EDITORIAL

Preimplantation Genetics¹

YURY VERLINSKY² and ANVER KULIEV²

Yury Verlinsky

The present Special Issue is based on the materials from the Second International Symposium on Preimplantation Genetics, Chicago, September 18–21, 1997. In contrast to the previous Special Issue in 1996, which was devoted to the deliberations of the 5th Annual Meeting of the International Working Group on Preimplantation Genetics (1), the present one is much broader in its contents and includes the progress in basic and clinical research in preimplantation genetics, as well as the experience in the application of preimplantation genetic diagnosis (PGD) to clinical practice. Approximately 400 participants from as many as 30 countries gathered at the Symposium to discuss the most recent developments in preimplantation genetics and the problems to be resolved in the near-future. Because PGD provides a very valuable option for genetically disadvantaged people to control the outcome of their reproduction from the very outset, instead

of facing the risk for abortion following prenatal diagnosis, PGD is currently applied all over the world, except for the African region. As many as 40 PGD centers have been established in 17 countries, including the United States, the United Kingdom, Belgium, Italy, Spain, Australia, Israel, Russia, Belarus, The Netherlands, South Korea, Sweden, Colombia, Cyprus, Finland, Greece, and Brazil. At the time of the Symposium, 28 of these centers from as many as 12 countries were involved in applying PGD clinically and had performed approximately 1200 clinical cycles, resulting in 231 pregnancies (20% pregnancy rate) and the birth of 166 healthy children. Although most of these clinical cycles have been performed by a few well-established centers, such as Chicago, Saint Barnabas, Brussels, and London, a handful of centers have now performed more than a dozen clinical cycles. More than two-thirds of clinical cycles have been performed in the United States, with the largest number (640 cycles) contributed by the Chicago center.

Although preimplantation diagnosis was initially offered to couples at high risk for having children with single-gene disorders, more than half of the cases have been performed for age-related aneuploidies (more than 700 cycles). The list of conditions for which PGD was performed is rapidly being extended and currently, in addition to age-related aneuploidies, includes cystic fibrosis, Tay–Sachs disease, hemophilia A and B, retinitis pigmentosa, sickle cell disease, thalassemia, Alport disease, α -1-antitrypsin deficiency, fragile X, Duchenne muscular dystrophy, Lesch Nyhan syndrome, myotonic dystrophy, rhesus (Rh D), Marfan's syndrome, familial adenomatous polyposis coli, Huntington's disease, and X-linked diseases by sex determination. Many centers presently concentrate on chromosomal aneuploidies and sex selection, using

¹ This Editorial reflects the personal opinions of Drs. Verlinsky and Kuliev and does not necessarily reflect the opinion of either this journal or the publisher.

² Reproductive Genetics Institute, Illinois Masonic Medical Center, 836 West Wellington Avenue, Chicago, Illinois 60657.

the fluorescent in situ hybridization technique, while others also perform single-cell polymerase chain reaction (PCR) analysis for single-gene disorders. On the other hand, some centers are involved in PGD of a particular group of conditions, such as PGD for thalassemias in Cyprus (2).

Because not all the papers presented at the Symposium could be included in this volume, only selected contributions have been considered, which, however, represent fully those areas that were discussed at the Symposium. First of all the paper by Van Blercom (3) summarizes the results of the author's longstanding research work on a possible effect of preovulatory maturation on the chromosomal normality of oocytes. These data have great potential significance for the development of methods for avoiding age-related aneuploidy. Another paper in this introductory section is devoted to genetic manipulation by nuclear transfer, which describes the original work by Wolf's group on the production of rhesus monkeys by nuclear transfer from preimplantation-stage embryos, in the light of recent reports on cloning in adult animals (4).

The next section consists of comprehensive analyses of the paternal and maternal contribution to chromosomal abnormalities in humans. The paper by Martin presents available data on cytogenetic analysis in human sperm, which is based heavily on the author's original work in the field (5). In addition to documenting the overall 6% paternal contribution, the paper presents strong evidence for an increased frequency of sex chromosome aneuploidy, as well as for aneuploidy of autosomes 1, 13, and 21, in infertile men, which has immediate clinical implications with the widespread introduction of intracytoplasmic sperm injection in assisted reproduction practices.

The possible nature of age-related aneuploidies in human oocytes is discussed in Hunt's paper, also using the author's original work in this field (6). The major emphasis of the paper is errors in the processes of spindle formation, which are very sensitive to increased maternal age and factors external to the oocyte. The paper demonstrates clearly the role of spindle formation errors in the origin of aneuploidies with age.

