Preimplantation Genetic Diagnosis - A Review

Abstract

Preimplantation genetic diagnosis (PGD) was introduced nearly a quarter-century ago. Since then, it has been expanding in scope and applications. It is an evolving technique that provides a practical alternative to prenatal diagnosis and prevents termination of pregnancy in couples with a high risk for offspring affected by a sex-linked genetic disease. Early protocols used blastomeres biopsied from cleavage-stage embryos. With further advancements, improved results have started to be seen with biopsy from first and second polar body or early trophectoderm.

Preimplantation genetic diagnosis was first carried out using polymerase chain reaction followed by fluorescence in situ hybridization. Now, newer molecular tools, such as DNA microarrays and next-generation sequencing are being tested. This advancement in the field of medicine has not only provided an insight into reproductive genetics and early human development but has also raised important new ethical, legal and social issues about assisted human reproduction in order to avoid inappropriate use of this technology.

Keywords: Preimplantation genetic diagnosis, Preimplantation genetic testing, Preimplantation genetic screening, Aneuploidy testing, Embryo biopsy, Sex selection.

Introduction

Preimplantation genetic diagnosis also termed as preimplantation genetic testing is a clinical diagnostic procedure. It has evolved from the gradual advances in assisted reproductive technology/ in vitro fertilization (IVF) that first took place nearly 25 years ago. Preimplantation genetic diagnosis (PGD) was originally developed to reduce the transmission of severe genetic diseases in couples with a reproductive risk. The same technology has recently been used for screening embryos for common age-related aneuploidies in order to improve IVF success. This has also been termed as preimplantation genetic diagnosis-aneuploidy screening (PGD-AS).

History

While preliminary experiments were carried out for several years, it was finally in 1990 that the first baby was born using this advanced technology. Since then, it has had a great impact on the legislation of various countries with respect to embryo research. The first successful application of preimplantation genetic testing was in parents who were carriers of an X-linked disease and had a risk of transmission of the disease to the male offspring. Using polymerase chain reaction, only the Y chromosomes were amplified to discriminate between male and female embryos following which only the female embryos were used for the IVF procedure.

What Is Preimplantation Genetic Diagnosis?

In PGD, cellular material harvested from oocytes or early human embryos that have been cultured in vitro (Fig. 1) are tested for a specific genetic abnormality. After diagnosis, only the unaffected embryos are selected for transfer to the uterus.
Figure 1. Principle of Preimplantation Genetic Testing—A single cell is biopsied and is further tested using suitable Genetic Testing. The unaffected embryos are then transferred to the Mother for a Potential Pregnancy

This, however, is different from prenatal genetic testing in which testing is carried out after implantation of the embryo. In comparison to other established methods of prenatal diagnosis, such as chorionic villus sampling and amniocentesis, PGD is not performed on an ongoing intrauterine pregnancy in the late first or early second trimester, but on embryos developing in the IVF laboratory prior to transfer to the uterus. Thus, preimplantation genetic testing is said to be an early form of prenatal genetic testing.5

Most common indications for preimplantation genetic testing are enlisted as follows:

- Recurrent pregnancy loss
- Advanced maternal age
- Unsuccessful IVF cycles (repeat implantation failure)
- Severe male factor
- Aneuploidy testing
- Sex-linked disorders—hemophilia, fragile X syndrome, neuromuscular dystrophies
- Structural chromosome rearrangements—translocations, inversions, and deletions
- Single gene disorders—cystic fibrosis, Tay-Sachs disease, sickle cell anemia, Huntington disease

**Recurrent Pregnancy Loss**

Recurrent pregnancy loss is defined as two or more consecutive pregnancy losses before 20 weeks period of gestation. Chromosomal abnormalities are seen in 50-80% of abortuses and thus genetic testing can benefit this subgroup of patients.6

**Advanced Maternal Age**

As women age, the chromosomes in the oocyte are less likely to divide properly. Or in other words, the risk of aneuploidy increases with increasing age. The most common of these is trisomy 21 or Down’s syndrome. The rate of aneuploidy rises from 20% in mothers aged 35-39 years to nearly 40% in mothers above 40 years. In such cases, PGD increases the chance of a healthy pregnancy.7

**Recurrent IVF Failure**

Recurrent IVF failure is usually defined as three or more failed IVF attempts. Studies have shown that this affected population has a higher number of chromosomally abnormal embryos.8

**Male Factor Infertility**

Genetic defects associated with male factor infertility include aneuploidy, most commonly Klinefelter syndrome, Robertsonian translocations, Y chromosome microdeletions, etc.9

**Techniques**

Preimplantation Genetic Diagnosis is a complex multistep procedure (Fig. 2), which requires
manipulations of the gametes and embryos. It is incorporated in the usual procedure of an otherwise commonly used technique known as in vitro fertilization (IVF). This helps in selecting unaffected embryos for transfer to the mother for a potential pregnancy.\textsuperscript{10}

The first step in this process is controlled ovarian hyperstimulation. For the recruitment of ovarian follicles from the mother, an injectable gonadotropin is used. This process is monitored using pelvic ultrasonography\textsuperscript{11} and when the size and number of the ovarian follicles is considered appropriate, oocyte maturation is triggered using hormones. Following this, the oocytes are retrieved by transvaginal needle aspiration.\textsuperscript{7} These oocytes are then inseminated in vitro for which two mechanisms have been mentioned:

1. **Conventional Insemination:** In this hundreds to thousands of sperms are placed around the oocyte and fertilization is allowed to occur spontaneously.

