Preimplantation genetic diagnosis

Preimplantation genetic diagnosis is a treatment for genetic disease which uses assisted conception to create embryos free from disease, which are available for implantation. This article gives an up-to-date review of an increasingly important area of medicine.

Preimplantation diagnosis (PGD) has now become an established technique as an alternative to prenatal diagnosis (PND) for couples carrying genetic conditions that may affect their offspring. PGD has some clear advantages over PND as it allows the diagnosis of an unaffected embryo before implantation, thus avoiding an affected pregnancy. It is offered in a few specialized centres worldwide and requires close collaboration between clinicians and scientists in the assisted conception unit, molecular biologists, cytogeneticists, clinical geneticists and clinicians from other specialities (Braude et al, 2002).

Before the advent of PGD there were few options for couples at risk of transmitting genetic disorders. These included accepting the risks of the condition and hoping for favourable odds, PND (amniocentesis or chorionic villous sampling) and then being prepared to terminate an affected pregnancy, the use of donor gametes, adoption or deciding to remain childless. In addition, for X-linked conditions, there is now the possibility of using fluorescent-activated cell sorting to separate X and Y bearing spermatozoa from the semen. Although this process is not totally reliable, it may be used to skew the result in favour of an unaffected pregnancy.

Why is PGD used?

Patients choose PGD for a variety of reasons. They may already have an affected child, have lost affected children, or experienced affected family members as a result of the genetic condition and wish to avoid further risk. Couples may have had several terminations of affected pregnancies or they may have personal, including religious, objections to termination of pregnancy. They may have suffered multiple miscarriages as a result of a chromosome rearrangement and see PGD as an option to reduce the risk of further miscarriage. Some may carry a dominant disorder and are either already affected or will become affected by that condition, and so wish to avoid the same fate for their offspring and future generations (Grace et al, 2004).

In order for a preimplantation genetic test to be performed, a representative sample of the embryo is required. A single cell can be removed from a late cleavage stage embryo (8–16 cells), a few cells may be taken from the trophectoderm of a blastocyst, or one or both of the polar bodies can be removed from the unfertilized egg or early zygote. All couples regardless of their fertility status have to undergo assisted reproductive techniques (ART) in order to obtain unfertilized eggs or embryos.

PGD is offered for X-linked disorders, chromosomal arrangements and single gene disorders. In X-linked conditions such as Duchenne muscular dystrophy, haemophilia, Hunter syndrome and incontentia pigmenti where a specific test at the single cell level is not available, couples may opt to have sex selection of the embryos using fluorescence in-situ hybridization (FISH) in order to replace female embryos only, thereby preventing the condition. This does not eliminate the disease from the family in the future as half of these female embryos may be carriers and the fact that half the non-transferred male embryos could be normal may raise ethical objections. In general, this test is robust, as misdiagnosis would have to involve two errors; loss of a Y signal and gain of an X.

PGD can be used to detect a variety of chromosomal arrangements such as translocations, inversions, deletions or insertions on interphase nuclei using FISH. Reciprocal translocations (where there is an exchange of terminal segments from two different chromosomes) occur in about 1 in every 500 of the general population. They are more common in infertile couples (0.6%), men with oligoasthenoteratospermia (3%) and in couples with repeated ART failures (3.2%). In fertile couples with three or more consecutive first trimester miscarriages they are present in almost 10% of cases. A carrier of a balanced reciprocal translocation is phenotypically normal but 50–70% of their gametes are likely to be unbalanced. Since each translocation is unique the specific effects of the translocation such as miscarriage, affected liveborn risk and reproductive failure will be particular to the specific chromosomes involved and their breakpoints. The most common indication for PGD for translocations is to prevent repeated miscarriage. Other causes of miscarriage such as antiphospholipid syndrome or intrauterine abnormalities must first be excluded as they cannot be prevented using PGD but may be present in addition to the chromosome rearrangement.

In single gene disorders such as cystic fibrosis, spinal muscular atrophy, sickle cell disease, Huntingdon disease and myotonic dystrophy where a specific mutation, which may be recessive or dominant can be detected, the molecular abnormality is tested, following amplification by polymerase chain reaction (PCR) of DNA extracted from single cells (Sermon et al, 2004).

