

PREIMPLANTATION GENETIC DIAGNOSIS

INTRODUCTION

1. Inherited genetic diseases have been a dreaded problem for some families attempting to conceive a child. If affected parents or carriers of genetic disorders wished to avoid transmitting a condition to their child, they can choose to have prenatal diagnosis of their fetus. Amniocentesis or chorionic villus sampling enables cells from the fetus to be collected and sent for genetic analysis. They could then choose to terminate the pregnancy if the fetus is affected.
2. Preimplantation genetic diagnosis (PGD) is the prevention of the birth of affected children in couples at genetic risk by sampling and genetic testing of nuclear material obtained from blastomeres or polar body biopsy of the embryo thus enabling selection and transfer of only normal embryos to achieve a normal pregnancy and birth of a healthy baby. In this way, couples do not have to experience the agony of aborting affected fetuses.

BACKGROUND

3. The first clinical PGD was reported by Handyside and co-workers¹ who described the sexing of preimplantation embryos at risk for sex-linked disease by performing embryo biopsy at the cleavage stage and sexing with Y-specific DNA amplification. A few years later, the introduction of fluorescent in situ hybridization (FISH), a method in which fluorescent labeled, chromosome-specific probes are hybridized to metaphase or interphase chromosomes were reported, allowing sexing of embryos as well as aneuploidy screening². Single gene disorders have been diagnosed with the polymerase chain reaction (PCR). DNA analysis is performed either on biopsied blastomeres or on sampled first and second polar bodies.

BIOPSY METHODS

(i) Polar Body Biopsy

4. The first and second polar bodies contain the complementary genotype to the oocyte. To remove the polar bodies, the oocyte is held with a holding pipette with the polar body at the 12 o'clock position. Using a sharp needle, a slit is made in the zona pellucida tangentially to the polar bodies. With a thin pipette, the polar bodies are removed from under the zona and transferred to a PCR tube or glass slide for analysis.

(ii) Cleavage Stage Biopsy

5. This is the most widely used technique. The advantage of cleavage stage biopsy is that the genetic constitution of the embryo is completely formed and thus comparable to genetic material obtained at prenatal diagnosis. Embryos are usually obtained after intracytoplasmic sperm injection (ICSI). This avoids contamination with sperm, which is important when PCR is used and reduces the possibility of failure of fertilization with insemination. A hole is made in the zona pellucida of the embryo by applying Acid Tyrode's solution or using a laser. A pipette is inserted through the hole and one blastomere is aspirated and removed from the embryo for analysis. Diagnosing one or two cells isolated from 8-16 cell embryos may occasionally fail to detect mosaicism.

METHODS OF DNA ANALYSIS

(i) In Situ Hybridization

6. In situ hybridization permits the analysis of genetic material of a single nucleus in metaphase or interphase, by incubating a fixed dried cell with a specific probe, which binds to the gene of interest. The gene probe is labeled with fluorescent markers (FISH) and allows numerical chromosome analysis.

7. The advantage of FISH is that, since the cells do not have to be in metaphase, interphase nuclei and even arrested cells can also be analysed. The choice of appropriate probes allow the exact identification of the chromosomes. Unfortunately, only limited numbers of chromosomes can be analysed at one time. However, new developments in the near future eg. Comparative genomic hybridization (CGH), spectral karyotyping (SKY) and DNA chips will allow analysis of all chromosomes.

(ii) Polymerase Chain Reaction

8. Polymerase chain reaction (PCR) allows amplification of well-defined DNA sequences enzymatically in an exponential way. The boundaries of the amplified fragment are determined by a couple of primers which anneal to the denatured template DNA and which then form the starting point of a DNA polymerase to synthesize the complementary strand. The gene of interest is thus amplified for identification.

9. Contamination is an important problem in single-cell PCR : when the sample contains only two copies of the DNA under investigation, one copy of extraneous DNA can lead to misdiagnosis. Two sources of contamination can be distinguished. The first, from cellular sources, contains whole genomic DNA, while the second is carry-over contamination from products of former PCR reactions.

10. Another problem encountered with PCR is allele drop-out (ADO) where an affected allele may fail to amplify during PCR. ADO would create a particular problem

for the correct diagnosis of autosomal dominant diseases if the affected allele would fail to amplify and in compound heterozygotes when autosomal recessive diseases were concerned³.

INDICATIONS

11. Although PGD is an early form of prenatal diagnosis, it will not be an alternative for chorionic villus sampling or amniocentesis in all cases. There are several situations in which PGD would be beneficial:

- (i) In parents who have a genetic diseases or are carriers and have concurrent fertility problems necessitating treatment with IVF
- (ii) Some parents have personal histories of prenatal diagnosis followed by termination of pregnancy for affected fetuses. Some may feel they cannot cope with another failure and would prefer IVF and PGD
- (iii) Another group of patients have moral, emotional or religious objections to termination of pregnancy and see PGD as the only way to have unaffected children

CURRENT STATE OF THE TECHNIQUE

12. Since the first report of clinically applied preimplantation genetic diagnosis¹, the numbers of fertility centers performing PGD and the numbers of PGD treatments have risen steadily.

13. The European Society of Human Reproduction and Embryology (ESHRE) formed a PGD Consortium in 1997 to study the long-term efficacy and clinical outcome of PGD. Their latest published report includes data from 886 couples, 1318 PGD cycles and 162 babies⁴. The data was collected from 27 in-vitro fertilization (IVF) centers who are actively practicing PGD (Table 1). Apart from these centres involved in the Consortium, other centres in the USA, Russia, Belarus, Colombia, Cyprus, Finland, Jordan and Turkey are performing PGD.

