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Current Status of Preimplantation Diagnosis

INTRODUCTION

Preimplantation genetic diagnosis (PGD) has been applied clinically at over 20 centers around the world. More than a hundred unaffected children have already been born following PGD in over 700 clinical cycles performed for single-gene and chromosomal disorders. This progress was reviewed by the 6th Annual Meeting of the International Working Group on Preimplantation Genetics, organized in association with the 9th International Congress of Human Genetics, Rio de Janeiro, August 18, 1996. Major emphasis was on preimplantation diagnosis of chromosomal aneuploidies using FISH analysis.

PREIMPLANTATION DIAGNOSIS OF CHROMOSOMAL DISORDERS

At present more than half of PGD cycles have been performed for age-related aneuploidies. FISH analysis with chromosome-specific probes was used in all cases, as metaphase analysis is still too unreliable to be accepted for clinical application. Using FISH analysis of chromosomes X, Y, 13, 18, and 21, 523 PGD cycles have been performed in 413 couples, mostly over age 35 years. This has resulted in 115 clinical pregnancies, from which 56 normal babies have already been born (Chicago, Cornell, Atlanta, St. Barnabas). PGD has also been applied for translocations in eight patients (St. Barnabas, Barcelona, Bologna, Hammersmith, University College London), resulting in three births of

unaffected children (triplet) and in one ongoing twin pregnancy (St. Barnabas). The following chromosomes were involved in translocations: t(13;14), four cases; t(13;21); t(6;21); t(12;13); and t(12;20).

Despite the obvious progress in PGD of chromosomal disorders, there are still problems to be resolved. There are difficulties in interpretation of high rates of abnormalities in polar body analysis (1-3) and high rates of mosaicism at the cleavage stage (4-6). The possibility of overestimating abnormalities in polar bodies was suggested by the greater number of missing signals compared to the number of extra signals found in the first polar bodies: 6% of abnormal first polar bodies showed missing chromosomes, and 52% showed missing chromatids, compared to 2% showing extra chromosomes and 19% showing extra chromatids (Chicago). Other artifactual results are due to fragmentation of the first polar body in some cases. It is thus important to develop criteria for recording signals in polar bodies, so these clearly differ from those in somatic cells and sperm. The accuracy of genetic analysis of the oocytes may be improved also by testing of both first and second polar bodies. In the presented material only half of the oocytes selected for transfer were tested by both polar bodies; 31% of the oocytes were tested only by the first, and 19% only by the second polar body (Chicago). Even incomplete information on the genotype of the oocytes may increase the chances of avoiding the transfer of abnormal oocytes, particularly when the first polar body is analyzed; however, undetected aneuploidies origi-

nating in either the first or the second meiotic divisions cannot be excluded.

Although most aneuploidies originate in maternal meiosis, it is important to evaluate the paternal contribution to chromosomal abnormalities (7). This cannot be detected by the polar body approach. To this end, review of the data on FISH analysis of human sperm has demonstrated that most autosomes have a similar disomy frequency of approximately 0.1%, with the exception of chromosome 21 (0.3%) (Calgary). Assuming an equal nullisomy frequency, extrapolating results from the limited number of autosomes studied to all autosomes yields a conservative estimate of a 4.8% frequency of aneuploidy. Sex chromosomes have a significantly higher frequency of disomy than autosomes (0.4%). The increased aneuploidy frequency of sex chromosomes and chromosome 21 from FISH data mirrors the results obtained previously by karyotyping. Thus the overall conservative estimate of the frequency of aneuploidy in human sperm is 5.6%. This also corroborates the research on the paternal origin of aneuploidies in human liveborns and spontaneous abortions, which has demonstrated that, depending on the chromosome, 0–17% of autosomal aneuploidies have a paternal origin: The paternal contribution is higher for sex chromosomes (47,XXX, 7%; 45,X, 80%; 47,XXY, 50%; and 47,XYY, 100%). Thus, particularly for sex chromosomes, the possibility of a paternally derived aneuploidy must be kept in mind.

The biological significance of the high frequency of mosaicism observed at the cleavage stage remains unclear. The experience with applying FISH analysis using chromosome specific probes to cells from cleaving embryos, presented in detail at the meeting, demonstrated that a sizable proportion (12%) of mosaicism observed in cleaving embryos is constitutional (St. Barnabas) (6,8). Such embryos plausibly should be avoided in transfer, but it is possible that a considerable proportion may be part of a normal process at this stage. For example, in diploid embryos with one or few tetraploid cells, if a normal cell is biopsied, and the embryos transferred, it will probably not produce an abnormal fetus since tetraploidization seems to be a normal phenomenon in blastocyst formation. But if the tetraploid cell is biopsied, the embryo will not be transferred, regardless of whether few or all the other cells of the embryo are abnormal. Mosaicism is significantly more frequent in dysmorphic embryos, being as high as 43% in arrested embryos (6);

therefore, transferring the embryos with the best morphology already eliminates some mosaic embryos. Another consideration derives from the 12% of clinically significant mosaicism in cleaving embryos having an average percentage of abnormal cells of approximately 40%; thus, the overall risk of misdiagnosis due to mosaicism should not exceed 7%, whatever the fate of the abnormal clones in the mosaic embryo (unpublished data from material in Ref 6). Clearly, more data are needed before appropriate guidelines may be developed regarding the evaluation of biologically significant mosaicism at the cleavage stage.

