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## Review Article

# New perspectives on preimplantation genetic diagnosis and preimplantation genetic screening



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## ABSTRACT

Preimplantation genetic diagnosis is a procedure that involves the removal of one or more nuclei from oocytes (a polar body) or embryos (blastomeres or trophectoderm cells) in order to test for problems in genome sequence or chromosomes of the embryo prior to implantation. It provides new hope of having unaffected children, as well as avoiding the necessity of terminating an affected pregnancy for genetic parents who carry an affected gene or have balanced chromosomal status. Polymerase chain reaction-based molecular techniques are the methods used to detect gene defects with a known sequence and X-linked diseases. The indication for using this approach has expanded for couples who are prevented from having babies because they carry a serious genetic disorder to couples with conditions that are not immediately life threatening, such as cancer predisposition genes and Huntington disease. In addition, fluorescent *in situ* hybridization (FISH) has been widely applied for the detection of chromosome abnormalities. FISH allows the evaluation of many chromosomes at the same time, up to 15 chromosome pairs in a single cell. Preimplantation genetic screening, defined as a test that screens for aneuploidy, has been most commonly used in situations of advanced maternal age, a history of recurrent miscarriage, a history of repeated implantation failure, or a severe male factor. Unfortunately, randomized controlled trials have as yet shown no benefit with respect to preimplantation genetic screening using cleavage stage biopsy, which is probably attributable to the high levels of mosaicism at early cleavage stages and the limitations of FISH. Recently, two main types of array-based technology combined with whole genome amplification have been developed for use in preimplantation genetic diagnosis; these are comparative genomic hybridization and single nucleotide polymorphism-based arrays. Both allow the analysis of all chromosomes, and the latter also allows the haplotype of the sample to be determined. The promising results of these two approaches will inspire further validation of these array platforms, even at the single-cell level. It remains to be decided which embryo stage is the best for biopsy. Moreover, if randomized controlled trials are confirmed to play a role in increasing delivery rates, this will be a major step forward for assisted reproductive technology patients around the world.

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## Introduction

Preimplantation genetic diagnosis (PGD) is a procedure that involves the removal prior to the implantation of one or more nuclei from oocytes [a polar body or bodies (PBs)] or embryos (blastomeres or trophectoderm cells) to test for mutations in the genome sequence or for chromosomal aneuploidy. In 1990, Handyside et al [1] first applied PGD to the detection of X chromosome-

linked diseases in order to achieve successful pregnancies. PGD techniques are widely carried out by many *in vitro* fertilization (IVF) centers and provide patients with new hope of having unaffected children, as well as avoiding the necessity of terminating an affected pregnancy for couples carrying affected genes or chromosome problems.

Preimplantation genetic screening (PGS) is defined as a test screening for aneuploidy by means of PGD in specific groups of patients. Both genetic parents have no known genetic or chromosomal problem. The first pregnancies using this approach were reported by Verlinsky et al [2], and these were obtained using embryos following aneuploidy screening using a fluorescent *in situ* hybridization (FISH) probe for chromosomes X, Y, 18, and/or 13/21.

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A woman's age is associated with increasing aneuploidy, which means that there is an increased risk of implantation failure and miscarriage. It is well known that maternal age significantly affects the incidence of aneuploidy in clinically recognized pregnancies; it dramatically increases from 2% to 3% in the 20- to 25-year group to 35% among women older than 40 years [3,4]. Therefore, PGS has been most commonly used in women of advanced age. In addition, PGS is also applied when women have a history of recurrent miscarriage, when women have a history of repeated implantation failure (RIF), and when women have a partner with severe male factor, because of the higher percentage of aneuploidy in embryos from these groups [5–13]. However, the efficacy of PGS in clinical practice remains under debate.

This review explores the technology and clinical application of PGD and the effect of PGS on the probability of live birth after IVF.

### **Biopsy procedure**

PGD is a procedure that requires the removal of one or more cells from embryos in order to have sufficient genetic material for diagnosis (Table 1). Biopsies are performed using a range of different methods including PB biopsy from an oocyte or zygote, cleavage stage biopsy, and blastocyst biopsy. Cleavage stage biopsy is the most common type of biopsy worldwide, and it has been reported that 90% of PGD is performed by removal of one or more blastomeres at cleavage stage [14].