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Disadvantages of PGD

PGD requires ART in couples who may not be subfertile and has associated risks, including ovarian hyperstimulation syndrome, infection, bleeding and the complications of multiple pregnancy. The success rate of this procedure (around 20% chance of livebirth) is lower than routine ART and for many couples the choice of conceiving naturally may give them better odds of an unaffected child. For couples with a history of recurrent miscarriage the chance of a livebirth following spontaneous conception in the next pregnancy is in excess of 65%, so the decision to use PGD may be debatable.

In the UK funding for PGD is not centrally organized. So for couples who are unsuccessful in their application for health authority funding, the cost is around £5–6000 per cycle.

As PGD is performed on a single cell or small number of cells it can only test for the condition affecting the couple. In PND many conditions can be tested allowing the detection of other spontaneous abnormalities. PGD is still a novel technique, the first pregnancy reported after PGD with PCR for an X-linked condition was in 1990 and the first live birth in 1992. The long-term effects of removing one or more cell that decreases the cellular mass of the embryo are unknown. The complications of pregnancy have been shown to be similar to intracytoplasmic sperm injection and there is no significant increase in neonatal complications. An increased rate of multiple pregnancy including triplets from double embryo transfer have occurred. Sequelae have not been fully evaluated.

The technology of single cell analysis is complex and demanding and misdiagnoses has been reported. Misdiagnoses in the PCR group, commonly a result of allele drop out (ADO) or amplification failure, are quoted as higher than those in the FISH group. The possibility of misdiagnosis in the PCR cases (around 4–7%) can be decreased by the use of multiplex fluorescent PCR techniques. Here simultaneous amplification of two or more loci, one containing the mutation and one or more containing informative polymorphic markers in close proximity to the mutation (acting as a minifingerprint), confirms the embryonic origin of the DNA hence the chance of ADO and risk of misdiagnosis is reduced (Sermon, 2002).

Ethical issues

The application of PGD is expanding rapidly and with it creating controversies and ethical dilemmas. According to UK HFEA (Human Fertilisation and Embryology Authority, 2003) guidelines PGD licences should only be considered if the genetic condition is serious, the recurrence risk is significant and PND would be considered under usual circumstances.

Some centres around the world are offering PGD for sex selection for couples with a preference for one sex over the other. This may be when only one child is allowed per family, or where male offspring are favoured over female for cultural or economic reasons. Alternatively it may be performed for 'family balancing' where there are already one or more children of the same sex in the family and there is a desire for a child of the other sex. However, as it is still an expensive technique that requires substantial expertise and equipment, it is not widely available and thus has little chance of altering the sex ratio compared to abortion or feticide, which is still practiced in a number of countries even though it is outlawed, such as the Indian subcontinent and Asia.

Preimplantation human leukocyte antigen (HLA) matching has been used to ensure that an embryo to be replaced after in-vitro fertilization (IVF) and PGD will be a suitable tissue match for an affected sibling, where it is intended that cord blood will be harvested at birth for use in bone marrow cell therapy (Verlinsky et al, 2004).

The first such case born was from an embryo tested not only to exclude it being affected by Fanconi anaemia, but that infant born should be a suitable match for his sibling who suffered from the condition and required a bone marrow transplant. In these situations a large number of embryos are likely to be discarded as only three in 16 embryos will both be unaffected by the recessive disorder and be a full HLA match. Several PGD cycles might have to be performed to achieve a match let alone a successful pregnancy. This method has also been applied for several other conditions including thalassaemia, hyperimmunoglobulin M syndrome, X-linked adrenoleukodystrophy and Wiskott–Aldrich syndrome. HLA typing without PGD in the absence of a high-risk genetic transmissible disease is even more controversial. Here there may be no pre-existing genetic condition to be avoided, but the PGD performed with the sole objective of pre-selecting HLA matched progeny for cell therapy treatment of siblings. Although the likelihood of match is higher (1 in 4 embryos is likely to be a suitable match), 75% of the created essentially normal embryos will be unsuitable and discarded. Issues of consent and protection of the children's autonomy are raised, especially should the cord blood fail to yield sufficient stem cells or if the cell therapy should fail.

The application of FISH as well as other more sophisticated techniques has been extended to detect embryos that contain major sporadic chromosomal or age-related aneuploidies that may result in failure of implantation or spontaneous miscarriage, and to remove then from the cohort available for transfer (*Figure 1*). This technique is variously called preimplantation genetic screening (PGS) or aneuploidy screening (PGD-AS). During PGS, individual embryos are biopsied, and single blastomeres are examined using between 5 and 14 FISH probes (most commonly X, Y, 13, 15, 16, 17, 18, 21 and 22). The technique has so far been advocated for advanced maternal age, repeated IVF failure and recurrent miscarriage (Munne, 2003).

