Potential usefulness of preimplantation genetic diagnosis in the control and prevention of genetic diseases

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SUMMARY Prenatal diagnosis of molecular mutations can be of immense value, since diagnosis followed by genetic counselling provides the most appropriate approach to genetic diseases control and prevention. However, ethical, psychosocial and religious considerations hamper adoption of prenatal diagnosis in communities where termination of a pregnancy may not be acceptable. Recently, preimplantation genetic diagnosis has attracted considerable interest. This involves *in vitro* fertilization, followed by genetic disorder diagnosis using polar bodies or cells extracted from a blastomere stage. The normal blastomere is implanted in the womb and pregnancy proceeds naturally. If an abnormality is diagnosed, the blastomere is not implanted, thus preventing pregnancy with the affected fetus. This paper outlines the potential usefulness of preimplantation genetic diagnosis in the control and prevention of genetic disease in our part of the world.

Introduction

Genetic diseases can have devastating effects on both patients and their families alike, and on the community at large. Efforts have been made at various levels to prevent and control genetic diseases. Preventing the birth of a child with genetic disease (intervention at the primary level) is the preferred approach. This involves such steps as screening of carriers and groups at risk, genetic counselling and prenatal diagnosis and aborting the abnormal fetus [*1*]. This latter strategy is associated with serious ethical dilemmas. Despite its high success rate in several countries in preventing different genetic diseases [*2*], in some communities it is not considered acceptable.

Preimplantation genetic diagnosis (PGD) is defined as "an approach to diagnose a genetic defect by *in vitro* body biopsy, blastomere or blastocyte analysis following *in vitro* fertilization (IVF)" [*3,4,14–16*]. If found to be normal, the fertilized embryo is implanted in a uterus and allowed to proceed normally. Thus PGD tests for a genetic disease before pregnancy.

PGD was first undertaken in 1989 to diagnose an X-linked disease [*3*], followed in 1990 to diagnose an autosomal recessive condition [*4*]. A number of children have since been born who were conceived fol-

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lowing this procedure [5–11]. With developments in molecular biology, several genetic conditions can now be diagnosed with precision using DNA from a single cell. Hence, the use of PGD in clinical practice is growing and its future appears bright [4,12,13]. The steps in PGD can be summarized as follows [14–16].

- Induction of superovulation to produce several follicles according to standard IVF methods;
- Micromanipulation to remove the first or second polar bodies or to biopsy cleavage-stage embryo (for X-linked disorders) or blastomere;
- Performing of genetic analysis by fluorescence *in situ* hybridization (FISH) or polymerase chain reaction (PCR) on the biopsied polar bodies or single blastomere.

PGD involves single-cell genotyping [12]. This has been perfected for a number of diseases and involves the use of PCR. The steps involved in single-cell genotyping are summarized in Figure 1 [12–16].