The first PB extruded from the oocyte prior to fertilization and the second PB extruded after fertilization are both not necessary for subsequent embryo development [15]. Therefore, the first or second PB can be biopsied without damaging embryo development. However, it has been shown that approximately 50% of oocytes with single chromatid errors in the first PB undergo normal embryonic development [16]. Postzygotic aneuploidy during embryogenesis cannot be detected by PB biopsy [17]. Hence, PB biopsy is limited to only maternal aneuploidies. Nevertheless, approximately 90% of human aneuploidy originated during maternal meiosis [18]. Because of this, the European Society for Human Reproduction and Embryology (ESHRE) PGS task force hopes to use PB biopsy to detect all chromosomes using molecular techniques [microarray comparative genomic hybridization (CGH), multiplex ligation-dependent probe amplification (MLPA), etc.] [19].

**Table 1**  
Differences between the biopsy methods used for preimplantation genetic diagnosis.

Stage of biopsy	Advantages	Disadvantages and limitations
Oocyte/zygote (polar body)	No effect on subsequent embryo development Enough time to perform analysis prior to transfer	Only one or two cells available for analysis Only maternal message obtained DNA liable to degenerate
Cleavage stage	Diagnosis of maternal and paternal inherited disease Possibility of sex determination Up to two cells available for analysis	Limited time for analysis High incidence of chromosomal mosaicism
Blastocyst	Fewer number of embryos to be biopsied and fewer specimens to process Three or more cells per embryo available to overcome allele dropout Less problem of mosaicism	Occasions when embryos failing to blastocysts Need for cryopreservation most of the time

Cleavage stage biopsy involves one or two cells being biopsied from embryos if six or more blastomeres are present. The time of biopsy is on the morning of Day 3 (68–72 hours) after insemination. Magli et al [20] recommended that very poor quality embryos are not suitable for PGD. The issue exists as to whether one or two blastomeres should be removed during cleavage stage biopsy. Human embryo biopsy for PGD should be envisaged as a balance between cell retrieval in order to allow a safe and correct diagnosis of the embryo on the one hand, and safeguarding the implantation potential of the biopsied embryo on the other. Removing one blastomere is less invasive than removing two blastomeres and results in greater numbers of top-quality blastocysts, good-quality blastocysts, and early blastocysts on Day 5 [21,22]. Nevertheless, some tests may require the biopsies of two cells to achieve an acceptable level of diagnostic accuracy.

In recent years, blastocyst biopsy has been increasingly used, especially over the past 5 years. The human blastocyst consists of an outer layer of trophectoderm cells and an inner cluster of cells, the inner cell mass. The procedures for blastocyst biopsy include zona opening on D3 or on D5 together with removal of trophectoderms. The first human blastocyst biopsy was performed in 1990 by Dokras and colleagues [23]. The advantages of blastocyst biopsy are that it can provide more than three blastomeres for analysis and can overcome the problems of allele dropout (ADO) and amplification failure that are encountered during single cell polymerase chain reaction (PCR) and FISH. The availability of more cells ought to increase diagnostic accuracy. Chen et al [24] suggested that blastocyst biopsy provides the advantages of being relatively cost-effective and being less labor-intensive compared with cleavage stage biopsy. However, the application of blastocyst biopsy depends on embryo development, and only 50–60% of embryos are able to reach blastocyst stage.

A recent systemic review by van Echten-Arends et al [25] showed that the rate of chromosomal mosaicism of cleavage embryos appears to be as high as 72%. Even at the blastocyst stage, Fragouli et al [26] demonstrated that 32.4% of blastocysts are mosaic. Mosaic diploid–aneuploid blastocysts with >30% normal cells account for <6% of the analyzed embryos. Although meiotic and postzygotic errors leading to mosaicism are common, most mosaic blastocysts contain no normal cells. They concluded that aCGH (array CGH) trophectoderm analysis is an accurate aneuploidy detection tool that can assist in the identification of viable euploid embryos with a higher implantation potential.

