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Review article

Preimplantation genetic diagnosis (PGD) An update review of biopsy sample, technique, and diagnosis method

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ABSTRACT

Preimplantation genetic diagnosis (PGD) is a genetic screening technique for embryos to be implanted in assisted reproductive technology or in vitro fertilization (IVF). PGD requires several stages and manipulation of gametes and embryos. Unfortunately, PGD is not always successful. This review was aimed to reveal the update of PGD, in term of biopsy sample, biopsy technique and its diagnosis technique. A computerized search of PubMed and google scholar database was conducted for general terms such as "preimplantation genetic diagnosis", "polar body", "cleavage" and "blastocyst" and followed by screening and evaluation of collected proper accordingly. In PGD, sample of biopsy can be obtained from (1) polar body of oocyte (2) blastomere of cleavage stage of embryo and (3) trophectoderm cells of blastocyst. The process of sloughing off the oocyte or embryo cell can be done through several techniques, namely Mechanical Partial Zone Dissection (PZD) which is carried out using two pipettes, namely holding pipette to hold the embryo and injection pipette to slit the blastomere/cell out of the embryo, while the Chemical Drilling Zone is done by using acid tyrode (pH 2.3) to create holes in the membrane, and Laser-assisted Dissection which is done by opening the zone through a laser that enters the located area of oocyte/embryo. Then, diagnostic methods are used to detect the abnormalities found, such as Polymerase Chain Reaction (PCR) which is used for diseases caused by single gene disorders, Fluorescence In Situ Hybridization (FISH) which is used to detect chromosomal abnormalities, Array Comparative Genomic Hybridization (Array CGH) which is used to investigate aneuploidy in oocytes, and gene expression could be evaluated on mRNA representing the genetic function of oocyte development. The most appropriate update of PGD is polar body for the sample, laser-assisted dissection for the technique and CGH for the diagnosis method.

Keywords: PGD, sample of PGD, Technique of PGD, Diagnosis method of PGD

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INTRODUCTION

Preimplantation genetic diagnosis (PGD) is a genetic screening technique for embryos to be implanted in assisted reproductive technology or in vitro fertilization (IVF). Single gene abnormalities and/or chromosomal abnormalities in embryos, preventing implantation of the fetus and/or limiting the risk underlying embryo transfer by carrying chromosomal abnormalities and causing implantation failure and miscarriage [1], This technology is also used to determine the chances of pregnancy, savior siblings and sex selection [2].

Preimplantation genetic diagnosis requires several stages and manipulation of gametes and embryos aimed at the selection of healthy embryos that do not carry genetic abnormalities for transfer and potential future pregnancies. The PGD stage begins with the use of IVF, which is a procedure that requires approach to oocytes and creating numerous embryos for testing. Stage of IVF is performed by controlling ovarian hyper ovulation using exogenous gonadotropins and other medications in expectant mothers. Some of the oocytes that developed and were eligible for further processing were collected by means of transvaginal needle aspiration. The oocytes were then inseminated in vitro to produce embryos which were then cultured for several days. Oocyte fertilization can occur within hours of oocyte retrieval by either (1) conventional insemination, in which several hundred thousand sperm are positioned around an oocyte prepared for spontaneous fertilization, or (2) intracytoplasmic sperm injection (ICSI), in which one sperm is injected to oocyte [3].

When IVF is successful, a biopsy procedure is needed to take cells for PGD examination. Currently, PGD is not always successful. Therefore, this article is structured to reveal sample

selection, use of biopsy techniques and appropriate diagnostic methods to ensure the success of PGD.

Indication of PGD

Initially, PGD was performed to determine the presence of Mendelian inherited disorders. Mendelian disorders are single gene disorders that are described based on pedigree patterns, namely autosomal dominant, autosomal recessive, X chain (recessive and dominant) and Y chain. Examples of disorders in Mendelian pattern are cystic fibrosis, beta thalassemia, sickle cell anemia, myotonic dystrophy, Huntington's disease, fragile X syndrome and spinal muscular atrophy [3,4].

Furthermore, the development of PGD technology allows for the examination of polygenic disorders whose character and phenotype depend on multiple loci which are non-Mendelian disorders. These polygenic disorders are more complex than single gene disorders and are often influenced by factors outside the locus of the nuclear genetic material, such as epigenetic modifications, mitochondrial (and mitochondrial DNA) abnormalities, post-translational modifications and environmental contributions. Examples of polygenic disorders are breast cancer, rheumatoid arthritis and multiple sclerosis. Meanwhile, cardiac abnormalities, cleft palate/lip and some behavioral-related diseases are still not fully understood because polygenic, epigenetic and environmental contributions to phenotype are not fully understood [3, 4, 5, 6].