The next two sections of the volume are devoted to actual PGD and its clinical application at different centers. A group of papers describes the problems and results of PGD of single-gene disorders. Because at least four misdiagnoses have already been reported, indicating a high risk of misdiagnosis due to possible DNA contamination and PCR failures, three papers concentrate on the current developments in detecting, controlling, and avoiding misdiagnosis, using multi-

plex, nested, and fluorescence PCR (7-9). A paper by Rechitsky *et al.* (7) presents original observations on allele dropout rates in single fibroblasts, blastomeres, and polar bodies and approaches introduced in actual single-cell diagnosis, including simultaneous detection of the mutant genes and linked polymorphic markers and sequential analysis of the first and second polar body. Two papers by Finlay *et al.* (8,9) present other significant improvements in the accuracy and reliability of DNA analysis in single cells by the use of fluorescent PCR. Another paper discusses the possibility of misdiagnosis due to mosaicism, which is one of the major problems when PGD is performed at cleavage stage (10). Finally, one paper in this section is devoted to PGD of Marfan's syndrome (11).

The section on PGD of chromosomal aneuploidies makes up one of the largest groups, reflecting the increasing proportion of cases presently performed for patients of advanced maternal age undergoing in vitro fertilization (IVF). Although PGD for chromosomal abnormalities was introduced as recently as 3 years ago, more than 700 cycles have been performed for this indication only, resulting in approximately 150 pregnancies and more than a hundred births of healthy children. The largest series have been performed using polar body analysis (12,13), involving more than 500 clinical cycles, the others being performed by blastomere biopsy (14-20); it is of interest that at least 25 cycles were performed for couples carrying balanced translocations, resulting in the birth of 10 unaffected children (13). A lot of new information has emerged from these data, requiring careful consideration, such as a high frequency of chromosomal aneuploidy in oocytes and preimplantation embryos, a high frequency of mosaicism at the cleavage stage, the clinical significance of preselection of aneuploidy-free oocytes and embryos, and the long-term safety of the application of micromanipulation and biopsy procedures in this early preimplantation development. Based on the study of thousands of human oocytes, using the first and second polar bodies, which allows us to follow sequential errors in the first and second meiotic divisions, it was demonstrated that sequential analysis of both polar bodies makes it possible reliably to predict and avoid the transfer of embryos resulting from aneuploid oocytes. In contrast to previous knowledge in this field, most detected aneuploidies were due to chromatid errors in the first meiotic division. The simultaneous involvement of more than one chromosome in meiotic errors and the significant number of oocytes with sequential errors in both meiotic divisions support the above-mentioned role of spindle formation errors

with age. The other observation on the relationship between errors in human meiosis and postzygotic errors in the cleaving embryo must be explored further in the future.

Finally, the last section of the issue is devoted to genetic expression in preimplantation development; they present mouse data on expression of the PED gene (21) and data on the construction and evaluation of human individual libraries from preimplantation embryos (22,23). Because of the high relevance of the expression of homeobox genes to preimplantation development the last paper in this section is devoted to the evolutionary classification of homeodomains (24).

In conclusion, this Special Issue presents the current status of human preimplantation genetics research activities, as well as the results of clinical application of PGD. The data show that, since its introduction in 1990, PGD has become a practical option for couples at high risk of producing children with single-gene and chromosomal disorders. Because the present measures for prevention of congenital diseases are based heavily on prenatal diagnosis and termination of pregnancy, a number of ethnic groups cannot consider the organization of preventive services. Thus, in these communities, PGD is the only way to test and avoid genetic disease before pregnancy. PGD will also be of great use for couples who have experienced two or more terminations of pregnancies following prenatal diagnosis. As the data presented demonstrate, PGD will be of particular significance for assisted reproduction practices to improve the efficiency of IVF in couples of advanced maternal age.

