Preimplantation Diagnosis: An Alternative to Prenatal Diagnosis of Genetic and Chromosomal Disorders

REPORT OF THE EIGHTH ANNUAL MEETING OF THE INTERNATIONAL WORKING GROUP ON PREIMPLANTATION GENETICS,^{1,2} IN ASSOCIATION WITH THE NINTH INTERNATIONAL CONFERENCE ON PRENATAL DIAGNOSIS AND THERAPY, LOS ANGELES, JUNE 7, 1998

Although prenatal diagnosis has become an integral part of prenatal care and prevention of genetic disorders, it requires termination of affected pregnancies, which is not universally acceptable. Preimplantation genetic diagnosis (PGD) was introduced 8 years ago to detect affected embryos before pregnancy, thus avoiding the need for termination of pregnancies in couples at risk for having children with genetic and chromosomal disorders (1,2). Since the first attempts in 1990, hundreds of PGD procedures have been performed, mainly in the framework of assisted reproduction practices and genetics services, demonstrating an increasing acceptance of PGD in different populations around the world. A recent tendency of wider use of PGD has been due to offer it to in vitro fertilization (IVF) patients of advanced maternal age and application of the fluorescence in situ hybridization (FISH) technique for possible preselection and avoidance of transfer of oocytes and embryos with common aneuploidies and translocations (3,4). The progress in PGD is annually reviewed by meetings of the International Working Group on Preimplantation Genetics, orga-

nized mainly in connection with Assisted Reproduction Technology Conferences and focused on technical issues related to embryology, micromanipulation, and single-cell genetic analysis (5–8). The present Eighth Annual Meeting was held in association with the Ninth International Conference on Prenatal Diagnosis and Therapy, which is of direct relevance to PGD, as PGD is gradually becoming an alternative to prenatal diagnosis.

One of the present features of PGD is that it can be performed, in principle, for any genetic condition for which there is sufficient sequence information. Based on this information, special primers or chromosome-specific probes may be developed for accurate selection of mutation-free embryos to ensure the establishment of a normal pregnancy. Because of apparent specialization of different centers in performing PGD for a particular group of diseases, there is already a need for a specialized directory of centers providing PGD for different groups of diseases. The other important feature is a growing acceptance of PGD of chromosomal abnormalities for assisted reproduction practices, oriented at improving the efficiency of IVF. The present annual meeting reviewed these developments, including (a) recent progress in PGD of chromosomal disorders, (b) advances in improving the accuracy and reliability of PGD, and (c) collaborative efforts in monitoring clinical outcomes of PGD.

The majority of PGD has presently been performed for chromosomal disorders. Overall approximately 900 clinical cycles were done, resulting in almost 200 pregnancies, from which more than 120 healthy children have already been born. Most of these cycles (659 IVF cycles) were performed at the Chicago center, using first and second polar body sampling for patients of advanced maternal age, demonstrating the clinical significance of the procedure for improving the chances of IVF patients to become pregnant (9). The data confirmed a high frequency (43.1%) of aneuploidies in oocytes from IVF patients of advanced maternal age, making obvious the requirement for genetic testing of oocytes in assisted reproduction practices for older women. Although first meiotic division errors were

¹ Participants in the Eighth Annual Meeting of the International Working Group on Preimplantation Genetics: Yury Verlinsky, Anver Kuliev, Joe Leigh Simpson, Luca Gianaroli, Inge Liebaers, Charles Strom, Ian Findlay, Muhterem Bahce, Svetlana Rechitsky, Karen Sermon, Ulli Weier, Joyce Harper, Carmen Marquez, Patrizia Ciotti, Dorit Manor, Jingly Fung, Magnus Nordenskjöld, Caroline Ogilvie, Norman Ginsberg, Jon Sherlock, David Cram, Dagan Wells, Stuart Lavery, David Levran, Igra Vanden Veyver, Pramila Dandekar, Melody White, Hung-Chin Kuo, and Genady Tsukerman.

² Correspondence should be addressed to Yury Verlinsky, Reproductive Genetics Institute, 836 West Wellington Avenue, Chicago, Illinois 60657.

predominant, further evidence was presented for errors originating also from the second meiotic division, making imperative genetic testing of oocytes by both first and second polar body studies. As reported previously the most frequent abnormalities were chromatid errors in the first meiotic division. As expected, the majority of chromosome-13 and -21 errors originated from the first meiotic division. However, most chromosome-18 anomalies were also due to first meiotic division errors, contrary to what was expected from molecular studies of an extra chromosome 18 in postnatal cases shown to originate predominantly from second meiotic non-disjunction. Finally, despite the average mean maternal age of 38.8 years, the pregnancy rate observed (21.3%) was similar to that in younger women, resulting in the overall birth of 88 healthy children.