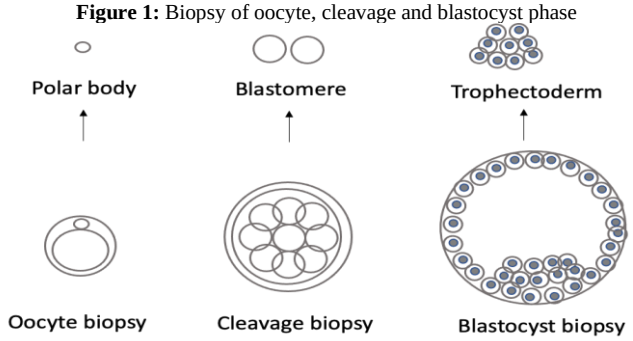
Meanwhile, PGD is also indicated for chromosomal abnormalities. Abnormalities in the number and structure of chromosomes allow spontaneous miscarriage and fetuses to carry abnormalities, although most are caused by abnormalities in the number of chromosomes. Abnormal numbers found are polyploidy, monosomy and trisomy. While structural abnormalities such as translocations, inversions, deletions and other chromosomal structural abnormalities, have a lower incidence of causing miscarriage [7, 8].

Mitochondrial abnormalities are an indication for PGD, although research in this area still needs further development. Mitochondrial abnormalities are caused by mutations in mitochondrial DNA, whose phenotype and affected tissues are difficult to recognize until a number of mitochondrial mutants reach the cell's tolerance limit. Many cells were identified with mitochondrial abnormalities due to heteroplasmy, i.e. a certain percentage of normal and mutant mitochondria exist together in one cell. Mitochondrial abnormalities are usually inherited maternally. Examples of mitochondrial disorders are Leigh syndrome, or neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) [3, 7, 9, 10].

Sample of biopsy

Biopsy can be performed from various samples, namely (1) polar body I and/or II biopsy of mature oocytes and/or zygote (to examine embryo genotype), (2) blastomere biopsy at the 4-8 cell division stage of the embryo (3 days after birth). fertilization) and (3) blastocyst biopsy (days 5-6 after fertilization) to collect trophectoderm cells [11].

Figure 1: Biopsy of oocyte, cleavage and blastocyst phase



Polar body biopsy is perhaps most valuable for examination by the fluorescence in situ hybridization (FISH) technique which detects aneuploidy with the assumption that most cases are caused by errors in cell division in the oocyte during meiosis. However, it is recommended that biopsies be performed on polar bodies I and II due to the substantial alterations for the occurrence of errors in meiotic divisions I and II. Polar body biopsy can be applied to examine maternally inherited single gene disorders but necessitates a more complicated technique and frequently requires biopsy of both polar bodies [12, 13].

The majority of PGD cases use the embryo biopsy technique, either at the blastomere stage or at the blastocyst stage. Even though it is still under debate, some researchers agree that the uptake of one or more cells at the division stage (4-8) of embryonic cells can cause damage to their development and viability; even without a second cell to analyze, no results, inaccurate or imprecise results may increase. While the blastocyst stage biopsy is more promising because of the higher number of cells and it is safer to collect but with limited time available for genetic testing because it is necessary to transfer the embryo to the uterus on the same day or the next day (days 5-6 after fertilization). In addition, there are fewer embryos that survive in vitro that extend the blastocyst stage, so less embryos are accessible for biopsy at the blastocyst stage. In contrast, the ability of the embryo to survive to the blastocyst stage makes it possible to produce a larger live birth, which is a major goal in patients undergoing PGD [3, 12, 13].

Because polar bodies can be biopsied with less risk of harming the oocyte compared to blastomere biopsy and blastocyst biopsy, polar bodies can be used for indirect genetic analysis of oocytes, which are part of IVF.

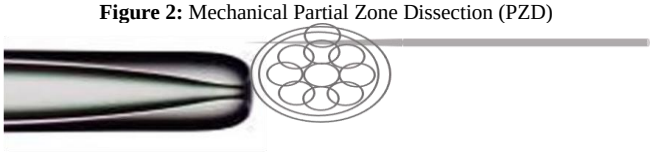
Techniques of sampling

The process of sloughing off the oocyte or embryo cell membrane on a biopsy, can be done through several techniques, namely

Mechanical Partial Zone Dissection (PZD)

The PZD procedure is performed using two needles to create slits perpendicular to each other towards the zone. The cross-shaped slit allows access to the peri vitelline space and simultaneously transfers the two polar bodies even when the two polar bodies are located apart [14].