To date there are no randomized controlled trials examining the outcome of PGS in terms of livebirth per cycle started and evidence at present suggesting that there is any benefit is poor. This is an expensive and so far unproven test that is taken up by women desperate to gain a pregnancy when this test might actually decrease their chances of pregnancy by reducing the cohort of normal embryos available for transfer. There does not appear to be an indication for PGD-AS for couples with unexplained miscarriage when examined in a prospective cohort study (Platteau et al, 2005).

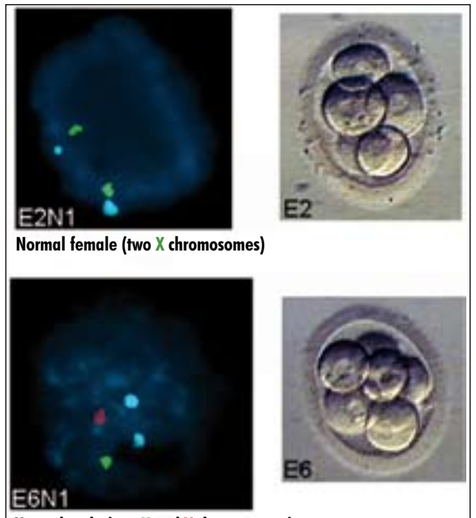

Figure 1. Fluorescent in-situ hybridization for a sexing case, where fluorescent probes have been hybridized onto the X chromosome (green), the Y chromosome (red) and chromosome 22 (blue). Embryo 2 has two green signals signifying the presence of two X chromosomes hence diagnosing the embryo as female. Embryo 6 has one red and one green signal confirming the presence of one Y and one X chromosome hence diagnosing the embryo as female. The presence of two chromosome 22s confirms that each embryo is diploid.

New developments

The ideal is to be able to perform analysis of the whole genome and several techniques have been developed. Comparative genomic hybridization (CGH) is where molecular techniques are used to perform a quantitative analysis of all 46 chromosomes, so some embryos diagnosed as normal can be diagnosed as abnormal for other aneuploides. However, at present this technique requires 2–3 days for diagnosis, and is therefore unsuitable for routine use on cleavage stage embryos or blastocysts which need to be cryopreserved until the diagnosis has been made. Several ongoing pregnancies and live births have been reported, but at present the damage caused by the freezing and thawing of biopsied embryos probably outweigh the benefits of CGH (Wilton, 2005).

Faster more robust techniques such as DNA microarrays may remove the need for embryo freezing. This is a method of molecular analysis primarily used for gene expression analysis. However, it could also be applied to routine PGD to screen for mutations in any one gene, or screening several genes for several mutations. Microarrays might also be useful in PGD of specific conditions that are genetically heterogeneous and for which there are few common mutations such as Duchenne muscular dystrophy, where it could provide a generic testing procedure applicable to all patients carrying the gene. The ability to screen mutations in one gene or several mutations for different genes would allow embryos to be tested for serious susceptibility traits loci, such as the breast and ovarian cancer susceptibility gene (BRCA1) (Wells, 2004).

The desire to undergo PGD in order to minimize the risks of an affected pregnancy in couples is fully appreciated. Preparation of the couple requires substantial workup both clinically and in the laboratory. It is essential for couples to be counselled before undergoing a PGD cycle of the potential pitfalls, the risks and chance of disappointment with the procedure. The application of PGD has been extended to more controversial uses raising moral and ethical issues. Rapid advances in molecular genetics will allow efficient examination of the entire chromosomes of the embryo. **BJHM**

Conflict of interest: none.

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KEY POINTS

- Preimplantation genetic diagnosis (PGD) has become increasingly available as a method of detecting genetic disorders before pregnancy is established as an alternative to prenatal diagnosis and termination of pregnancy.
- Preliminary information and results so far suggest that PGD is probably safe and reliable.
- Although most couples requesting PGD do not have fertility problems, they will require assisted reproductive technology to create embryos for testing in the laboratory.
- The procedure is complex and expensive and the success rate is low (20% livebirth rate per cycle started).
- The range of genetic conditions for which PGD is offered is constantly increasing and new molecular techniques will allow rapid and efficient analysis of the whole genome.