14. Apart from aneuploidy diagnosis, several genetic diseases have been tested for. These include autosomal dominant, autosomal recessive and sex-linked disorders (Table II).

15. The data for PGD for aneuploidy screening showed that a total of 6025 oocytes were retrieved, a fertilization rate of 62% was achieved, biopsy was successful in 99% of cases and 63% of embryos undergoing FISH had a diagnosis. Only 36% of embryos were deemed suitable for transfer.

16. The data for PGD of inherited disorders showed that from a total of 10267 oocytes collected, a fertilization of 63% was attained, 81% of embryos were suitable for

biopsy and 96% were successfully biopsied. The diagnosis was obtained in 86% of these biopsied embryos and 43% were suitable for transfer. From the initial number of oocytes collected, only 18% were finally diagnosed as suitable for transfer, which confirms the need for the retrieval of large numbers of oocytes for a successful PGD cycle.

PROBLEMS ENCOUNTERED WITH PGD

17. Couples wishing to avail themselves to PGD will have to undergo in-vitro fertilization (IVF). This involves time, expenses and at the end of a cycle, the uncertainties of success at a pregnancy. It is a process of decreasing numbers as the embryos diagnosed as suitable for transfer will be few.

18. The possibility of a misdiagnosis will be dependent on the experience, care and technical expertise of analysis. Sources of error include mosaicism, contamination of DNA material for PCR and allele drop-out. Hence, most centres still recommend that couples having PGD undergo a confirmation test with prenatal diagnosis.

FUTURE APPLICATIONS OF PGD

19. In future, improved genetic and DNA analysis techniques will improve the accuracy of diagnosis of the preimplantation embryo. There will also be more genes that can be identified and some other applications would include diagnosis of Mendelian disorders using linked polymorphic markers and structural chromosomal abnormalities using centromeric and telomeric probes.

20. As deranged chromosome complements have been identified in first trimester pregnancy failures, aneuploidy screening and transfer of euploid embryos may in future be used to improve assisted reproductive technology (ART) success especially in older patients with repeated IVF failures and recurrent abortions.

21. It is possible that with improved genetic diagnosis, other less fatal or debilitating genetic disorders may be presented as choices for PGD eg. HLA screening, BRCA gene testing for cancer predisposition.

*Dr. Christine Yap
Consultant
Department of Obstetrics & Gynaecology
Singapore General Hospital*

REFERENCES

1. Handyside AH, Kontogianni EH, Hardy K, Winston RML (1990). Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature*; 344:768-770.
2. Griffin DK, Handyside AH, Penketh RJA, Winston RML, Delhanty JDA (1991). Fluorescent in-situ hybridization to interphase nuclei of human preimplantation embryos with X and Y chromosome specific probes. *Hum Reprod*; 6:101-105.
3. Ray P, Winston RML, Handyside AH (1994). Single cell analysis for diagnosis of cystic fibrosis and Lesch-Nyhan syndrome in human embryos before implantation. *Miami Bio/Technology European Symposium, advances in Gene Technology : Molecular Biology and Human Genetic Disease*; 5:46.
4. ESHRE PGD Consortium Steering Committee (2000). ESHRE Preimplantation Genetic Diagnosis Consortium : data collection II (May 2000). *Hum Reprod*; 15(12):2673-2683.

TABLE I. Centres Involved in ESHRE PGD Consortium

1	Sydney IVF
2	University of Adelaide
3	Melbourne IVF
4	Centre for Medical Genetics, VUB, Brussels
5	Centre for Preimplantation Genetic Diagnosis, Aarhus University Hospital, Aarhus
6	Hopitaux Beclere et Necker, Paris
7	Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg
8	St Sophia's Childrens Hospital, University of Athens
9	IVF and Genetics, Athens
10	Department of O&G, Rambam Medical Centre, Haifa
11	IVF and Infertility Centre, University of Bologna
12	SISMER, Bologna
13	PGD Working Group, Maastricht
14	Stichting Klinische Genetica Zuid-Oost Nederland, Maastricht
15	Department of O&G, Samsung Cheil Hospital, Sungkyankwan University, Seoul
16	Instituto Dexeus, Barcelona
17	Unitat de Biologia Cellular, Univ. Autonomia, Barcelona
18	Department of Clinical Genetics, Karolinska Hospital, Stockholm
19	Assisted Conception Unit, St. Thomas' Hospital, London
20	Department of O&G, University College, London
21	Institute of O&G, RPMS, Hammersmith Hospital, London
22	School of Biology, University of Leeds, Leeds
23	Department of O&G, Baylor College of Medicine, Houston, Texas
24	Department of O&G, University of Florida, Florida
25	Jones Institute for Reproductive Medicine, Norfolk, Virginia
26	New York University Medical Center, New York
27	Institute of Reproductive Medicine and Science, St Barnabas Medical Center, New Jersey

TABLE II. Genetic diseases that have been tested with PGD

<ul style="list-style-type: none"> • Autosomal recessive 	<ul style="list-style-type: none"> • Cystic fibrosis • Beta-thalassaemia • Spinal muscular atrophy • Tay-Sachs disease • Rh Isoimmunization • Gaucher disease • Sickle cell anaemia
<ul style="list-style-type: none"> • Autosomal dominant 	<ul style="list-style-type: none"> • Myotonic dystrophy • Huntington's disease • Charcot-Marie-Tooth disease • Neurofibromatosis type I • Marfan syndrome • Osteogenesis imperfecta
<ul style="list-style-type: none"> • Sex-linked 	<ul style="list-style-type: none"> • Duchenne and Becker's muscular dystrophy • Haemophilia • Fragile-X syndrome • Mental retardation • Wiskott-Aldrich syndrome • Charcot-Marie-Tooth • Retinitis pigmentosa

