PREIMPLANTATION GENETIC DIAGNOSIS

Technology that allows couples with a family history of monogenic disorders, x-linked diseases and chromosomal abnormality to have a healthy baby.

About Preimplantation genetic diagnosis

Pre-implantation genetic diagnosis (PGD) allows couples with a family history of monogenic disorders, x-linked diseases and known chromosomal abnormality to avoid the transfer of embryos with these specific genetic disorders. The first preimplantation diagnosis was performed in 1989 for sex selection due to an X-linked disease. Currently, there are an estimated 10,000 children who were born after preimplantational biopsies.

PGD essentially consists of several steps:

1. ovarian superstimulation
2. aspiration of ovarian follicles
3. oocyte retrieval
4. intracytoplasmic injection of oocytes with processed sperm
5. in vitro culture of fertilized oocytes
6. blastomere biopsy on D3 or trophectoderm biopsy on D5
7. genetic testing
8. transfer of a genetically normal embryo

If the blastocyst is not transferred to a receptive uterus until the 5th or 6th day, it loses the ability to produce an embryo. To preserve this possibility, it must be vitrified for later transfer.
Indications of PGD

Indications are similar to conventional prenatal diagnosis with regard to:

a) genetic risks with monogenic or chromosomal causes
b) major predisposition to tumors
c) non-genetic risks
d) selection of the best embryos in IVF laboratories

As PGD involves both an IVF or intracytoplasmic sperm injection (ICSI) procedure and a genetic study, it is mandatory to predict the number of unaffected embryos obtainable for transfer prior to realizing the procedure. The number depends on the embryogenic potential of the fertilized oocytes and the implicated risk according to the genetic disorder. The embryogenic potential depends mainly on the woman's age and the absence of factors that facilitate the production of incompetent gametes. Generally, when a woman is younger than 35 years and the male produces good quality sperm, the embryogenic potential is approximately 50%. The embryogenic potential decreases when a woman's age increases or when sperm is of inferior quality. However, the genetic risk depends on the type of disorder (recessive, dominant, sex-linked) or if the disorder is chromosomal. Table 1 shows the estimated number of embryos needed to have the chance to transfer some unaffected embryos, based on the reasoning of PGD.

PGD technology has several primary applications:

1) Single gene disorders

a) Recessive monogenic disorders

Examples of recessive disorders are congenital disorders such as cystic fibrosis, Tay-Sachs, and thalassemia, which involve two mutated chromosomes from each healthy carrier parent. When the disorder is molecularly characterized, the mutation may be analyzed in cells removed from a cleavage embryo or blastocyst. Minisequencing is the method of choice. However, when the mutation is not known, it might be determined by a linkage study.

In cases where the mutation has not been identified in one of the parents, the use of polymorphic markers linked to the gene of interest could help to provide a better diagnosis and allow to have more transferable embryos; otherwise, embryos carrying the known mutation would be considered as affected when they could be healthy carriers. Today, with the availability of SNParrays, the characterization of individual mutations is no longer needed.
b) Dominant monogenic disorders

Examples of autosomal dominant disorders are myotonic dystrophy, fascioscapulohumeral dystrophy, retinoblastoma, Von Hippel Lindau, MEN I and II, Huntington's disease, osteogenesis imperfecta, and achondroplasia.

When the patient has a "de novo mutation" it is necessary to sequence the entire gene to identify the mutation. Once the mutation has been characterized, this sequence can be targeted in the cells removed from the embryo. In contrast, when there are several affected members in the family, PGD can also be addressed with polymorphic markers linked to the respective gene.

Usually, Huntington's disease develops late in life or when the offspring are of child-bearing age. Many of them do not want to perform the genetic study because they do not want to know their genetic status in advance, but they want to make sure that their children do not have the mutation. Unlike prenatal diagnosis, PGD for Huntington's disease avoids disclosure of the status of the carrier of the mutation.