### **Methods of genetic diagnosis**

FISH uses DNA probes labeled with distinctly colored fluorochromes that can bind to specific DNA sequences on chromosomes. FISH is used to detect chromosome status, which includes X-linked diseases, aneuploidy screening, and other structural rearrangements. Over the past decade, FISH has been widely applied for the detection of chromosome abnormalities [26–31]. FISH allows the evaluation of many chromosomes at the same time, specifically up to 15 chromosome pairs using a single cell [32]. However, the application of FISH for PGD has several technical limitations including hybridization failure (lack of FISH signal), signal overlap, signal splitting, and poor probe hybridization, as well as problems associated with cell loss and variable cell fixation [33–36]. Some have reported that the error rate for FISH detection of chromosomal translocation accounts is in the range of 6% [33,34,37].

Different PCR-related methods, such as amplification refractory mutation system, restriction endonuclease digestion, heteroduplex analysis, single-strand conformational polymorphism analysis, denaturant gradient gel electrophoresis, fluorescent PCR, and multiplex PCR, have been developed to provide the ability to detect

single-gene defects within known mutation sequences and in relation to X-linked disease [38]. Usually, a specific protocol is required to carry out PGD when detecting a known genetic disease [39]. Table 2 summarizes the most common indications for single-gene defects based on the data collected by the ESHRE PGD Consortium [40]. In Taiwan, the most common indications for single-gene disorders are  $\alpha$ -thalassaemia and  $\beta$ -thalassaemia [41]. The indication for PGD has developed from the original driving force, which was couples who were at risk of having a baby carrying a serious genetic disorder to the conditions that are not immediately life threatening, such as cancer predisposition genes and Huntington disease. In the latter situation, the couples consider the genetic predisposition as serious enough to undergo IVF treatment with PGD in order to avoid the birth of affected children who will in the future probably suffer from the disease. In the ESHRE PGD Consortium report, it is clearly stated that the accuracy of PCR is as high as 94.1% [42]. However, the application of PCR techniques continues to encounter various problems, such as amplification failure, ADO, and contamination leading to misdiagnosis. The incidence of amplification failure can be as high as 10% [43]. The ESHRE guidelines recommended that the rate of ADO needs to be controlled and should be as low as 10% [44]. ADO is a phenomenon whereby only one of the two alleles is successfully amplified and is equally likely to affect either of the alleles in a heterozygous cell. The ADO frequency can be estimated, but it is not possible to predict which allele will be affected in a given reaction. ADO may be a problem when examining heterozygous embryos. When the genetic disease is dominant, the ADO of the affected allele will lead to misdiagnosis. When the genetic disease is recessive, the ADO of the normal allele will also lead to a misdiagnosis. Both of the above factors may affect the usefulness of a PCR-based molecular technique.

CGH is a technique that utilizes molecular genetics and cytogenetics [45]. DNA from the test sample and DNA from a normal control DNA are amplified respectively using whole genome amplification (WGA). Different WGA approaches have been reported, such as degenerate oligonucleotide primer PCR [46,47] and primer extension preamplification (PEP) PCR [48], as well as newer methods such as iPEP (improved PEP) [49], multiple displacement amplification [50,51], and GenomePlex [52]. So far, there seems to be no universal WGA. The amplified DNAs are then differentially labeled with one of two fluorochromes, for example, red for the test DNA and green for the control DNA. After labeling, both DNAs are mixed together in equal proportions and are allowed to competitively hybridize to either the metaphase spreads from a normal

male control cell line (m-CGH) or onto an array platform containing small pieces of chromosome (a-CGH). Both m-CGH and a-CGH have been applied in clinical practice [18, 44, 53–56]. However, the time-consuming nature of m-CGH has hindered the wide application of this approach to PGS. By contrast, the protocol for a-CGH has been made user-friendly and the time needed to complete the analysis has been shortened; as a result, it is being increasingly used in clinical practice. Traversa et al [57] demonstrated the reliability and feasibility of the CGH approach for the detection of aneuploidy in patients with RIF and recurrent miscarriage. Nonetheless, there are still some inadequacies associated with the CGH approach. These include the inability to differentiate between balanced translocations and inversions, and the inability to detect some types of specific ploidy status, such as polyploidy or monoploidy. Furthermore, the systems cannot detect changes in DNA sequences (point mutations, intragenic insertions or deletions, triplet repeat expansion, etc.). Finally, they are unable to find gains or losses in regions of the genome not covered by the array [58].

