamamah. CNRS UPR 1142, Montpellier; Laboratoire de Assisted Reproduction, Motol Hospital, Prague, Czech Republic; Department of Reproductive Biology B. Arnaud de Villeneuve, Montpellier, France; Department of Reproductive Biology B, Arnaud de Villeneuve Hospital, Montpellier, France.

OBJECTIVE: Demonstrate the efficiency and the reliability of PNAs for the in situ chromosomal analysis of human oocytes and blastomeres

DESIGN: Peptide Nucleic Acids (PNAs) are synthetic DNA mimics based on an uncharged polyamide backbone. Because PNA probes present multiple advantages in terms of affinity and specificity, we have tested this new type of probe on isolated human oocytes, polar bodies and blastomeres, in order to assess the possibility of using it for preimplantation diagnosis of aneuploidy.

MATERIALS AND METHODS: Using centromeric PNA probes specific for chromosomes 1, 4, 9, 16, X and Y, we performed multicolour labelling PNA reaction, sequential PNA reaction and combined PNA and fluorescence in situ hybridization (FISH) in 34 unfertilized oocytes and 27 blastomeres. Each PNA probe consists of a mixture of several short synthetic sequences and/or sub-telomeric regions specific for the centromeric sequence of the targeted chromosomes. The PNA probes were directly fluorochrome labelled and supplied ready to use in hybridization buffer. The hybridization timing of PNA probes was 40 - 60 minutes.

RESULTS: In 27 oocytes, simple multicolour PNA assays were done. A sequential PNA procedure was experimented in 5 oocytes. Two other oocytes were utilized to test the combined PNA and FISH protocol. In 17 cases, corresponding polar bodies were obtained through the fixation procedure. Twenty-six oocytes (76.5%) displayed a normal pattern of signal according to the probes used. Eight oocytes (23.5%) displayed numerical abnormalities for the targeted chromosomes. Twenty-three blastomeres from 10 embryos were processed for PNA experiments. In 3 cases, the FISH technique was used as control in one blastomere. Only three embryos appeared to be diploid for the chromosomes tested. Four others displayed aneuploidy, and 3 embryos displayed mosaic pattern. Both rates and types of abnormalities scored in oocytes and blastomeres are in good agreement with the data of previous FISH studies.

CONCLUSION: The present study describes the first use of PNAs on isolated human oocytes and blastomeres for in-situ chromosomal identification. PNA probes allow a reliable chromosomal analysis and consequently, can provide an interesting adjunct to FISH for diagnostic analysis. The PNA technology possesses undeniable advantages for in-situ recognition of complementary DNA sequences. Because of the simplicity and the rapidity of the PNA procedure, these preliminary results point out the potential application of PNA in PGD program.

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Monday, October 18, 2004
5:00 P.M.

O-75

PGD of aneuploidy to reduce multiple gestations and maintain high pregnancy rates in women 35 and older. M. Oter, M. Sandalinas, P. Colls, T. Escudero, X. Zheng, S. Munne. Reprogenetics LLC, So. San Francisco, CA; Reprogenetics Spain, Barcelona, Spain; Reprogenetics LLC, West Orange, NJ.

INTRODUCTION: Triplet pregnancies are 37% premature, have a 15% pregnancy loss rate, 5% morbidity, and reducing from triplets to twins results in 5–15% fetal mortality. Higher multiple pregnancies are even worse. It is estimated that each triplet pregnancy costs to the system $300,000. Preimplantation Genetic Diagnosis for aneuploidy (PGD-A) can increase implantation and pregnancy rates in women 35 and older, by selecting those embryos with a higher chance of implanting. Thus PGD may allow the replacement of fewer embryos to achieve acceptable pregnancy rates without the risk of multiple pregnancy.

MATERIAL AND METHODS: As part of an ongoing research study, patients undergoing PGD-A were blindly matched to controls before pregnancy results were obtained. The matching was based on maternal age (39.9 years for both control and PGD-A groups), number of oocytes (13.7 and 14.3, respectively), number of zygotes (8.3 and 8.5, respectively), previous number of IVF cycles (1.4 and 1.7 respectively) and date of retrieval. Of the PGD group we selected those that had only one or two embryos replaced and compared them to their respective controls regarding implantation rates, pregnancy rates and multiple conception rates. The PGD-A method consisted on the biopsy of a single cell per embryo, followed by FISH using probes for 6 to 9 chromosomes (XY, 13, 15, 16, 17, 18, 21, 22).

RESULTS: The Control and PGD groups consisted of 108 cycles each. A total of 396 embryos were replaced in the control (average 3.7 embryos) resulting in a 33% pregnancy rate per retrieval (36/108) and an implantation rate of 12% (47/396). There were two triplet pregnancies in the control group. In contrast, 165 embryos were replaced in the PGD-A group (average 1.5), resulting in 30% (32/108) pregnancy rate and a 24% (40/165) implantation rate, with no triplet pregnancies. While there were no differences in pregnancy rates, the implantation rate was significantly higher in the PGD-A group (p<0.001).

CONCLUSION: The present results indicate that offering PGD and replacing a maximum of two embryos, PGD-A can provide similar pregnancy rates as cycles with unlimited embryos replaced, while reducing the risk of triplet pregnancies. The cost to the system is more than compensated. In this case, the cost of 108 PGD-A cycles would be about $324,000 (at $3000 a cycle) compared to $600,000 for two twin pregnancies. Although two-embryo limit was not applied to the control group it is easy to extrapolate that without PGD the pregnancy rate in the control group would have been halved. Thus, in countries with rules and legislations stressing two embryos replaced, PGD-A should be of great help in order to maintain acceptable pregnancy rates in women 35 and older.

Monday, October 18, 2004
5:15 P.M.