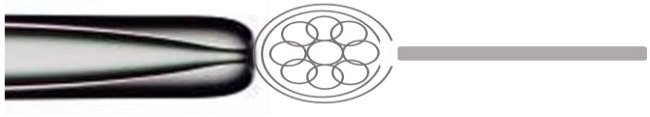
Figure 2: Mechanical Partial Zone Dissection (PZD)



Chemical Drilling Zone

This procedure is performed using acid tyrode (pH 2.3) to create large, round holes in the zona pellucida area. (Figure 3) For this chemical zone drilling, oocytes or embryos are manipulated individually and immediately washed to avoid a higher pH difference as local acidification of the culture medium or acid deposition into the peri vitelline region which may cause cell lysis or another small cell damage [15].

Figure 3: Chemical Drilling Zone



Laser-assisted Dissection

This technique is most relevant procedures in sampling are non-traumatic, timely opening of the zona pellucida, removal of the complete polar body and accurate diagnosis of the disease. The introduction of the zone opening is assisted by a laser that enters the area where the polar body and the embryo to be biopsied are located. Simultaneous removal of the polar body and embryo can reduce the overall biopsy time. This biopsy procedure should be performed by a person trained for polar body biopsy to avoid any damage [5,9,16].

Methods of diagnosis

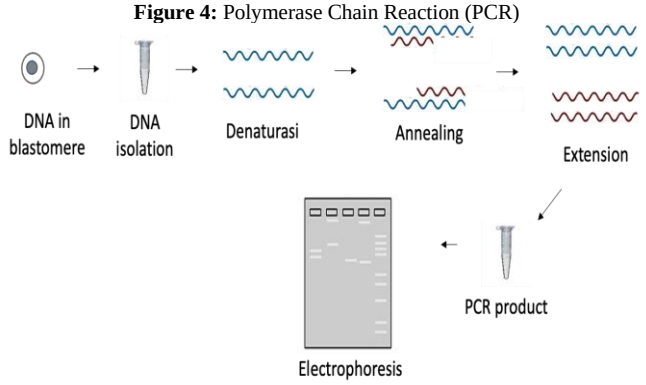
Furthermore, after obtaining a biopsy sample, a diagnostic method is used to detect the abnormalities found.

Polymerase Chain Reaction (PCR)

This PCR technique is used for diseases caused by single gene disorders caused by mutations in DNA [6,7]. The main principle of PCR is to amplify a specific segment of DNA, two primers having a sequence complementary to that DNA segment are used. (Figure 4) The two primers will hybridize to opposite strands of the target DNA, so that DNA polymerase can extend the sequence between the two primers. Each cycle produces a complementary DNA strand of the target gene. This causes the product of each cycle to be doubled, giving an exponential increase in the total number of DNA copies synthesized [17].

Despite the risk of contamination associated with single-cell PCR, problems inherent in this method such as exclusive amplification of a single cell in the allele carrying the disease under investigation (allele drop-out) and amplification failure which occurs in 10-20% of cases, if not noticed, it can lead to misdiagnosis. Another problem is the polar body biopsy, which only carries the maternal genome so that only abnormalities of maternal origin or X sequences can be examined [5,17].

Figure 4: Polymerase Chain Reaction (PCR)

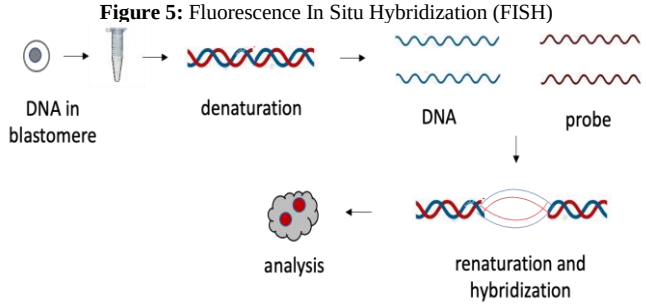


Fluorescence in Situ Hybridization (FISH)

The FISH technique can be used to detect chromosomal abnormalities caused by failure of chromosome distribution during meiotic division. Failure of chromosome distribution causes monosomy and trisomy which is often found during pregnancy and causes miscarriage. With the FISH technique, it can determine how many chromosomes or chromatids are examined from polar bodies I and II [5,9, 17].

FISH results on polar body biopsies indicate indirect conclusions that can be drawn to describe the number of chromosomes present in oocytes. With the current FISH technique, up to 6 probes can be performed in one assay. The failure that can occur using this technique is the presence of FISH drop-out, which is caused because one probe fails to detect the chromosome to be examined and the chromosome actually exists. The frequency of FISH drop-outs is estimated at 2-3% per examination (Figure 5) [5.9, 17]