It is well known that people with certain genetic disorders live in communities, such as mute communities for congenital deafness or persons with achondroplastic dwarfism, and that these couples desire PGD to increase their likelihood of having similarly affected offspring. This is a situation in which it is difficult to satisfy the parents because the medical team cannot help them.

c) Sex linked genetic disorders

X-linked disorders are transmitted by the healthy carrier mothers to their sons, while the affected males transmit the condition to their grandchildren through their healthy carrier daughters but not through their sons. When the mutation is characterized, it is recommended to perform PGD by minisequencing the mutation. Some reprogeneticists carry out embryo sexing to avoid the birth of males, in such cases, but, this is not recommended. Examples of recessive X-linked diseases are hemophilia, Fragile X, and Duchenne muscular dystrophy.

In contrast, dominant X-linked diseases are transmitted by affected women to 50% of their daughters and sons, but affected males do not transmit it to their sons. Examples of diseases linked dominant X are Rett syndrome, incontinentia pigmeni, pseudohyperparathyroidism, and vitamin D-resistant rachitism.

As an example of Y-linked disorders, there are some AZF region microdeleted
in the long arm of the Y-chromosome. In this case, the only option to avoid transmission to the offspring is female sex selection.

2) **Chromosome rearrangements** - constitutional chromosomal abnormalities are present in up to 0.9% in newborns, and are associated with 50-60% of first trimester miscarriages. Most of these aneuploidies are a result of a meiotic non-disjunction event, but about 1/500 individuals carry a balanced structural rearrangement as reciprocal and Robertsonian translocations. Although most present with normal phenotypes, they often suffer from repeated spontaneous abortions and/or fertility problems, and have an increased risk of delivering children with congenital anomalies and/or intellectual disability. Several studies have shown that PGD improves the pregnancy outcome for translocation carriers, especially in patients with recurrent pregnancy losses.

3) **PGD for Human Leukocyte Antigen HLA typing** - people affected by malignant conditions, such as leukaemia, lymphoma or some other disorders as beta-thalassaemia, sickle cell anaemia, Fanconi anaemia, Wiscott-Aldrich syndrome, X-linked adrenoleukodystrophy and hypogammaglobulin syndromes, may benefit from allogenic haematopoietic stem cell transplantation (allo-HSCT), using an HLA-matched, related donor, most often a sibling child. PGD techniques are helpful in two situations: (a) one child has a non-inherited disease such as leukaemia and the parents want to have PGD with HLA typing alone to allow the newborn to be a donor to the sick child; or (b) one child has a heritable disorder and the parents need PGD in order to avoid another affected child, and, at the same time, HLA typing brings the hope of saving the already affected sibling. For ethical reasons, in some countries, only the latter procedure is deemed acceptable.

4) **PGD for Rh blood group typing**

PGD can also be indicated in women who are Rh negative and are highly sensitized with antibodies against Rh factor. If Rh genotyping in the male shows that he is heterozygous, it is feasible to perform a PGD to avoid possible erythroblastosis fetalis and intrauterine blood exchange transfusion. PGD has also been used in women sensitized by other blood factors, such as the Kell/Cellentano group.

5) **Sex selection**

Some of the clinics that offer PGD provide it for sex selection for non-medical reasons. Nearly half of these clinics perform it only for "family balancing", which is where a couple with two or more children of one sex desire a child of the other, but half do not restrict sex selection to family balancing. In India, this practice has been used to select only male embryos although this practice is illegal. Opinions on whether sex selection for non-medical reasons is ethically acceptable differ widely, as exemplified by the fact that the ESHRE
Task Force could not formulate a uniform recommendation.