Similar results were reported from the SISMER center, comparing pregnancy and implantation rates in different age groups, following PGD of aneuploidies, using blastomere biopsy and FISH analysis with specific probes for chromosomes X, Y, 13, 18, and 21 in 114 IVF patients (10). The study showed that IVF patients of ages ranging from 38 to 39 years appeared to be the most appropriate candidates to derive benefit from implementation of PGD. The extension of FISH analysis at the cleavage stage to chromosomes not commonly observed in spontaneous abortions was reported from the St. Barnabas center, providing evidence for a significant contribution of chromosomal abnormalities to embryonic loss prior to recognition of pregnancy (11).

Considerable progress was reported for PGD of translocations from the St. Barnabas center (12,13). Most PGD cycles were performed by first polar body removal and FISH analysis using whole-chromosome painting probes. In all but one cycle, embryos resulting from normal or balanced oocytes were preselected for transfer. The method has recently been improved by the introduction of centromeric or telomeric probes for one or both translocated chromosomes applied simultaneously with whole-painting probes. In a few cycles, PGD for translocations was also performed by blastomere biopsy and specially designed spanning or flanking probes. The accumulated data further confirmed a significant reduction of spontaneous abortions after PGD of translocations. In contrast to this encouraging experience, there was another report on PGD for translocations from University College London demonstrating failed attempts at detection of either normal or balanced embryos for transfer in a number of clinical cycles, performed by blastomere biopsy and the FISH technique.

A status report was presented on the preparation of case-specific probes for detection of normal and derivative chromosomes in interphase cells, to facilitate PGD of translocations if commercial probes for detection of such translocations are not available (13). This is based on routine screening of the insert DNA libraries generated by the International Human Genome Project and isolation of probes for loci of interest to prepare case-specific DNA probes relevant to the chromosomal rearrangements in question. The turnaround time for preparation of such probes is presently 3–4 weeks, with an extra 3 weeks required for optimization in high-performance cases. The cost for preparation of the case-specific probes is still high (\$5,000 per case) but might be reduced with a decreased turnaround time.

Exciting new developments in karyotyping techniques for the first and second polar body were reported. One of these techniques was based on chromosome identification by spectral karyotyping (SKY), reported from the St. Barnabas center (14). A hybridization efficiency as high as 95.2% was achieved in SKY of 63 unfertilized oocytes. A chromosome non-disjunction rate of 13.3%, detected by this method, was also confirmed by sequential FISH analysis, demonstrating that the SKY technique can be applied as an alternative method for karyotyping of the second meiotic metaphase and first polar body chromosomes. A new method for second polar body karyotyping was reported from the Chicago center, which is based on the nuclear transplantation technique combined with the induction of nuclear breakdown by acadaic acid (15).

Improving accuracy and reliability is still one of the important problems for PGD of single-gene disorders. Extensive experience on the optimization of polar body DNA analysis for PGD of different genetic conditions was presented from the Chicago center (16). Because allele dropout (ADO) is the major source of possible misdiagnosis in PGD of single-gene disorders, the emphasis was on the development of approaches for a maximum detection rate of ADO. This was applied in dozens of clinical PGD cycles for cystic fibrosis, α -1-antitrypsin deficiency, retinitis pigmentosa, hemophilias A and B, thalassemias, Alport, Gaucher's, Tay Sachs's and sickle cell disease, long-chain acyl-CoA dehydrogenase deficiency, and epidermolysis bullosa. Most of these cycles resulted in embryo transfer, and approximately a quarter resulted in a clinical pregnancy. PGD for each of these mutations was performed following preclinical single-cell polymerase chain reaction (PCR) experiments, to develop a multiplex

nested PCR system for detection of ADO in the polar body or blastomere analysis. It was demonstrated that the risk of misdiagnosis due to ADO may be significantly reduced by introduction of sequential analysis of the first and second polar body in combination with multiplex PCR, involving simultaneous amplification of the gene and linked polymorphic markers. With two or more polymorphic markers in the PCR, depending on their availability, as much as 100% of ADO may be detected, excluding the risk of misdiagnosis in PGD. This was applied in 48 clinical cycles, resulting in embryo transfer in 44 cycles and clinical pregnancy in 17 of them. Overall, more than 600 oocytes were studied by sequential analysis of both the first and the second polar body for as many as 15 loci, with a 96% diagnostic accuracy rate.

