

An Introduction to Preimplantation Diagnosis

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Introduction

Assisted reproduction techniques (ART) stood the test as a major mean in alleviating the misery of infertility. Millions of babies have been brought to this world through assisted reproduction techniques (ART). ART also carries new hope to otherwise "fertile" patients at high risk of transmitting a serious genetic disorder to their offspring. This last group of patients often have a complex and difficult reproductive history that may include a genetically abnormal family member, a termination of an affected pregnancy, death of an affected child, or recurrent miscarriage^{1,2}. Their subsequent reproductive options are limited and include natural conception followed by birth of genetically abnormal offspring, or prenatal diagnosis and the possibility of further terminations of affected pregnancies, or remaining childless, adopting, and undergoing gamete or embryo donation; an option which is not acceptable in all societies. All such possibilities carry an enormous potential psychological burden. For couples who find these alternatives unacceptable, preimplantation genetic diagnosis (PGD) provides a further option³⁻⁸.

Definitions

PGD, in the broadest definition, means performance of chromosomal and/or genetic diagnosis on pre-conception embryos prior to their transfer to the uterus.

PGD of embryos could be used in ART cycles in a special group of infertile couples to enhance pregnancy rate and reduce the incidence of miscarriage and as an optional therapy in cases of repeated IVF failure. In such group of patients, it has been recommended, from the clinical point of view, to describe the procedure as preimplantation genetic screening (PGS)⁹ and to reserve the term PGD to those undergoing the procedure to avoid a specific genetic or chromosomal risk. However, PGS and PGD involve similar clinical and laboratory steps, and for simplicity the term PGD will be used indiscriminately to describe different aspects of the procedure regardless of indications.

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Development of the field of PGD

The first successful PGD cycle was reported back in 1990^{4,5}. By the turn of the century, the estimated number of apparently healthy children born after PGD worldwide has surpassed 1,000.

PGD is a highly specialized and rapidly evolving technique. The developments in the field necessitated the establishment of a governing body to provide guidance, evaluation and rules to this novel area of human biology.

HFEA (Human Fertilisation and Embryology Authority), in the UK plays a governing role and provides public assurance of sound ethical as well as scientific standards concerning ART in general and PGD technology in particular. Similarly, the European society of human reproduction (ESHRE) has founded the 'ESHRE/PGD consortium' at the ESHRE annual meeting in Edinburgh back in 1997 to collect detailed data on the practice of PGD⁹⁻¹². Centres all over the world were asked to become members and to give data voluntarily on the patient referrals, PGD cycles, pregnancies and babies. The consortium guidelines are looked at as evidence of good practice, short of any obligatory status. The consortium has already published three reports the latest appeared in human reproduction journal November 2004.

Reasons for Referral for PGD

ESHRE consortium report⁹, studied the results of 36 PGD units (Report IV, second half of year 2002). The following were the commonest indications for referral for PGD arranged according to frequency.

1- Chromosomal Abnormalities

A- Structural Chromosomal Abnormalities

As Reciprocal and Robertsonian translocations, inversions and deletions.

B-Numerical Chromosomal Aberrations

As Aneuploidy risk, 47 XXY, 47 XYY, Sex chromosome mosaicism and Male meiotic abnormalities.

2- Referrals for X-linked disorders

3- Referrals for autosomal recessive diseases.

4- Autosomal dominant diseases.

5- *More than one indication.*

6- *Mitochondrial diseases.*

7- *Y- chromosome deletions.*

8- *Social sexing of embryos.*

Inclusion and Exclusion Criteria for PGD

Not all the patients with chromosomal or genetic disorders could be assisted through the use of PGD.

ESRH/PGD consortium has set certain criteria for patient's selection for both PGS and PGD procedures⁹⁻¹².

Inclusion Criteria for both PGS/PGD (ESHRE Recommendations)

1. The genetic diagnosis should be practically possible with reliability more than 90%.
2. ICSI treatment should be considered applicable with a fair chance of success.
3. High recurrence risk exists should natural conception occurs. (10% in chromosomal rearrangements and 25-50% for monogenic disorders).
4. Recurrent miscarriage (>2) especially if recurrent miscarriage related to parental structure chromosome abnormality is expected should natural pregnancy occur.
5. Repeated implantation failure (>3 IVF/ET cycles).
6. Advanced maternal age (>36 years).
7. Serious health problems are expected as a direct consequence to the disorder.
8. HLA typing of embryos is acceptable when a previous child with malignant disease or genetic disorder, is likely to be cured or his life expectancy to be substantially prolonged by stem cells transplantation from cord blood of an HLA identical sibling. It is essential in such circumstances to possess the necessary techniques to combine HLA typing with PGD to exclude the reoccurrence of the same abnormality in the new sibling.