**Genetic methods used in PGD**

There are different approaches for examination of the genetic constitution in PGD. Currently, most PGD are using biochemical techniques based on **polymerase chain reaction (PCR)**, wherein the disease-linked loci are amplified from blastomeric DNA using targeted primers designed specifically for the mutation of interest. PCR diagnosis from a single cell is used for the diagnosis of single gene defects or triplet repeat disorders. **Microarray-based comparative genomic hybridization (aCGH)** is a molecular cytogenetic technique which is performed for detection of chromosomal rearrangements (and also for aneuploidy screening in PGS). The principle of aCGH is complex analysis of chromosomal constitution of the cell using fluorescently labeled nucleotides spotted on a microchip. The result is compared with control DNA which is known to have no genetic alterations. Array CGH has proven to be a specific, sensitive, fast and high-throughput technique, but a main disadvantage is its inability to detect structural chromosomal aberrations without copy number changes, i.e. low-level mosaicism, balanced chromosomal translocations, and inversions. The latest microchip-based technology – **karyomapping** – targets approximately 300,000 of the most informative markers in the genome for efficient genome-wide coverage, meaning that any single gene disorder can be screened for. Karyomapping is a modern, very fast and progressive method, which allows the detection of mutations combined with aneuploidy screening in one test. This new approach enables two-stage genetic selection of embryos, increasing chances for selecting the most suitable embryo for transfer.

**Biopsy techniques used for PGD**

There are several biopsy techniques for pre-implantation genetic testing used to evaluate the DNA of embryos before day 6 of conception.

They are the following:

- **Polar-body biopsy** (assessing female gametes), in which oocyte genotype can be deduced by analysis of the first and/or second polar body. Oocytes considered genetically "normal" can then be utilised for IVF. As it is performed before fertilisation, polar-body analysis offers the possibility of preconception diagnosis without reduction in cell number of the embryo. One clear disadvantage is the inability to assess paternal genotype. However, as 95% of aneuploidies arise in maternal meiosis, there is little loss in efficiency for aneuploidy testing.

- **Blastomere biopsy** (aspiration), assessing the 3-day, six-to eight-cell cleavage embryo, in which the zona pellucida is traversed to extract
one or two cells.

- **Trophectoderm biopsy**, assessing the 5-to-6-day, 120-cell blastocyst. Because more cells can be removed at this stage, it potentially facilitates more accurate diagnosis. These additional 2–3 days in culture, beyond that required for an eight-cell embryo, allow some time for natural deselection of non-progressing or aneuploid embryos.
- **Blastocentesis**, in blastocysts on D5/D6, as a new type of noninvasive embryo biopsy based on the presence of cells and DNA in the blastocoelic cavity.

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**Success or failure factors**

It has been recommended a **minimum of 6 to 8 good-quality embryos when using PGD**. Otherwise, it can be difficult to understand how patients at a mean age ≥38 years (advanced age), routinely produced a total minimum of 6 embryos to be submitted to PGD/PGS as the method for embryo selection. This may be seen as denying proven aspects such as the reduction in ovarian reserve and the response to stimulation with aging.

Not all people who want to perform PGD are able to do so. There are two fundamental requisites: the couple should be fertile and it should have the genetic characterization of the disorder which is intended to be diagnosed. This last point is very important because couples assume that they will have a normal healthy child, whereas it is only possible to offer to minimize the risk for the disease for which they have a great risk of transmitting to their offspring. As there is a small risk of misdiagnosis due to the existence of mosaicism or limitations of the techniques used, couples should always be offered the possibility of an amniocentesis to double check the results. It should be remembered that all the other prenatal diagnoses, both the non-invasive prenatal test (NIPT) and the chorionic villi sample, as well as PGD are screening methods because these analyses are based on cells from the trophectoderm. The procedure should always be explained in detail during the entire treatment to ensure that couples are informed about the potential risks in the short, medium and long term.

Because the majority of patients for PGD are advised by a geneticist, the patients are usually more informed about the risk at birth or during pregnancy, from the end of the first trimester and beginning of the second, which are completely different from the risk during the preimplantation phase, particularly with regard to the risks of aneuploidies and abnormal segregations in carriers of chromosomal rearrangements. During preimplantation development, the risks are much higher. To benefit patients with a more predictable PGD, doctors should ensure that the couple produces a sufficient quantity of embryos to obtain non-affected embryos for transfer.”
Complications

Embryonic mosaicism

A major limitation to the efficiency of PGD/PGS is embryonic mosaicism. Approximately 29% of all embryos may be mosaic, but many of these will not implant successfully. Of those that do implant, some will subsequently miscarry, but a small percentage may be ongoing, so even women who have undergone PGD/PGS should still follow routine local protocols for antenatal aneuploidy screening. There are no data supporting whether either day 3 (cleavage stage) or day 5 (trophectoderm) biopsy improves the detection of mosaic embryos. Biopsy at the cleavage stage may reduce implantation rates from 50 to 30% (relative reduction of 39%), while biopsy at the blastocyst stage does not affect the probability that an embryo will implant and progress to delivery, and therefore appears to be safer.