2. **Intracytoplasmic Sperm Injection (ICSI):** In this technique, under controlled conditions supervision, one sperm is mechanically injected into an ovum for fertilization.\textsuperscript{12}

Several studies have been carried out to test the efficacy of these two techniques. ICSI holds several advantages over conventional techniques. Men with reduced sperm quality like oligosperma, poor motility or abnormal sperm morphology become suitable candidates for ICSI. This is also recommended in all cases in which polymerase chain reaction (PCR) is required for preimplantation genetic diagnosis. This is mainly because PCR is a highly sensitive technique and the supernumerary sperms embedded in the zonapellucida after IVF might lead to contamination of PCR reactions with paternal DNA and, therefore, to a possible misdiagnosis.\textsuperscript{1} Further, the failure rates with ICSI are lower than the conventional techniques due to which most institutes prefer this technique.\textsuperscript{13}

Following fertilization, the embryos are allowed to grow in a culture medium. A specialized biopsy procedure is required to remove the cells for preimplantation genetic testing. The timing of this biopsy is extremely critical. This biopsy procedure can be carried out at three points in the embryonic cycle: (1) polar body biopsy, (2) cleavage stage embryo biopsy, and (3) blastocyst embryo biopsy.

**Polar Body Biopsy**

The first and second polar bodies are byproducts of oogenesis following meiosis (Fig. 3).
The first polar body that contains a complement of 23 bivalent maternal chromosomes is formed alongside the mature oocyte. The second polar body is formed on fertilization and contains a complement of 23 paternal chromatids that are expelled out of the oocyte.\(^1\) The sepolar bodies are then retrieved from the developmental cycle and used for genetic testing. The optimal time for polar body biopsy is said to be 6-9 hours post fertilization\(^1\) as after this, degenerative changes begin making DNA-based testing difficult.

The main advantage of a polar body biopsy over other techniques is that polar bodies are extra-embryonic material and do not eventually take part in formation of the embryo. Thus there is no reduction in the embryonic cell number and its removal will not have any adverse effect on the fetus.\(^1\) Also, the ethical constraints over sampling of embryos are overcome by this technique.\(^{15,16,17}\) Another promising advantage of this technique is that since it is performed early in the IVF process, it allows extra time for genetic testing to be performed prior to the transfer.\(^5\)

On the other hand, a striking disadvantage of this technique is that as it only tests maternal disorders while paternal information is completely lacking. This can still result in formation of an affected embryo.\(^{18,19}\) Despite this, the clinical utility remains widespread. For women who carry recessive single-gene mutations or are affected with a dominant genetic disorder, if the mutation is present in the polar body DNA, it is assumed that the egg carries the normal allele and will lead to a normal embryo.\(^{20}\) Also, first polar body biopsy provides pre-conceptual information on the egg, which can guide clinicians as to which oocytes should be selected for fertilization.\(^{21}\) For example, if the polar body shows two copies of chromosome 8, the corresponding mature oocytes will have no copies, and an embryo will be monosomic for chromosome 8. Such oocytes are then dropped from the IVF pool.

**Cleavage Stage Biopsy**

Cleavage stage day-3 biopsy is the most widely used biopsy technique for preimplantation genetic diagnosis.\(^5\) Day 3 is the critical time preferred for this biopsy. This is because at day-3 (16 cell stage), the individual cells (blastomeres) of the cleaving embryo are distinct and discernible. However, after the 16-cell stage, compaction begins, tight junctions form\(^{22}\) between these blastomeres and cellular apposition makes the biopsy extremely challenging. Further, biopsy prior to day-3 (2-4 cell stage) removal of even a single cell results in loss of a large proportion of the cellular mass.
of the embryo. This can have detrimental effects on further developmental potential.\textsuperscript{23,24} Another controversial issue with respect to this is the removal of single or two blastomeres. While removal of two cells reduces the cell mass significantly, but at the same time it improves the diagnostic accuracy.\textsuperscript{25,26}

The main advantage of cleavage-stage biopsy over polar body biopsy is that it can detect both maternally and paternally derived chromosomal defects. Further, biopsy on day-3 embryos allows 2-3 days for genetic analysis to be completed if a fresh embryo transfer is desired.\textsuperscript{5}

Cohen et al.\textsuperscript{27} pointed out that the biopsy of a single cell at the 8-cell stage would lead to a decrease in implantation of 12.5%, and a 2-cell blastomere biopsy would produce a 25% decrease in implantation. This high implantation failure rate has proposed multicellular trophectoderm biopsy on a day-5 blastocyst as an alternative to cleavage stage biopsy.