Exclusion Criteria for both PGS/PGD (ESHRE Recommendations)

1. If the diagnosis of the genetic disorder is not possible with current techniques.
2. The diagnosis of the illness is not certain.
3. High body index (30kg/m²).
4. The presence of a contraindication for IVF.
5. Maternal age above 45 years.
6. A condition with a low recurrence risk (<10%).

7. Affected spouse has serious mental or psychiatric consequences related to the disorder.
8. In HLA typing the affected child is seriously ill with limited life expectancy to allow enough time for the procedure. The procedure is not guaranteed to provide recovery.
9. A woman with serious autosomal dominant or X-linked disorder which contra indicate IVF treatment.
10. Low total antral follicle count (poor responders).
11. Poor embryo quality.

Basic Principles of PGD

PGD would simply mean that a suitable portion (a polar body or a blastomere) is removed from a preimplantation embryo produced through assisted reproduction (in vitro fertilization). This embryo portion is examined for the specific abnormality with a specific test to rule out the disease. If the embryo proved to be normal, it is subsequently transferred to the woman's uterus.

PGD for chromosomal abnormalities mostly depends on chromosomal studies or using specific markers to detect the abnormal chromosome.

Single gene defects, on the other hand, would require identification of the normal and abnormal gene following amplification of the limited available DNA extracted from a single blastomere.

For single gene X-linked disorders where single gene analysis is not possible sex selection of embryos could be performed. This allows the transfer of only the desired sex, free of the disorder.

Applied Steps in the Procedure of PGD include ART cycle, Embryo Biopsy Techniques, Techniques for the Diagnosis of Chromosomal and Genetic Disorders

ART Cycle

The female partner undergoes a routine cycle of ovarian super stimulation. Oocyte collection is performed and mature oocytes are identified under stereomicroscope. Metaphase II oocytes at this stage would be suitable for first polar body removal and analysis.

Oocytes are inseminated with the male partner sperms as usual or preferably injected with a single sperm as is the case in ICSI (ESHRE consortium recommendation). The later, has the advantage of avoiding contamination with semen material that might cause problems with polymerase chain reaction (PCR) and lead to false results.

Injected oocytes are examined for fertilization 18 hours after ICSI. Upon penetration of the egg by the sperm (fertilization), but prior to the joining of the sperm's genetic material with the egg's genetic material, the second polar body is produced. This second polar body carries only half the number of the oocyte chromosomes and gives good indication about what is left behind to merge with the male chromosomes. The appearance of the second polar body at the proper site under the zona pellucida (ZP) is reassuring of good fertilization. The polar bodies have no known function except to assist in cell division and expulsion of excess chromosomes. They are simply "by-products" of the egg's division. The polar bodies disintegrate and are not part of the developing embryo. However, by testing the first and second polar bodies, the genetic make-up of the egg, and maternal genetic contribution in the resultant embryo, can be determined. In some instances, it is necessary to confirm a diagnosis based on polar body testing by performing a sequential blastomere biopsy.

The fertilized oocytes are further incubated in sequential media and examined for division on the following day. On the third day embryos are expected to have reached 6-8 cells stage and are then ready for blastomere biopsy (discussed later).

Following biopsy, embryos are returned to their sequential media and incubated for another 2-3 days. At this early point of embryo development, all of the cells are equivalent and thus a biopsy of a cell from the embryo does not hinder proper future development.

Selected embryos are transferred to the uterus on the fourth or fifth day.

With the current state of art, Pre-natal diagnostic procedures, if available, are recommended in order to confirm the results of PGD (ESHRE consortium recommendation).

Embryo Biopsy Techniques²⁰⁻²⁴

Various methods have been described to create an opening in the ZP of oocytes and embryos (zona drilling). Such hole is used for the purpose of obtaining specimens for genetic diagnosis. Openings have been created mechanically by sharp instruments, by tearing with the use of glass hooks or glass micro-needle, by chemical digestion with acidified Tyrode's solution, and by drilling with the use of a controlled laser beam.