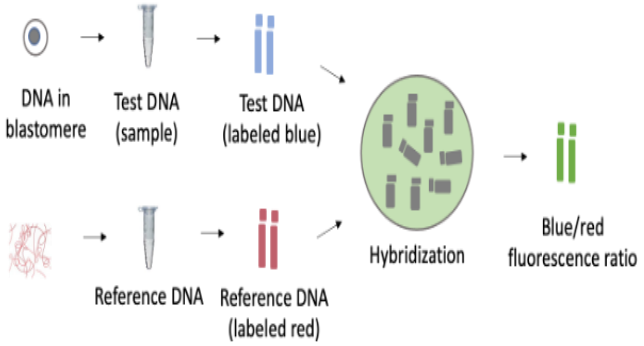
Figure 5: Fluorescence In Situ Hybridization (FISH)



Array Comparative Genomic Hybridization (Array CGH)

Since detection of aneuploidy in the cell division phase is not the best approach due to the high incidence of chromosomal mosaicism, the European Society of Human Reproduction and Embryology (ESHRE) initiated the use of CGH arrays in polar body biopsies, to analyze chromosomal complement in polar bodies I and II (Figure 6) [17].

Figure 6: Array Comparative Genomic Hybridization (Array CGH)



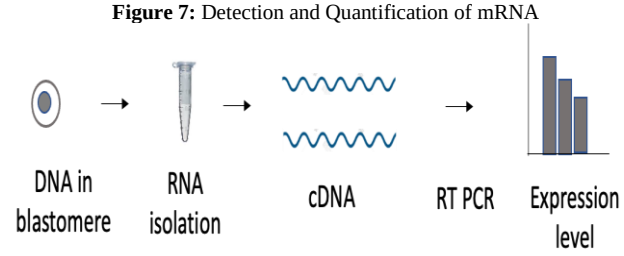
CGH arrays make it possible to detect entire chromosomes simultaneously. Although this technique has been applied to investigate aneuploidy in oocytes and polar bodies in some cases, this technique has not been routinely performed, due to the length of time required (according to the original protocol). However, now a faster CGH protocol has been developed which is around 12 hours and 18 hours for all stages of analysis prior to evaluation [9, 17].

The faster time with the CGH array indicates that the detection of aneuploidy chromosomes from the oocyte is reliable with times that can be estimated by the CGH array of the two polar bodies. The results showed that 94% of the polar body examinations were positively correlated with the condition of the oocytes.

Detection and Quantification of mRNA

Mature oocytes have unique properties, namely the ability to incorporate foreign DNA, directly regulate gene expression and early embryo differentiation. These functions require changes in the mRNA population. Gene expression of oocytes can be evaluated on mRNA which represents genetic function and epigenetic downstream effects that affect oocyte development. The important function of cytoplasmic factors in oocytes is supported by the observation that oocytes can divide parthenogenetically without sperm and that enucleated oocytes can fully support embryonic development after nuclear cell transfer (Figure 7) [17].

Figure 7: Detection and Quantification of mRNA



The ability to detect and compare individual differences in gene expression in oocytes without disturbing the oocyte can be accomplished by detecting mRNA in single-cell polar bodies.

Polar bodies resulting from meiosis II are processed first to obtain mRNA and reverse transcription (RT) PCR is performed to obtain cDNA. The cDNA product was then specifically and exponentially amplified for each candidate gene (12 candidate genes) with high expression in meiosis II oocytes. Gene expression was then analyzed using Real-time TaqMan qPCR. The result of mRNA quantification in polar bodies was that more than half of the candidate genes replicated. This indicates the presence of mRNA in the polar body [19,20].

Detection and quantification of mRNA in polar bodies is possible and reflects the transcript profile of oocytes resulting from meiosis II division. The resulting transcripts have varying expression in different oocytes and this variation can be reflected in the polar body. This variation probably reflects the many factors seen in oocytes during their development including nutritional status and the disturbances that occur. Genes with high expression in oocytes could be detected in polar bodies, suggesting that failure to identify specific mRNAs in polar bodies is associated with critical oocyte transcript levels [19,20].

CONCLUSIONS

Preimplantation genetic diagnosis is an assisted reproductive technology for embryo screening by detecting defects caused by gene and/or chromosomal abnormalities. The ultimate goal of this screening is to prevent implantation of the fetus or limit the risk of transferring embryos carrying genetic disorders.

Polar body I and II biopsy are one way to detect genetic disorders that occur in oocytes more safely, the risk of harming the oocyte is less and causing no damage to oocyte development and viability compared to blastomere biopsy and blastocyst biopsy techniques. Polar bodies have the same genetic material as oocytes and the results obtained from the diagnosis of polar bodies are a reflection of the condition of the oocyte.

Several techniques can be used for biopsy of polar bodies I and II such as Mechanical Partial Zone Dissection (PZD), chemical zone drilling and laser-assisted dissection. The methods that can be used for the diagnosis of polar bodies are PCR, FISH, CGH arrays, detection and quantification of mRNA and polar body fragmentation. The selection of the inspection method is adjusted to the purpose of the examination.

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