Cost of PGD

Costs of assisted reproductive techniques are high all over the world, and the inclusion of PGS/PGD only increases this cost. In countries where IVF procedures are not publicly funded or covered by insurance, these expenses can be prohibitive for many couples.

Ethical issues

PGD has raised ethical issues, although this approach could reduce reliance on fetal deselection during pregnancy. The technique can be used for prenatal sex discernment of the embryo, and thus potentially can be used to select embryos of one sex in preference of the other in the context of "family balancing". It may be possible to make other "social selection" choices in the future that introduce socio-economic concerns. Only unaffected embryos are implanted in a woman’s uterus; those that are affected are either discarded or donated to science.

Prognosis

Although noninvasive techniques, such as time-lapse analysis and noninvasive assessments of embryos and oocytes, are being developed in order to generate an alternative to PGD, invasive diagnostic techniques still remain the
most reliable methods to eliminate affected and chromosomally abnormal embryos. In the last couple of decades, preventive medicine has gained more attention worldwide. This, together with the implementation of more powerful, comprehensive, and cost-effective techniques, such as NGS, will strengthen the place of PGD in ART and increase the demand for PGD.

Currently, the choice of technique depends mostly on the indication (whether the purpose is either mutation testing or chromosomal analysis) and on the cost, the availability, and the applicability of the technique. As the list of conditions and indications for PGD testing is continuing to extend enormously, the techniques have been evolving toward a universal method for the simultaneous diagnosis of multiple types of genetic conditions such as monogenic disorders, HLA typing, rearrangements, aneuploidy screening, and the selection of the best embryo that has the highest implantation capacity. The development of such a method will be possible in the near future.

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Diagnoses

Azoospermia
Complete absence of sperm in the ejaculate of a man.
Learn more at: [www.fertilitypedia.org/therapy/diag/azoospermia](http://www.fertilitypedia.org/therapy/diag/azoospermia)

Cryptozoospermia
Male infertility diagnosis characterized by extremely low concentration of sperm in semen.
Learn more at: [www.fertilitypedia.org/therapy/diag/cryptozoospermia](http://www.fertilitypedia.org/therapy/diag/cryptozoospermia)

Hypogonadism
A medical term which describes a diminished functional activity of the gonads – the testes and ovaries.
Learn more at: [www.fertilitypedia.org/therapy/diag/hypogonadism](http://www.fertilitypedia.org/therapy/diag/hypogonadism)

Kallmann syndrome
A genetic condition where the primary symptom is a failure to start puberty or a failure to fully complete puberty.
Learn more at: [www.fertilitypedia.org/therapy/diag/kallmann-syndrome](http://www.fertilitypedia.org/therapy/diag/kallmann-syndrome)
Non-obstructive azoospermia
Complete absence of sperm in the ejaculate due to testicular failure.
Learn more at: www.fertilitypedia.org/therapy/diag/non-obstructive-azoospermia

Obstructive azoospermia
Absence of sperm in the ejaculate despite normal spermatogenesis, caused by an obstruction of the genital tract.
Learn more at: www.fertilitypedia.org/therapy/diag/obstructive-azoospermia

Sertoli cell-only syndrome
The absence of any developmental stage of sperm cell in the testes.
Learn more at: www.fertilitypedia.org/therapy/diag/sertoli-cell-only-syndrome

XX male syndrome
The male sex chromosomal disorder characterized by a spectrum of clinical presentations, ranging from ambiguous to normal male genitalia.
Learn more at: www.fertilitypedia.org/therapy/diag/xx-male-syndrome

Y-chromosome deletions
A family of genetic disorders caused by missing gene(s) in the Y chromosome.
Learn more at: www.fertilitypedia.org/therapy/diag/y-chromosome-deletions

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