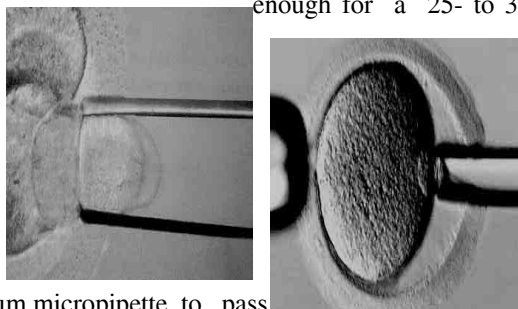
In selecting the optimal method for creating an opening in the ZP, preference should be given to an approach that is simple and efficient, and that minimizes the time the oocyte or embryo is outside the incubator. It is important, in this context, to stress on the steps taken to reduce pH and

temperature changes. The chosen method should also be universal in that it can be applied at any stage of development and good enough to create an opening sufficient in size to accomplish the successful removal of polar bodies or blastomeres with minimal trauma.

For polar bodies extraction the mechanical techniques are considered the safest and most widely practiced. They avoid the damaging effect of acid Tyrode's solution on the oocyte spindle.

Microtool requirements consist of one holding pipette opposite one microneedle and a micropipette; preferably a double pipette holder.

A "V"-shaped cut is created in the ZP to provide a single triangular flap opening that is large enough for a 25- to 30-



um micropipette to pass

Figure 1. Showing steps of mechanical Zona drilling and blastomere biopsy

through to remove a polar body or blastomere(s). To briefly describe the technique (figure 2), the oocyte or embryo is held in position, loosely, by gentle suction from the holding pipette and rotated with the micro-needle until the area with the largest perivitelline space is visible at the 12-o'clock position. Additional suction is applied to hold the oocyte firmly in position, and the microneedle is passed through the ZP starting at the 1 to 2 o'clock position and advanced tangentially through the perivitelline space and out the other side at the 10 to 11 o'clock position. The oocyte is released from the holding pipette and held by the microneedle. The microneedle is brought to the bottom of the holding pipette and pressed to it, pinching a portion of the ZP. By gently rubbing the microneedle against the holding pipette with a sawing motion, the first cut is made and the oocyte is released. The result is a single slit opening. To make the second cut, the oocyte is rotated vertically until the slit is clearly visible at the 12 o'clock position and then rotated horizontally, backward, until the slit is slightly out of focus, reaching the end of the slit, which is then at the 12 o'clock position. This allows the second intersecting cut to be positioned at the end of the first cut, creating the V-shaped opening. The second cut is completed by entering into the first slit, advancing tangentially through the perivitelline space, and ending at the 10 to 11

o'clock position. The oocyte is released from the holding pipette. A V-shaped cut can be seen when the embryo is rotated.

To complete the oocyte/embryo biopsy procedure, the embryo is rotated until the V-shaped opening is at the 3 o'clock position and then held firmly by suction from the holding pipette. The micropipette is brought into focus and slight upward pressure is applied with the micropipette to the triangular flap. The flap is opened and the micropipette is inserted. Blastomeres are removed without distorting the blastomere configuration of the embryo. Polar bodies are removed through the created opening in the ZP by aspiration²⁵, cleavage stage blastomeres are removed by aspiration²⁴, extrusion²⁶ or displacement techniques. Trophectoderm cells are removed from blastocysts by mechanical excision or mechanical stitch and pull technique. The triangular flap moves back after the micropipette is removed protecting the embryo.

Chemical zona breaching for embryos and blastocysts is commonly practiced by PGD centers. Embryos are placed in a drop of a biopsy medium (commercial or house made calcium free and magnesium free medium) under mineral oil. Holding the embryo with a holding pipette, the ZP is locally digested, by releasing acidified Tyrode's solution directly to a small area on its surface. At the end of the procedure embryos should be removed and carefully washed.

The third possible option is to breach the ZP with laser energy. The 1.48- μ m infrared diode laser offers a fast and extremely convenient alternative for ZP dissection, with apparently minimal compromise to the embryo when compared to other forms of ZP opening. Blastocysts are removed through the hole created with laser in a similar way to the above procedure and trophectoderm are removed easily from blastocysts by herniation following laser²⁷. Critics of the technique express concern regarding the damage inflicted on embryos brought by the release of laser energy.