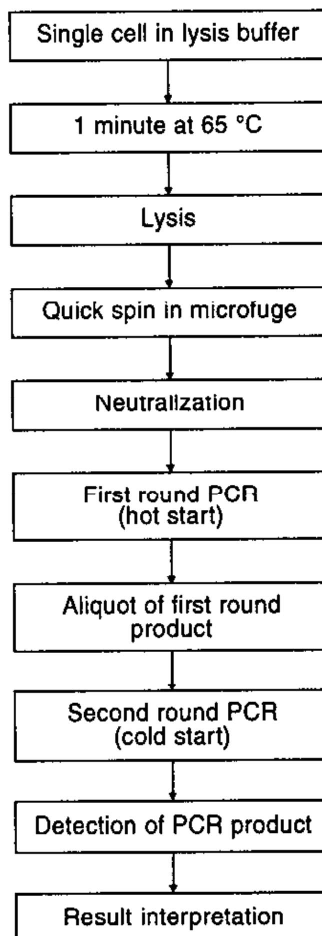


Figure 1 **Steps for the DNA genotyping of a single cell**

Detailed steps in PGD

Initial procedures

Follicular stimulation is brought about by injection of human chorionic gonadotropin (HCG). Oocytes are recovered by ultrasound-guided aspiration and are grown in culture. The oocyte preparation is then subjected to micromanipulation. The first polar body is removed for biopsy and genetic diagnosis. The oocyte is subjected to intracytoplasmic sperm injection for zygote formation. The second polar body is removed for another genetic diagnosis. The zygote is allowed to grow to the six–eight-cell blastomere stage and one or two cells are removed by biopsy for analysis. The DNA from the cells is removed and subjected to PCR using special primers to amplify the gene or DNA region for the condition under investigation. These steps are schematically presented in Figure 2.

Polar body analysis

For single-gene disorders, the first and second polar bodies are analysed along with the cells from the oocyte. The strategy is

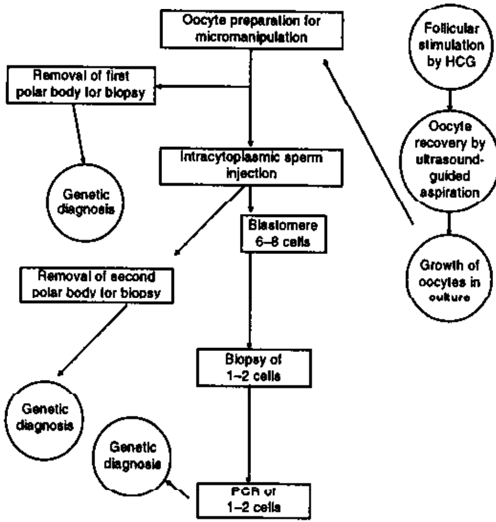


Figure 2 Schematic presentation of the detailed steps in preimplantation genetic diagnosis

depicted schematically in Figure 3. If a cell is heterozygous in meiosis I, then the first polar body will either be normal or affected. If normal, the oocyte will be affected after meiosis II and the second polar body will also be affected. On the other hand, if the first polar body is affected, the oocyte will be normal. This can be confirmed by analysis of the second polar body, which will be normal. If crossing-over has taken place, then the first polar body, like the oocyte, will be heterozygous for the abnormal condition and confirmation will depend on the analysis of the second polar body, which if affected, will mean that the oocyte is normal (Figure 3).

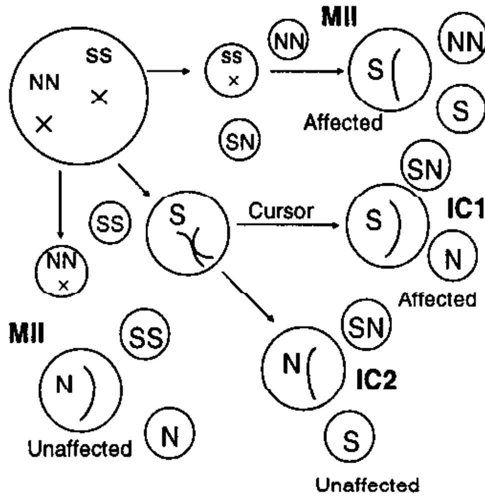
According to the experiences of some investigators [*8,17*], the polar-body-inferred PGD appears to be accurate in predicting genotype and selecting unaffected oocytes for fertilization and transfer [*11–18*].

Molecular biology techniques useful in PGD

Developments in molecular biology technology have played a major role in advancements in PGD. These advances have made it possible for a single cell to be used to diagnose genetic abnormalities, either single- gene or chromosomal, with efficiency and accuracy [*11,18*].