O-76

Application of HLA STRs haplotyping in preimplantation HLA matching. F. Fiorentino, A. Biricik, H. Karadayi, C. Magli, L. Giaroli, S. Kahrman. EMBRYOGEN, Rome, Italy; ISTANBUL MEMORIAL HOSPITAL, Istanbul, Turkey.

OBJECTIVE: Preimplantation Genetic Diagnosis (PGD) of single gene disorders, combined with HLA matching, has recently emerged as a tool for couples at risk of passing on a genetic disease, to select embryos both free of the disease and of a compatible Human Leukocyte Antigen (HLA) tissue type with an existing affected child. In this study, our aim was to optimize flexible preimplantation HLA matching protocol appropriate for the analysis of a wide spectrum of possible HLA genotypes, precluding the design of case specific protocols each time.

DESIGN: An indirect single-cell HLA typing protocol, based on a multiplex fluorescent polymerase chain reaction (PCR) of microsatellite markers located in HLA complex, was designed. The strategy was applied on 23 PGD cycles.
MATERIALS AND METHODS: External and internal primers for amplification of polymorphic STR markers scattered throughout the HLA complex were designed. The primer sequences of the individual STRs were chosen to work in a multiplex fluorescent PCR format, in combination with the other markers and the primers used to identify the disease. A panel of 29 different STRs were studied during the set-up phase to evaluate their informativity for HLA matching in family members (father, mother, and affected child). The PGD strategy involved a multiplex external/nested PCR amplification of the causative mutations. STR markers linked to these regions for ADO detection, and a range of 10–15 informative HLA markers simultaneously from biopsied blastomeres, Mutation analysis was carried out using the minisequencing technique.

RESULTS: The strategy was clinically applied for HLA matching in 23 cycles (20 for β-thalassemia, 1 for Wiscott-Aldrich syndrome and 2 for leukemia) from 19 couples overall involving the testing of 398 blastomeres in combination with a genetic disease, and of 6 blastomeres for HLA matching only. In 377/404 (93.3%) of blastomeres amplification products for at least one locus were produced. A total of 357 blastomeres (94.7%) gave positive amplification for all the loci investigated. Amplification failure for all the markers resulted in 27 (6.7%) blastomeres. A reliable HLA genotype was obtained in 356/357 (94.4%) of the blastomeres with positive PCR results. In total, 32 (15.8%) embryos revealed an HLA match with the affected siblings, 24 (11.8%) of them resulted unaffected and 20 (9.9%) have been transferred back to patients. In 8 (3.9%) embryos, a recombination event occurred; in 3 of these embryos the genotype was still unaffected and HLA compatible, but was not considered for transfer because of recombination event. Aneuploidy of chromosome 6 has also been observed in 17 (8.3%) of the embryos. Five clinical pregnancies were obtained, 2 of them have already born. Two pregnancies are still ongoing and were confirmed as healthy and HLA identical with the affected children by prenatal diagnosis. One pregnancy of a β-thalassaemia case resulted in early abortion.

CONCLUSION: Multiplex PCR of HLA STR markers has revealed a useful diagnostic tool for indirect HLA matching evaluation. The major advantage of this approach is that the validation of a single assay can be performed once and then used for the majority of the patients, reducing notably the time needed for preclinical set-up of each case. The current data confirm the feasibility of preimplantation HLA matching as a part of PGD, providing a realistic option for couples desiring an HLA-compatible child for the treatment of affected siblings.

Supported by: None

Monday, October 18, 2004
5:30 P.M.

O-78


OBJECTIVE: To ascertain if the results of a Preimplantation Genetic Diagnosis (PGD) cycle are predictive for the next. This is important for those patients that fail to conceive in the first cycle due to high rates of chromosomally abnormal embryos.

DESIGN: Retrospective study.

MATERIALS AND METHODS: 152 patients from our database of 2500 cycles from 71 IVF centers were selected following these criteria: a) patients with have two or more cycles in less than two years, more than two years past between the first and second cycle could increase the proportion of abnormal embryos due to age; b) patients which do not involve polar body biopsy considering that post-zygotic abnormalities will not be observed; c) patients which do not involve PGD for single gene defects because the number of repeated cycles was too low for a meaningful analysis; d) patients with more than four embryos analyzed in their cycles, because small cohorts of embryos are difficult to compare. PGD was performed for numerical abnormalities or for translocations following protocols previously published by our center. For numerical abnormalities the PGD procedure consisted of the FISH analysis of eight chromosome pairs (XY, 13, 15, 16, 18, 21, 22 plus either 1, 7, 14 or 17). Abnormalities detectable with this system are monosomies, trisomies, polyploidy, haploidy, chaotic embryos and a fraction of mosaic embryos. For PGD of translocations, usually one or two telomeric probes for the chromosomes involved in the translocation were analyzed with two or one centromeric probes for the same chromosomes, respectively. We defined that the results of a PGD cycle are predictable of the next if there was a difference of 0–20% points in the rate of normal embryos from one cycle to the next. For example, if in one cycle a patient had 30% normal embryos and in the next it had 30% to 50% normal embryos, we would consider for that the PGD results for the first cycle were “predictable” of the second cycle.

RESULTS: The results are reported in Table 1 according to the PGD indication. 

In addition to Table 1, it was found that when all embryos were abnormal in the first cycle, the chance of finding at least one normal one in the second cycle was 95% for PGD of aneuploidy and 66% for PGD of translocations.

CONCLUSION: The results of a PGD cycle of translocation are 90% predictable for the next. This is valuable data for patients with this condition to decide if they want to attempt a new PGD cycle. We have reported in the past that if 70% or more embryos are abnormal for the translocation, it is really difficult to conceive since in addition there is a baseline of 50%