Regardless of the employed technique for biopsy, PGD involves analysis of a limited number of embryonic cells. Some units use one cell analysis. Others extract two or more cells from an embryo, in order to obtain a firm diagnosis. It has been argued, that removal of more than one cell from a certain embryo is not likely to affect implantation or future development²⁴. However, animal studies as well as other recent data on human embryos demonstrated the opposite. Furthermore, units employing single cell biopsy techniques were noted to achieve better pregnancy rates. There is no definite consensus of opinion

regarding the number of blastomeres to be removed. However, it has been recommended by ESHRE consortium report to use 8 cells or more embryos in cases where more than one blastomere are to be removed.

Techniques for the Diagnosis of Chromosomal and Genetic Disorders

Obviously, routine chromosomal banding would not be a suitable approach to diagnose polar bodies or blastomeres chromosomal abnormalities. In view of the very limited material available, and lack of enough metaphase material, the investigator resorts in such circumstances to more sophisticated tests.

The development, however, of in situ hybridisation and radio labelled DNA probes in the late 1970s and early 1980s introduced us to a new era of molecular technology. It became possible to trace the radio active probe attaching itself to a specific chromosomal site. However, the disadvantages in using radio active probes are needless to stress. With the subsequent emergence of non isotopic probes for in situ hybridisation, particularly Fluorescence in situ hybridisation our diagnostic abilities expanded tremendously [Fluorescent in Situ Hybridization (FISH)]²⁸⁻³¹.

This diagnostic tool combines conventional cytogenetics with molecular genetic technology. It is based on the unique ability of a "man made" portion of a single stranded DNA (a specifically designed probe with fluorescent signal) to combine with its complementary target sequence on the tested strand of DNA. The signal is subsequently traced with fluorescent microscope. A number of DNA probes each labelled with a different fluorochrome in the same procedure means that separate loci on certain chromosome could be identified, comparisons could be made and relationship to centromeres could be identified.

FISH is a relatively rapid and simple test. FISH also, (to the contrary to routine chromosomal analysis) could be performed on cells in the resting interphase.

Recently, whole chromosome paint probes have been designed in which a mixture of probes is used in a single test (single hybridization) with fluorescent painting of the whole chromosome. Multicolor spectral karyotyping (SKY) and multicolour FISH (M-FISH) are the latest developments in FISH technology. They utilize pools of whole human chromosome paint probes to provide a multicolour human karyotype in which each pair of homologous chromosome is given a certain fluorescent colour. Computer programmes made it even easier to read such tests. SKY and M-FISH proved indispensable in the diagnosis of subtle chromosomal disorders.

Some authors argue against probing for many chromosomes at one time. ESHRE recommendation is to use a probe set of at least five chromosome pairs from 13, 14, 15, 16, 16, 21, X and Y. However, a trend towards higher pregnancy rates was noted in probing for a maximum of 5 chromosomes compared to 9 chromosomes. This may indicate that with increased number of chromosomes analyzed there is a greater decrease in specificity with a higher number of false-positive abnormal embryos, resulting in fewer embryos available for transfer. Therefore, FISH using a 9 chromosome panel was noted to offers a little added value over the 5 chromosome panel with a potential adverse effect on pregnancy rates. Nevertheless, rehybridization is considered acceptable by ESHRE consortium opinion. Rehybridization simply means to use the FISH technique to stain and visualise certain chromosome with its specific colour indicator and to "wash" this probe or colour indicator off the chromosome by heating at 50 centigrade and use the same cell with hybridization to another FISH probe. This process (sequential FISH analysis), could be repeated several times without compromise to the diagnostic potential³².

When FISH is used for sex selection as the case in X-linked disorders a "probe set" containing at least one probe specific for each centromere region of the X and Y chromosomes, and one autosome, are recommended.

ESHRE consortium reports comment on specific fixation and staining techniques and are referred to for further reading.