REFERENCES

1. Special Issue on Preimplantation Diagnosis. *J Assist Reprod Genet* 1996;13:87-192
2. Kuliev A, Rechitsky S, Verlinsky O, Ivakhnenko V, Evsikov S, Wolf G, Angastiniotis M, Geoghiou D, Kukhareno V, Strom C, Verlinsky Y: Preimplantation diagnosis of thalassemia. *J Assist Reprod Genet* 1998;15:219-225
3. Van Blerkom J: Epigenetic influences on oocyte developmental competence: Perifollicular vascularity and intrafollicular oxygen. *J Assist Reprod Genet* 1998;15:226-234
4. Wolf D, Meng L, Ely JJ, Stouffer RL: Recent progress in mammalian cloning. *J Assist Reprod Genet* 1998;15:235-239
5. Martin RH: Genetics of human sperm. *J Assist Reprod Genet* 1998;15:240-245
6. Hunt PA: The control of mammalian female meiosis: Factors that influence chromosome segregation. *J Assist Reprod Genet* 1998;15:246-252
7. Rechitsky S, Strom C, Verlinsky O, Amet T, Ivakhnenko V, Kukhareno V, Kuliev A, Verlinsky Y: Allele dropout in polar bodies and blastomeres. *J Assist Reprod Genet* 1998;15:253-257
8. Findlay I, Corby N, Rutherford A, Quirke P: Comparison of FISH, PRINS, and conventional and fluorescent PCR for single cell sexing: Suitability for preimplantation genetic diagnosis. *J Assist Reprod Genet* 1998;15:258-265
9. Findlay I, Tóth T, Mathews P, Marton T, Quirke P, Zoltán P: Rapid trisomy diagnosis (21, 18, and 13) using fluorescent PCR and short tandem repeats: Applications for prenatal diagnosis and preimplantation genetic diagnosis. *J Assist Reprod Genet* 1998;15:266-275
10. Kuo HC, Ogilvie C, Handyside A: Chromosomal mosaicism in cleavage-stage human embryos and the accuracy of single cell genetic analysis. *J Assist Reprod Genet* 1998;15:276-280
11. Blaszczyk A, Tang YX, Dietz HC, Adler A, Berkeley AS, Krey LC, Francomano C, Grifo JA: Preimplantation genetic diagnosis of human embryos for Marfan's syndrome. *J Assist Reprod Genet* 1998;15:281-284
12. Verlinsky Y, Cieslak J, Ivakhnenko V, Evsikov S, Wolf G, White M, Lifchez A, Kaplan B, Moise J, Valle J, Ginsberg N, Strom C, Kuliev A: Preimplantation diagnosis of common aneuploidies by the first and second polar body FISH analysis. *J Assist Reprod Genet* 1998;15:285-289
13. Munné S, Morrison L, Fung J, Márquez C, Weier U, Bahçe M, Sable D, Grundfeld L, Schoolcraft B, Scott R, Cohen J: Spontaneous abortions are reduced after preconception diagnosis of translocations. *J Assist Reprod Genet* 1985;15:290-296
14. Magli MC, Gianaroli L, Munné S, Ferraretti AP: Incidence of chromosomal abnormalities from a morphologically normal cohort of embryos in poor-prognosis patients. *J Assist Reprod Genet* 1998;15:297-301
15. Boada M, Carrera M, De la Iglesia C, Sandalinas M, Barri PN, Veiga A: Successful use of the laser for human embryo biopsy in preimplantation genetic diagnosis: Report of two cases. *J Assist Reprod Genet* 1998;15:302-307
16. Manor D, Stein D, Itskovitz-Eldor J: Preimplantation diagnosis by FISH: The Rambam experience. *J Assist Reprod Genet* 1998;15:308-309
17. Vidal F, Giménez C, Rubio C, Simón C, Pellicer A, Santaló J, Egozcue J: FISH preimplantation diagnosis of chromosome aneuploidy in recurrent pregnancy wastage. *J Assist Reprod Genet* 1998;15:310-313
18. Smith SE, Toledo AA, Massey JB, Kort HI: Simultaneous detection of chromosomes X, Y, 13, 18, and 21 by fluorescence in situ hybridization in blastomeres obtained from preimplantation embryos. *J Assist Reprod Genet* 1998;15:314-319
19. Bowman M, de Boer K, Cullinan R, Catt J, Jansen R: Do alterations in the sex ratio occur at fertilization? A case report using fluorescence in situ hybridization. *J Assist Reprod Genet* 1998;15:320-322
20. Fung J, Hyun W, Dandekar P, Pedersen R, Weier HUG: Spectral imaging in preconception/preimplantation genetic diagnosis of aneuploidy: Multicolor, multichromosome screening of single cells. *J Assist Reprod Genet* 1998;15:323-330
21. Warner CM, McElhinny AS, Wu L, Cieluch C, Ke X, Cao W, Tang C, Exley G: Role of the *Ped* gene and apoptosis genes in control of preimplantation development. *J Assist Reprod Genet* 1998;15:331-337
22. Morozov G, Verlinsky O, Rechitsky S, Kukhareno V, Golts-

- man E, Ivakhenko V, Gindilis V, Strom C, Kuliev A, Verlinsky Y: Sequence analysis of libraries from individual human blastocysts. *J Assist Reprod Genet* 1998;15:338–343
23. Adjaye J, Daniels R, Monk M: The construction of cDNA libraries from human single preimplantation embryos and their use in the study of gene expression during development. *J Assist Reprod Genet* 1998;15:344–348
24. Gindilis V, Goltsman E, Verlinsky Y: Evolutionary classification of homeodomains. *J Assist Reprod Genet* 1998;15:349–358