**Trophectoderm (Blastocyst) Biopsy**

Blastocyst biopsy was first reported by McArthur and the Sydney IVF group.\textsuperscript{28,29} The human blastocyst contains approximately 130 cells distributed between the inner cell mass and the surrounding trophectoderm cells. Biopsy procedure involves removal of the trophectoderm cells while cells from the inner cell mass are avoided. Thus it is believed to be less harmful than blastomere biopsy. In addition, the inner cell mass that is destined to become the fetus proper is unlikely to be damaged, thereby reducing possible ethical concerns.\textsuperscript{30}

Trophectoderm biopsy has the advantage of collecting multiple cells thereby increasing diagnostic accuracy. In addition, practically since only about 50% of fertilized embryos ultimately progress to blastocysts, fewer embryos are biopsied compared to day-3 blastomere or polar body biopsy.

**Diagnostic Techniques**

Once the biopsy has been obtained using any of the above techniques, the sample is subjected to one of the diagnostic modalities to test for either monogenic disorders or chromosomal abnormalities. Usually, PCR-based methods are used for monogenic disorders and FISH is used for chromosomal abnormalities.

**Polymerase Chain Reaction (PCR)**

PCR is used for the diagnosis of single gene defects, including dominant and recessive disorders. PCR amplifies a specific DNA sequence in a logarithmic manner into billions of copies in order to facilitate its analysis. It is a relatively fast and convenient way to test DNA. The method has been used in a variety of preimplantation genetic testing protocols. However, it requires ample amounts of a pure sample of DNA, which is difficult to obtain from a single cell such as a polar body. Also, laboratory contamination is a possible complication. Thus, the laboratory environment must be strictly controlled to avoid contaminants.

Misdiagnosis can also occur in PCR due to a common phenomenon called allele dropout. This is the preferential amplification of one allele over another during the PCR process. This is mainly a problem when two different mutations are carried out and only one mutation is being studied.

**Fluorescence in situ Hybridization (FISH)**

FISH is used for the determination of sex for X-linked diseases, chromosomal abnormalities, and aneuploidy screening. Specific fluorescent-labelled probes are used for this purpose. These are then visualized under a fluorescent microscope. Chromosomes that are most commonly analyzed with FISH include X, Y, 1, 13, 16, 18, and 21.

**Comparative Genomic Hybridization**

Despite advancements in the field of genetics, none of the diagnostic modalities can test all 23 pairs of chromosomes. Recently with the advent of comparative genomic hybridization, this limitation has been overcome. Not only does this enumerate all chromosomes but also provides a more detailed picture than FISH.\textsuperscript{31}

**Embryo Preservation and Transfer**

Cryopreservation of surplus embryos is now a routine practice in IVF procedures.\textsuperscript{32} This is done either by slow freezing or vitrification. The main principle is slow penetration of the cryoprotectant through the intact zona pellucida. These protocols are, however, difficult to apply when the zona pellucida has been breached thereby making cryopreservation of a biopsied embryo even more difficult. The initial attempts at cryopreservation after biopsy resulted in a reduced survival rate of embryos compared with non-biopsied embryos.\textsuperscript{33} Recently, however, pregnancies have been reported in cryopreserved embryos after biopsy and thus is being considered as a promising tool in the near future.
Risks Associated with Preimplantation Genetic Diagnosis

When cleavage stage biopsy is performed, all of the cells in the embryo at that stage are totipotent. Therefore, removing one of these cells can definitely reduce the growth rate of the embryo but is not known to cause any anatomical defect. However, preimplantation genetic diagnosis is not a fool-proof technique and has certain risks of misdiagnosis. These can either be a human error or a spurious result obtained through various diagnostic modalities. All these diagnostic methods have their inherent diagnostic limitations. However, with current methods like comparative genomic hybridization and DNA microarrays, the limitations can be overcome. To minimize this risk, all laboratories must have multiple layers of confirmatory checks.

The greatest risk of misdiagnosis stems from cellular mosaicism within the developing embryo. Various studies have shown that as many as 50% of cleavage stage embryos possess a mixture of normal (euploid) and abnormal (aneuploid) cells. Consequently, a biopsied cell from a cleavage stage embryo might not represent the actual chromosomal status of the fetus.

To conclude, preimplantation genetic diagnosis is an evolving technique that provides a practical alternative to prenatal diagnosis and prevents termination of pregnancy in couples with a high risk for offspring affected by a sex-linked genetic disease. PGD was first carried out using polymerase chain reaction followed by fluorescence in situ hybridization, DNA microarrays and next-generation sequencing. This provides an insight into genetics as well as reproductive medicine. However, legal issues need to be kept in mind to avoid misuse of this advanced technology.

Conflict of Interest: None

References

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