PGS, as a tool to improve implantation, reduce miscarriage rate, or improve the IVF cycle outcome³³, is becoming more applicable. Fluorescent in situ hybridization (FISH) of a single-blastomere chromosomes may turn out to be only the end of the beginning for the field of PGD, a stepping-stone, possibly, to technologies currently being refined and adapted for PGD purposes. Assays such as comparative genomic hybridization, DNA microarray "dot" technology, and others hold the promise of more precise means to predict, at minimum, the "implantability" of each embryo produced by a patient. This brings the goal of "one embryo transferred, one healthy baby born," closer, and could lower the high multiple pregnancy rate commonly seen in ART.

The Problem of Mosaicism

However, at times there can be an aberration in the cell division in which one or more of the "daughter" cells ends up being slightly different from the parent cell (mosaicism). Mosaicism means that it is possible that the cell that is biopsied may

not be representative of the entire embryo. For example, if during PGD, a blastomere biopsy is performed and the cell that is obtained is abnormal, the entire embryo would be considered abnormal even though the remaining cells in the embryo may be normal. The opposite is also true. An embryo with 7 abnormal cells and one normal can be considered normal if the "eighth" cell happens to be the one that is biopsied. the mosaicism issue will, hopefully, be better understood and techniques will be developed to minimize its impact on pregnancy and miscarriage rates encountered in PGD cycles.

Clinical Applications of FISH

FISH technology is used to identify chromosomal anomalies of biopsied polar bodies or blastomeres.

PGD can be offered to carriers of *balanced translocations* as an alternative to prenatal diagnosis and pregnancy termination of unbalanced fetuses.

Analysis by FISH chromosome painting of first polar bodies so far has been the most successful method; it uses two chromosome-painting probes applied to the translocated metaphase chromosomes in the polar body shortly after egg retrieval. With this approach, the frequency of spontaneous abortions in pregnant patients was significantly reduced ($P < .001$), from 95% lost fetuses in natural cycles to 12.5% after PGD.

The methods used hitherto either are useful only for female carriers of translocation or involve the use of sophisticated micromanipulation techniques that require a high level of skill to fix the metaphase chromosomes for proper analysis. An alternative to the analysis of metaphase chromosomes is the use of FISH on interphase blastomeres. This method can be applied to *translocations of parental origin* or for *inversions*.

Specific *spanning probes* can be developed that expand the breakpoints of each translocation or inversion. Probes can be used for translocations or inversions. For Robertsonian translocations, enumerator Δ -satellite or locus-specific probes can be used to detect aneuploid embryos. *Only spanning probes can differentiate between balanced and normal embryos*. Unfortunately, the production of case-specific breakpoint-spanning probes is expensive and time consuming. Using commercially available telomere probes instead would considerably simplify PGD of translocations and inversions.

*FISH technique is commonly used in PGD to diagnose X-linked single gene disorder, e.g.*³⁴:

- Duchenne muscular dystrophy.

- X linked developmental delay.
- Fragile X syndrome.
- Hemophilia A and B.
- Becker muscular dystrophy.
- Hunter syndrome.
- Ornithine transcarbamylase deficiency.
- Anderson-Fabry disease.
- Adrenoleukodystrophy.
- Pelizaeus-Merzbacher disease.
- Hypospadias.
- Wiskott-Aldrich syndrome.
- Lebers optic atrophy (mitochondria).

Polymerase Chain Reaction (PCR)³⁵⁻³⁷

PCR allows analysis of DNA from any cellular source containing nuclei, including specific diagnosis of X-linked diseases. DNA is uniquely designed for every individual. The sequence of nucleotides on DNA strands determine the type of proteins to be sensitized and all specific characteristics of different individuals.

Genetic defects are caused by alteration in the sequence of amino acids in the nucleotides on the DNA strands. With development in molecular biology the abnormal genes responsible for different types of genetic disorders are becoming gradually disclosed.