The analysis of DNA from a single cell has been possible as a result of an effective amplification technique known as polymerase chain reaction (PCR) [*19*]. Using specific primers, particular regions of the genome can be amplified to provide millions of copies of the DNA region of interest. This can then be tested by various mutation detection techniques such as dot blot analysis, denaturing gradient gel electrophoresis (DGGE), single-strand conformational polymorphism, reverse dot blot, amplification refractory mutation system (using mutation specific primers) and others. PCR coupled with automated fluorescent detection of the PCR product by a highly sophisticated gene scanner has also greatly increased the reliability of genetic analysis [*20*]. Comparative genomic hybridization, a technique originally developed for molecular cytogenetic analysis of tumours, also seems to hold much promise and great potential for PGD [*21*].

The combination of PCR and molecular cytogenetic methods has been of significant value in the study of chromosomal anomalies [*17–26*]. This involves fluorescence in situ hybridization (FISH) analysis of the chromosomal anomalies following PCR in the same cell fixed to a small piece of glass, to detect single-gene defects. Cytogenetic analysis of the first and second polar body chromosomes using FISH has proven reliable for the diagnosis of trisomies, and for



N = normal chromosome; S = sickle-cell carrying chromosome; MI and MII represent meiosis I and II. If the first polar body is normal and the second is affected, the oocyte is affected. If the first polar body is heterozygous and the second is normal, the oocyte is affected. If the first polar body is heterozygous and the second is affected, the oocyte is normal. If the first polar body is affected (homozygous) and the second is normal, then the oocyte is normal.

Figure 3 **Schematic presentation of the strategy for polar body analysis**

sex selection using chromosome specific signals [*21–26*].

Difficulties associated with PGD

Although PGD has been applied successfully in many centres in different countries, and many children have been born free of genetic diseases screened through PGD, there are several difficulties associated with the technology. These include:

- misdiagnosis of chromosomal anomalies due to mosaicism;
- restricted ability to provide confirmation due to limited availability of genetic material;
- high rate of fetal loss;
- higher frequency of chromosomal abnormalities;
- possible contamination with sperm;
- high cost of the procedure;
- frequent requirement for confirmation by chorionic villus sampling or DNA amniocentesis;
- limited number of centres performing PGD;
- high failure rate of transferred embryo to result in pregnancy;
- low pregnancy rate, especially in women of advanced reproductive age;
- unavailability of probes for several diseases;
- high rate of crossovers;
- risk of multiple pregnancies;
- the (still) experimental nature of the technique.

Advantages of PGD

Preimplantation genetic diagnosis has several advantages These include:

- an expanded treatment choice for high-risk couples;
- elimination of the need for pregnancy termination;
- reduced risk of miscarriage associated with prenatal diagnosis;
- a means by which couples can avoid the heartbreak of having a child with a genetic disease;
- fewer ethical considerations;
- a reduction in psychosocial trauma associated with genetic diseases.

Potential usefulness of PGD in prevention and control of genetic disorders

The list of diseases which have been successfully diagnosed during PGD is growing. Single-gene and chromosomal disorders have been successfully diagnosed, and as new primers, probes and techniques are standardized, further increases in the number of diseases diagnosed by this technique are anticipated. Some of the diseases diagnosed using PGD are shown in Box 1.

PGD is therefore set to play a significant role in the control and prevention of genetic diseases. Because the diagnosis is made prior to conception, PGD gives rise to fewer ethical, psychosocial and religious concerns. It has been successfully applied in a large number of high-risk populations for the diagnosis of several diseases. As a result, large numbers of children have been born free of any genetic defect and are living healthy lives.

Box 1 **Examples of genetic diseases diagnosed by preimplantation genetic diagnosis**

Single gene disorders
- Sickle-cell disease
- Thalassaemia
- Cystic fibrosis
- Tay–Sachs disease
- Lesch-Nyhan syndrome
- α1-antitrypsin deficiency
- Duchenne muscular dystrophy
- Retinitis pigmentosa
- Myotonic dystrophy
- Familial adenomatous polyposis coli
- Haemophilia, A and B
- Fragile X syndrome

Chromosomal abnormalities
- Numerical
  - Trisomy 21
  - Trisomy 13
  - Trisomy 18
- Kleinfelter syndrome
- Turner syndrome
- Structural

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