PREIMPLANTATION DIAGNOSIS OF SINGLE-GENE DISORDERS

Although the global experience with PGD of single-gene disorders had not changed considerably compared to the previous year (9–12), some important developments were reported. First, further data have been presented confirming the importance of the problem of failure of allele-specific amplification, referred to as allele dropout (ADO). A special study performed in preparation for PGD of thalassemias demonstrated different efficiencies of amplification and ADO rates in single cells for different thalassemia mutations: IVS1-110, IVS1-1, IVS1-6, IVS1-2, IVS2-745, and the nonsense mutation in codon 39 (Chicago). Simultaneous detection of the mutant gene together with the two highly polymorphic markers strongly linked to the β -globin gene (STRS at 5' and HUMTHO1) has been introduced to avoid a possible misdiagnosis due to ADO or DNA contamination in performing single-cell DNA analysis for clinical PGD of thalassemias. Single-cell amplification of two thalassemia mutations (IVS1-1 and IVS1-5) was also performed by the Hammersmith group, demonstrating 100% accuracy in homozygous cells and 14% ADO in the heterozygous state.

Additional data have been collected on ADO in CF (Hammersmith). Using spare embryos not selected for transfer in 18 PGD cycles for cystic fibrosis, the efficiency and accuracy of single blastomere amplification were shown to be 91.5%. Errors included embryos diagnosed homozygous unaffected instead of heterozygous carrier and embryos diagnosed normal instead of affected. The accuracy of diagnosis was higher in homozygous normal and affected blastomeres than in heterozy-

gous blastomeres. The overall accuracy of PGD in this series was 91.5%, resulting in six homozygous unaffected healthy births (12–14).

A system of multiplex PCR involving two separate regions of the CF gene (mainly ΔF -508 in exon 10 and a region in intron 6 containing a tightly linked polymorphism to the ΔF -508 mutation) was developed to minimize the potential misdiagnosis in PGD of CF (Chicago). The initial PCR reaction contains two pairs of outside primers. Following the first round of PCR, two separate aliquots are amplified using the inside primers specific for each side. Only when both the polymorphic site and the mutation agree are embryos transferred. This dual amplification allows detection of ADO and prevents transfer of misdiagnosed affected embryos.

Extensive preparatory work was undertaken for PGD of familial adenomatous polyposis coli (APC) (Hammersmith, University College London). It was demonstrated that PCR primers and conditions worked out in single lymphocytes and lymphoblastoid cells were suitable for DNA analysis of single blastomeres. To avoid the risk of misdiagnosis due to ADO, the value of linked polymorphic markers has also been tested following whole-genome amplification by primer extension preamplification. With combined analysis of both specific mutation and linked polymorphism, the mutation was detected in 96% of single carrier lymphocytes, making it possible to undertake one clinical PGD for a couple in whom one partner was a carrier of APC. Only one embryo was diagnosed as normal of four normally fertilized embryos following ICSI. The transfer of this single normal embryo did not result in a pregnancy, while all three embryos not transferred were confirmed to be affected (Hammersmith, University College London).

A two-step approach for polar body diagnosis of single-gene defects was introduced (Chicago). Because the genotype of the embryos that would develop from oocytes heterozygous at the first polar body could not be predicted, sequential analysis of both first and second polar bodies was performed. Follow-up analysis of blastomeres predicted to contain the mutant maternal allele because of a heterozygous first polar body and a hemizygous normal second polar body confirmed the accuracy of the technique. This was applied to couples at risk for CF and sickle cell anemia, resulting in two clinical pregnancies and one birth of an unaffected child.

The first successful lavage clinical cycle for hemophilia A was reported, although the embryo was male and could not be transferred (Baylor). In addition, four PGD cycles were performed at the cleavage stage, one resulting in an ongoing pregnancy following PGD for X-linked Charcot-Marie-Tooth by PCR sexing.

Among other reports demonstrating progress in PGD of single-gene disorders were the following. (a) Thirteen PGD cycles were undertaken for myotonic dystrophy. Since the expanded allele cannot be amplified in this disease, the diagnosis relied on the presence of the healthy allele of the affected parent. The ADO rate in these cycles reached 30% but did not matter because only the presence of the healthy allele of the affected parent would lead to the diagnosis of unaffected embryos and hence to transfer. Unaffected embryos were available for transfer in 11 cycles, but no pregnancy was established (Brussels). (b) Eleven PGD cycles for X-linked diseases were performed, resulting in four ongoing pregnancies (Barcelona). (c) A successful PGD cycle for X-linked hydrocephaly was carried out, resulting in the birth of unaffected twins (Gainesville).

SAFETY OF PREIMPLANTATION DIAGNOSIS

The importance of follow-up of outcomes and long-term surveillance of PGD was noted (Melbourne), leading to a strong recommendation to initiate a follow-up study and establish an International Registry on the safety of PGD, similar to that organized in relation to ICSI. Each PGD center should be following up clinical outcomes and the health status of the children born after PGD and reporting the results of their observations to the International Working Group on Preimplantation Genetics, together with their overall activity on PGD.

FUTURE MEETINGS

The 7th Annual Meeting of the International Working Group on Preimplantation Genetics will be held in June 1997, in association with ESHRE '97. It was also proposed to organize the 2nd International Symposium on Preimplantation Genetics, in Chicago, September 18–21, 1997.

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6th Annual Meeting of The International Working Group
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