For disorders with known gene constitution, PCR could be used to identify the abnormal gene in a certain DNA specimen. Since DNA is a double strand structure with hydrogen bonds binding both strands, *heating of the DNA structure will result in de-annealing (separation) of the two strands*. The specimen of DNA is then treated with *restriction enzymes* in order to break it into smaller segments and then pooled with medium containing specific primers to the genetic disorder to be tested for. Previous knowledge of the target DNA (the abnormal DNA sequence) is needed in order to generate the necessary *primer (amplimer)*. One of the amino acids on the primer is labelled with markers (e.g. radioactivity or fluorescent activity). The primer will bind to the complementary DNA in the tested sample (if the abnormal gene is present). With the help of a heat stable '*Taq*' *polymerase* provided in the medium and the availability of the necessary amino acids in the medium, extension of the primer on the DNA strand will occur by adding amino acids with the formation of a complementary copy of the whole strand of DNA. Cooling will result in reannealing of the appropriate strands of DNA to form the normal double strand DNA. *Repeating the same process for several times will result in the formation of several copies of the targeted DNA*. Thirty to 35 successive cycles of PCR could

produce 1 million copies (amplicons) of a specific DNA target sufficient for direct visualization by gel electrophoresis, by ultra violet fluorescence following ethidium bromide staining, or radioactivity detection, without the need to use indirect detection techniques.

Recent real time PCR machines have cut down the time needed to complete the process of diagnosis making to less than one hour. And fluorescent technology is being used to monitor the generation of PCR products during each cycle, thus eliminating the need for gel electrophoresis.

The reader is again referred to ESHRE report for further reading regarding recommendations concerning validation, Allelic drop outs (ADO), contamination control and work practice control.

Slide PCR

Both PCR and FISH have specific advantages and disadvantages. In an attempt to combine the advantages of both methods and minimize the disadvantages, a slide PCR method has been developed that allows the performance of PCR on blastomeres fixed on microscopic slides. A plastic ring is attached to the slide to form a well, and the biopsied blastomere was then fixed inside the well. The plastic ring not only facilitates slide PCR by holding the solution for amplification, but it further allows recycling of the same blastomeres for sequential PCR, and FISH. In addition, the plastic ring limits the area for fixation, thereby reducing loss and increasing efficiency. However, it has been reported that the efficiency of slide PCR is lower than that with a blastomere in suspension (solution PCR).

With better primers and optimal amplification conditions, the efficiency of slide PCR could be enhanced. Fixation with HCl-Tween 20 and selecting good quality blastomeres are other key factors to increase the efficiency of slide PCR. Fixation with HCl-Tween 20 will dissolve completely the cytoplasm and make the nuclei accessible for amplification and thus improve the amplification efficiency.

Recycling for sequential PCR allows the detection of multiple genes, when different sets of primers are used in each cycle.

Clinical Applications of PCR in PGD³⁸

PCR is mostly used in the diagnosis of (autosomal), *single gene defect*.

Specific PCR-based diagnosis could be carried out for:

Autosomal Dominant Disorders as

- Huntington disease.

- Myotonic dystrophy.

Autosomal Recessive Disorders as

- Cystic fibrosis.
- Spinal muscular atrophy (SMA).
- Epidermolysis bullosa.
- Ectodermal dysplasia/skin fragility syndrome.
- Sickle cell anaemia.

For single-gene disorders, where the heterozygous state could be distinguished (cystic fibrosis and epidermolysis bullosa), unaffected embryos are preferentially transferred, but heterozygous embryos could be transferred if no unaffected embryos were available, or in some cases if the quality of heterozygous embryos was superior (after discussion and with patient consent). However, in the case of cystic fibrosis with CBAVD, only unaffected embryos are to be transferred.

With PCR various mutations could be detected, e.g.: mutation $\Delta F508$ within the CFTR gene, the most common mutation associated with the disease cystic fibrosis, the presence of exon 7 of the survival motor neurone gene SMNt, deletions of which are associated with the disease SMA, and mutations in the PKP-1 gene, ablation of which is associated with a severe genodermatosis. In the absence of allele drop out (ADO), the assays used for detection of $\Delta F508$ and PKP-1 mutations are able to discriminate homozygous affected, homozygous unaffected and heterozygous embryos.

The above are only examples of the widening list of applications for PCR in PGD. With the tremendous development in molecular technology many of the present health problems are being linked with genetic basic defects. PGD is likely to acquire a larger scope of application both in preventive as well as therapeutic medicine in the near future.

PGD for Stem Cell Production

Recently, there have been a very growing number of high profile cases whereby PGD has been used not only to select embryos unaffected for the genetic disorder for which the pregnancy is at risk, but also to provide an HLA tissue type match for a genetically defective individual³⁹. "A newborn child can function as a stem cell (derived from umbilical cord blood at birth) donor for a matched person" has become more of an applied tool than a research fantasy.

