30. Handyside AH: Abnormalities of human preimplantation development in vitro. *Reprod Fertil Dev* 1992;4:481-495

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Progress in Preimplantation Genetics¹

At the conclusion of the First International Symposium on Preimplantation Genetics, Chicago, Illinois, September 14-19, 1990, statements and predictions were made, most of which appeared to be true. There were also some concerns, for example, regarding the accuracy and reliability of diagnoses based on single-cell genetic analysis and ethical and legal problems, which still exist. However, some other predictions appeared to be quite the opposite, because of dramatic technological developments, such as in situ hybridization techniques.

It would be fair to state that the past 7 years have been quite fruitful. More than 1200 Preimplantation Genetic Diagnosis (PGD) clinical cycles have been performed, versus a few dozen in 1990, by approximately three dozen centers all over the world. The number of PGD cycles was increasing steadily, but a particular jump was observed after the FISH technique was introduced for sex selection and aneuploidy detection in 1994. What is most encouraging is that 166 healthy children have been born, suggesting the reliability and safety of the procedure. It was agreed that a standardized follow-up study of the outcomes of PGD had to be established, to collect a representative sample for evaluating the possibility of long-term effects.

As predicted, there is a high risk of misdiagnosis due to possible DNA contamination and PCR failures, which has led to at least four misdiagnoses.

This has encouraged the development of sophisticated systems for controlling and avoiding misdiagnosis, using multiplex, nested, and fluorescence PCR. That ICSI has been widely introduced to PGD cases has helped to avoid possible contamination by sperm DNA. ICSI has become a well-established procedure for assisted fertilization and seems to have no short-term or long-term side effects, however, more data are needed on the possible contribution of ICSI to well-documented paternally derived sex chromosomal and, possibly, autosomal abnormalities.

A concern raised regarding possible recombinations at the first meiotic division, which might complicate the first polar body analysis, appeared to be true. As predicted, PGD cannot be performed without sequential analysis of the corresponding second polar body. Moreover, it was demonstrated that a reliable diagnosis can be made only in those cases in which crossover occurred, making it possible to follow-up both alleles through meiosis I and II, eliminating the possibility of undetected ADOs. On the other hand, sequential analysis of heterozygous oocytes by the second polar body not only makes it possible to pick up the resulting hemizygous normal oocytes, but also confirms the diagnosis of the abnormal ones.

The other observation concerning polar body diagnosis is that this approach appears extremely useful for PGD of translocations. It may also be noted that more than half of all PGD cycles have presently been performed by polar body analysis.

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In contrast to previous predictions, the age-related risk of common aneuploidies not only became an indication for PGD, but is presently the most common application of PGD, accounting for more than 60% of PGD cycles performed. The second largest application of PGD was sex selection for X-linked disorders, which is performed mainly by application of the FISH technique.

Despite predictions, uterine lavage and blastocyst biopsy have not been widely used. However, to what extent PGD based on trophectoderm biopsy would be representative for the embryo should still be demonstrated in light on the recent data about mosaicism.

The observation of mosaicism in cleaving embryos is, of course, one of the most intriguing subjects, as it may provide useful information for the understanding of early stages of human preimplantation development. As preliminary data show, the improvements of in vitro culture conditions will probably eliminate a certain proportion of mosaicism that is nonconstitutional and may be controlled; however, it will be of interest to follow the dynamics of the karyotypes of embryos resulting from oocytes with known genotypes, those tested by polar body analysis and not transferred because of being abnormal.

Many interesting questions have also been raised by FISH analysis in human gametes. It is clear that sequential analysis of the first and second polar bodies makes it possible to avoid the transfer of embryos resulting from aneuploid oocytes. The question is whether the observed high frequency is related to the FISH technique, micromanipulation, and/or IVF procedure. Follow-up results clearly show that more than two-thirds of the abnormalities detected in the first and second meiotic divisions by FISH are real events. In contrast to previous knowledge in this field, the majority of chromosomal aneuploidies in the egg is due to chromatid errors, observed at a high frequency at both the first and the second meiotic divisions. The introduction of a cocktail of FISH probes in the future will help us to investigate if several chromosomes are simultaneously involved in aneuploidy due to errors in the processes of spindle formation, which, according to the data presented, is very sensitive also to factors external to the oocyte and, particularly, to increased maternal age. In the meantime, improvement of the FISH technique and the introduction of other accurate methods for cytogenetic analysis will allow more information about the types and

frequencies of errors in the first and second meiotic divisions, and their diagnostic significance, to be acquired.

With the introduction of the FISH technique, a lot of data about the aneuploidy frequency in human sperm, which is approximately 6%, have been collected. It is well documented that the distribution is different for chromosome 21 and sex chromosomes. Thus, the paternal contribution to chromosomal aneuploidy in humans varies for different autosomes and sex chromosomes, the latter accounting for more than 50% of the paternal contribution. The data corroborate with studies of the outcomes of ICSI, demonstrating an increased number of sex chromosome aneuploidies in these patients. The observations of an increased disomy frequency for autosomes 1, 13, and 21 in infertile men has considerable clinical importance, as the application of ICSI may lead to the production of trisomic fetuses, suggesting strong indications for preimplantation or prenatal diagnosis in these patients.

The clinical significance of PGD for chromosomal aneuploidies is another important topic for further investigation. Although it seems quite obvious that preselection of normal embryos from the outset should considerably improve pregnancy and delivery rates, and also decrease the frequency of spontaneous abortions, the only way to test this hypothesis is to perform a randomized clinical trial, which is being planned at the present time.

Because the nature of age-related aneuploidies is still not clear, the progress of in vitro maturation of immature oocytes may make it possible to test whether in vitro matured oocytes have fewer age-related aneuploidies than those matured in vivo. Presentations made at the symposium clearly demonstrated that although it is still an experimental procedure, it is approaching the stage where the conditions of culture could be well controlled to synchronize the timing of nuclear and cytoplasmic maturational changes and achieve reproducible results in immature oocyte maturation in vitro.

Finally, considerable progress has been achieved in developing approaches for the study of genetic expression in mammalian preimplantation development, which will, of course, stimulate the research of genetic control in human preimplantation development. The development of experimental systems for the disruption of encoding of the genes known or suggested to be expressed at certain stages will be of particular importance, as demonstrated for

connexin genes and the genes involved in the plasma membrane sodium pump in the mouse. In addition to the antisense disruption, the use of knockout mice was of great importance for the understanding of the mechanisms of action of the *Ped* gene, the analogue of which may be expressed in a similar way in humans. Although progress in this field in humans has been limited, cDNA libraries have already been constructed from individual human preimplantation embryos and will definitely facilitate work in the future on testing the presence of known sequences suggested to play an important role in early preimplantation development, such as homeobox-containing genes (reviewed in an elegant way at the Symposium) and many others well studied in experimental systems.

It is understood that following recent publications about cloning, federal support of preimplantation genetics research is not foreseen to be substantial in the near future. However, because PGD will actually make preventive measures more ethically acceptable, there seem to be no obstacles for future developments in the field, to improve and apply this technique more widely in avoiding the risk of having affected children in couples at genetic risk.

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