The possibility of a high ADO detection rate in single-cell PCR analysis was also reported from the Leeds center, using fluorescent PCR (17). Using this method, DNA fingerprinting was applied to individually isolated blastomeres. In addition to mutation analysis (CFTR gene) and sex determination, fluorescent PCR analysis of six STR markers was performed. An ADO rate as high as 30% was detected in blastomeres for multiplex trisomy detection, compared to 20% ADO for multiplex DNA marker analysis. Simultaneous DNA fingerprinting was performed for markers on as many as eight chromosomes, providing the possibility for simultaneous sexing and determination of single-gene defect status, as well as testing for chromosomal aneuploidies. Reliability was 70–75% for trisomy multiplex analysis, compared to 80–85% for multiplex DNA fingerprinting. Neither ADO rates nor reliability of analysis depended on the fragment size tested. This makes fluorescent PCR techniques useful for application in PGD for multiple disorders.

Similar results were reported from University College London, by applying fluorescence PCR for multiplex mutation assay, including ΔF -508 deletion, β -thalassemia, myotonic dystrophy, sex determination, and aneuploidy detection. The method was reliably performed on as few as five cells, with a very low ADO rate but 25% preferential amplification. Further work was also performed for optimization of the whole genome amplification technique for production of sufficient copies of the initial templates for a large number of subsequent specific locus amplifications, but the method has yet to be tested for clinical application.

Experience with PGD of so-called dynamic mutations, such as myotonic dystrophy and Huntington's disease, was presented from the Brussels center. As the expanded allele of the affected parents is not ampli-

fied with an appropriate primer design, embryos with healthy alleles were selected for transfer, using initially conventional and then fluorescence PCR. Of 37 PGD cycles for myotonic dystrophy and Huntington's disease, resulting in the transfer of 64 unaffected embryos, three pregnancies were established in cases of PGD for myotonic dystrophy. Overall, the experience of this center includes PGD for single-gene and chromosomal disorders in 136 treatment cycles in 65 patients, resulting in 113 transfers (80%), 23 pregnancies, and 14 healthy children born, following confirmation of the diagnosis.

PGD oriented at a particular group of diseases was continued in Cyprus (18). Of 17 clinical cycles performed in nine patients at risk of having children with thalassemia, embryo transfer was possible in all but 1 cycle, based on nested multiplex PCR and sequential first and second polar body analysis. The work resulted in the establishment of seven pregnancies, with five healthy children born thus far.

Considerable progress was also reported from other PGD centers. The Rambam Medical Center explored the possibility of performing PGD at the blastocyst stage. However, of 75 embryos obtained from patients with repeated reproduction failures, only 25 (33%) reached the blastocyst stage and were transferred back to the uterus in 10 patients, resulting in three pregnancies. As many as 78% of embryos arrested at different stages of preimplantation development appeared to be chromosomally abnormal, which may explain the low percentage of embryos reaching the blastocyst stage and the primary difficulties these couples encountered in obtaining fertilization and pregnancy.

The University of Bologna center reported encouraging results involving the optimization of freezing/thawing procedures to obtain a better survival rate following PGD on thawed embryos presently performed at this center for X-linked diseases by the FISH technique. At the Baylor center PGD of blood type RhD was further refined, which presently involves nested PCR amplification with allele-specific primers for RhD and RhCE genes as an internal control. Progress in applying PGD was reported from centers in Stockholm (for translocations) and Clayton (sex determination and aneuploidies).

Although the code for PGD practice and licensing is not a problem in most countries, this has become the major obstacle for the development of PGD in the United Kingdom. A number of the centers there, which have previously contributed to the development of the procedure, were recently unable to apply PGD until the licensing process has just been completed.

Two approaches for assessing fetal outcome after PGD were proposed, using standard forms and definitions. One of these approaches was too detailed to be applied in practice, while the other was simple enough for realization but will require the incorporation of additional parameters to ensure sensitivity in picking up anomaly rates following polar body or blastomere biopsy. In approximately 200 children presently born following PGD performed worldwide, no particular anomaly seemed unusually prevalent. However, international standardized monitoring of anomaly rates after PGD will be of importance to avoid certain pitfalls enumerated, in the absence of required standardization in cohort or retrospective studies.

Although preimplantation testing of transcriptionally relevant abnormalities was not among the main issues under discussion, progress in some related areas is obvious from analysis of the stage-specific patterns of genetic expression (19) and mechanisms of genetic control of preimplantation development (20).

Finally, it was proposed that the next International Symposium on Preimplantation Genetics will be organized in Europe in the year 2000.

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Yury Verlinsky
Reproductive Genetics Institute
Chicago, Illinois