Couples who have children with certain medical problems may be interested in PGD for two reasons:

1. To facilitate the development of a genetically free child.
2. To create the possibility of treating an existing child with an otherwise untreatable condition.

The disorders where this technology has been used include:

- Fanconi anaemia.
- Thalassaemia.
- Diamond-blackfan anaemia.
- Acute lymphoid leukaemia.
- Acute myeloid leukaemia.

The thing that all these conditions have in common is that they can all be cured by bone marrow stem cell transplantation. Stem cells derived from an HLA compatible brother or a sister would be optimum denying the need for an immunosuppressive therapy. Through a blood test HLA markers (6 markers- 600 HLA molecules) present in the affected child are identified. PCR identify which embryos are a 100% HLA match (and free of the affected gene) and such ones are therefore transferred to the mother to produce the perfect match. Stem cells derived from the newborn cord blood are subsequently used to treat the sick child.

Ethics and Gender Selection in PGD

PGD is the only method for gender selection that is close to 100% accurate. (Other methods for gender selection attempt to separate the X and Y bearing sperm). To date, none of these techniques has proved to be successful.

There presently is little debate over the ethical validity of PGD for sex selection when its aim is to prevent the transmission of sex-linked genetic disease. In this case, sex selection does not prefer one sex over the other and does not contribute, as such, to gender bias. And when the genetic is severe, efforts to prevent it should be unhindered.

One the other hand, it is less easy to eliminate concerns regarding PGD and sex selection when it is aimed at serving social and psychological goals not related to the prevention of the disease.

It should be appreciated that sex-selective techniques used appear to have a clear claim on limited resources aimed for other medical procedures that are performed with the goal of eliminating disease and suffering.

The publication by the ethics committee of the American society of reproductive medicine (Fertility and Sterility Journal, May 1999) provided valuable ethical points for the interested reader.

Some Areas of Current Research

1- Blastomere culture^{40,41}

Since difficulties could be encountered in providing a conclusive diagnosis on the bases of a limited number of blastomeres. In vitro blastomere culture has been tried in order to increase the number of cells available for chromosomal analysis. Unfortunately, so far, the high frequency of nuclear defects and the occurrence of polyploidy and mosaicism among cultured blastomeres discourage the use of blastomere isolation and proliferation strategy for use in preimplantation genetic diagnosis.

2- Production of Metaphase Chromosomes from PB or Blastomeres

Verlinsky and Evsikov⁶, recently obtained chromosomes from human polar bodies by injecting the second polar body into a donor enucleated M-II oocyte. The oocyte is then activated to produce a pronucleus of the second polar body and cultured in Okadaic acid to produce metaphase chromosomes. A similar method applied to blastomeres was described recently, and is based on the observation that after blastomere fusion to a freshly matured, enucleated cow oocyte, the transferred nucleus becomes arrested in a configuration resembling M-II. This technique has already been applied clinically, resulting in chromosomally normal offspring.

3- Gene Therapy of Diseased Embryos^{42,43}

Approximately 3,000 human genetic diseases are believed to be the consequence of single gene defects¹⁶. To cure some, various methods have been used in attempts to introduce missing genes into affected cells. Such experimental treatments have suggested that to cure disease, the repair of a genetic defect does not have to be complete (we do not have to treat all cells of a certain embryo). This observation led to speculation that human embryos, which through the use of PGD have been diagnosed with a single gene defect, might be treatable through the transplantation of normal blastomeres, which contain the missing genes. If such transplanted blastomeres were to be integrated normally into the developing recipient embryo, a potential treatment protocol could be designed.

High-quality embryos appear to have the ability to integrate donor blastomeres. Because the treatment of single gene diseases does not require successful treatment of all cells, blastomere transplantation could be explored as a treatment option, which also would greatly enhance efficiency and utilisation of PGD.

Writing about PGD is a thrilling experience. It

combines an endogenous fear with a mixture of curiosity and hope. It shows us how small we are, exactly like the way we feel on looking through the window of an aeroplane cruising in the clouds. To utilise the acquired technology in a proper way good understanding and self control should be employed.

Adherence to governing bodies is mandatory and participation in the international group efforts is necessary. For the time being the latest ESHRE consortium principles and guidelines seem to be reasonable ways to regulate different aspects of PGD based on the standard and reliability of the available state of the art technology.

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