PREIMPLANTATION GENETIC DIAGNOSIS

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Introduction

Inherited genetic diseases have been a 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 foetus. Amniocentesis or chorionic villus sampling enables cells from the foetus to be collected and sent for genetic analysis. They could then choose to terminate the pregnancy if the foetus is affected.

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 foetuses.

Background

The first clinical PGD was reported by Handyside and co-workers[1] 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 hybridisation (FISH), a method in which fluorescent labelled, chromosome-specific probes are hybridised to metaphase or interphase chromosomes were reported, allowing sexing of embryos as well as aneuploidy screening.[2] 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

Polar Body Biopsy

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.

Cleavage Stage Biopsy

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 fertilisation 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

In Situ *Hybridisation*

In situ hybridisation 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 labelled with fluorescent markers (FISH) and allows numerical chromosome analysis.

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 allows 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, e.g. comparative genomic hybridisation (CGH), spectral karyotyping (SKY) and DNA chips will allow analysis of all chromosomes.

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.

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.

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 concerned.[3]

Indications

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 foetuses. 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

Since the first report of clinically applied preimplantation genetic diagnosis[1], the numbers of fertility centres performing PGD and the numbers of PGD treatments have risen steadily.

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 1318 PGD cycles and 215 babies.[4] The data was collected from 25 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.

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

The data for PGD for the years 1999-2001 showed that a total of 5985 oocytes were retrieved, a fertilisation rate of 62% was achieved, 48% were suitable for biopsy, biopsy was successful in 99% of cases and 85% of embryos had a diagnosis. Pregnancy rate was 19% per oocyte recovery and 23% per embryo transfer.

Problems Encountered with PGD

Couples wishing to avail themselves to PGD will have to undergo 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.

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.

Single cell genetic analysis of cleavage stage embryos is susceptible to extrinsic technical errors as well as intrinsic errors related to nuclear and chromosomal abnormalities (Table III). Several misdiagnoses have been reported in the literature. As errors can arise from diverse causes, it is clinically important to develop a model so that patients can be accurately counselled about the risks of misdiagnosis. This model should include source of variation from the cell chromosomes, recombination, contamination and amplification. Data on the frequency of haploid, diploid or more complex mosaic cells can be obtained through FISH studies. About 90% of cells have both parental chromosomes (diploid and tetraploid cells) and 10% of cells lack at least one parental chromosome.

Future Applications of PGD

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.

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 success rates, especially in older patients with repeated IVF failures and recurrent abortions.

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

Guidelines and Licensing

Legitimate concerns about potential misuse of embryo screening and selection make it essential that a sustained public debate about these issues occurs as technical progress

continues. Some of the discomfort that surrounds new uses of PGD stems from a sense in many countries that there is no effective oversight of its development and use.

In the UK, the Human Fertilisation and Embryology Authority (HFEA) has legal authority over which clinics are licensed to do PGD and for what indication. Additional uses of PGD may occur only if the HFEA is satisfied that the uses are within statutory guidelines and the clinic program is qualified to undertake the work. In addition, the HFEA uses a public consultation process to assess public attitudes and draw up guideline for new uses. The HFEA has provided a regulatory model that other countries could emulate.

In the US, no agency exists at the state or federal level that plays a role comparable to the HFEA. How PGD is used and for what indications is thus left largely to the discretion of providers offering those services and the patients who seek it.[6]

References

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4. ESHRE PGD Consortium Steering Committee (2002). ESHRE Preimplantation Genetic Diagnosis Consortium: data collection III (May 2001). Hum Reprod; 17(1):233-246.

5. Lewis CM, Pinel T, Whittaker JC, Handyside AH. Controlling misdiagnosis errors in preimplantation genetic diagnosis: a comprehensive model encompassing extrinsic and intrinsic sources of error (2001). Hum Reprod; 16(1):43-50.

6. Robertson JA. Extending preimplantation genetic diagnosis: the ethical debate (2003). Hum Reprod; 18(3):465-471.

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	ULB Erasme, Brussels
6	Centre for Preimplantation Genetic Diagnosis, Aarhus University Hospital, Aarhus
7	Hopitaux Beclere et Necker, Paris
8	Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg
9	St Sophia's Childrens Hospital, University of Athens
10	IVF and Genetics, Athens
11	SISMER, Bologna
12	PGD Working Group, Maastricht
13	Stichting Klinische Genetica Zuid-Oost Nederland, Maastricht
14	Department of O&G, Samsung Cheil Hospital, Sungkyankwan University, Seoul
15	Instituto Dexeus, Barcelona
16	Unitat de Biologia Cellular, Univ. Autonoma, Barcelona
17	Department of Clinical Genetics, Karolinska Hospital, Stockholm
18	Sahlgrenska University Hospital, Goteborg
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	Department of O&G, Baylor College of Medicine, Houston, Texas
23	Jones Institute for Reproductive Medicine, Norfolk, Virginia
24	New York University Medical Center, New York
25	Institute of Reproductive Medicine and Science, St Barnabas Medical Center, New Jersey

Table II. Genetic diseases that have been tested with PGD

Autosomal recessive	• Cystic fibrosis • Beta-thalassaemia • Spinal muscular atrophy • Tay-Sachs disease • Rh Isoimmunisation • Gaucher disease • Sickle cell anaemia
Autosomal dominant	• Myotonic dystrophy • Huntington's disease • Charcot-Marie-Tooth disease • Neurofibromatosis type I • Marfan syndrome • Osteogenesis imperfecta
Sex-linked	• Duchenne and Becker's muscular dystrophy • Haemophilia • Fragile-X syndrome • Mental retardation • Wiskott-Aldrich syndrome • Charcot-Marie-Tooth • Retinitis pigmentosa

Table III. Summary of Potential Diagnostic Errors with PGD using PCR

Source of Diagnostic Error	Possible Cause
Extrinsic errors: alleles	
Amplification failure	PCR failure
Allele drop-out	Degradation of target DNA
Extrinsic errors: contamination	
Related DNA	Maternal cumulus cells or paternal sperm DNA
Unrelated DNA	DNA in reagents or operator DNA
Carry-over DNA product	Amplified products
Intrinsic errors : nuclear abnormalities	
Binucleate	Failure of cytokineses or abnormal karyokinesis
Multinucleate	Abnormal karyokinesis
Anucleate	Cytoplasmic fragmentation
Intrinsic errors : chromosomal abnormalities	
Haploid	2nd polar body
Tetraploid	Failure of karyokinesis or derivation from binucleate cells
Higher order polyploidy	Endoreduplication/endomitosis
Aneuploidy	Non-disjunction, chaotic chromosomal segregation or chromosome loss