Single nucleotide polymorphisms (SNPs), which are variations at a single site in DNA, are the most common type of variation in the genome. SNP microarray analysis is used to detect polymorphisms that exist at a frequency greater than 1% within a population. SNPs not only can be applied for single-gene disorder detection as well as the detection of aneuploidy, but also can be used for determining which parental haplotype has been transmitted to the embryos [59–61]. However, there are several limitations to using SNPs in clinical practice. These include the fact that the association of copy number variants with clinical information remains unknown and that parental DNA is required for linkage analysis. The accuracy of SNPs has been reported to be also limited by contamination and ADO. Johnson et al [62] found that for the aneuploidy screening there was a false detection rate of 2.1% using SNP methods compared to 1% by traditional metaphase karyotyping. In addition, Bisignano et al [63] pointed out the poor quality of a considerable amount of SNP genotype information generated by whole-genome amplification as well as the difficulty of predicting haplotypes. Regardless of the method used for WGA, ADO rates for heterozygous SNPs can be as high as 40–50% [63]. Nevertheless, taking this into account, Handyside [64] believed that the SNP-based array approach with optimized protocols and parental support algorithms may be particularly suited to certain applications such as the PGD of single-gene defects and translocation chromosome imbalance combined with comprehensive detection of aneuploidy. In translocation cases, the normal embryos can be differentiated from balanced ones.

## Issues affecting PGS

To the best of our knowledge, the strategy of aneuploidy screening using PGS for improving the IVF outcomes remains controversial. Indications for PGS in clinical practice have included advanced maternal age (AMA;  $>36$  years), RIF (e.g.,  $\geq 3$  embryo transfers with high-quality embryos or the transfer of  $\geq 10$  embryos in multiple transfers, exact numbers to be determined by each center), and recurrent miscarriage ( $\geq 3$  miscarriages) [65]. When applying PGS to AMA, the results of a prospective randomized controlled trial (RCT) showed that PGS in women with AMA resulted in a significantly decreased clinical pregnancy rate compared to those without PGS (8.9% vs. 24.5%) [66]. By contrast, a retrospective study by Milán et al [67] has suggested that PGS can improve reproductive success in patients older than 40 years, with a more than 2-fold increase in ongoing pregnancy rate compared to non-PGS patients. When using PGS for RIF, one RCT study of nearly 200 patients [68] reported that PGS did not increase the implantation rate and pregnancy rate after IVF intracytoplasmic sperm

**Table 2**

The list of common indications for single-gene defects based on information from the ESHRE PGD consortium [40].

Disease
Cystic fibrosis
Myotonic dystrophy
Huntington disease
Beta thalassemia
Fragile X syndrome
Spinal muscular atrophy
Hemophilia A
Duchenne muscular dystrophy/Becker muscular dystrophy
Human leukocyte antigen
Others <sup>a</sup>

Note. From "The causes of misdiagnosis and adverse outcomes in PGD" by L. Wilton, A. Thornhill, J. Traeger-Synodinos, K.D. Sermon, and J.C. Harper, 2009, Hum Reprod, 24, p. 1221–8. The Authors 2009. Reproduced with permission.

<sup>a</sup> Others: sickle cell anemia, neurofibromatosis type 1, tuberous sclerosis, von Hippel Lindau, familial adenomatous polyposis, Charcot–Marie–Tooth disease type 1, Marfan syndrome, familial amyloidotic polyneuropathy.

injection in women with RIF. However, there is a lack of randomized control that assesses the efficacy of PGS on patients with recurrent miscarriage, although some retrospective studies appear to indicate that there is a positive effect on patients with recurrent miscarriage [68–70]. One recent meta-analysis of RCT by Mastenbroek et al [71] concluded that PGS did not have a beneficial effect on clinical pregnancy rate, miscarriage rate, and live born rate among patient with AMA and RIF. Therefore, it would seem there is no clear evidence to support any beneficial effect of PGS on IVF outcomes. The general consensus at the moment is that RCTs have shown no benefit of PGS using cleavage stage biopsy, and this is probably attributable to the high levels of mosaicism at the cleavage stage and to the limitations of FISH. Furthermore, it would therefore seem most useful for PGS RCTs to concentrate either on the PB or trophectoderm biopsy approach and a full chromosome count [72–74]. The ESHRE PGS task force is running a multicenter RCT to determine whether PGS truly does improve IVF outcome in patients with AMA using PB biopsy and a-CGH.

## Conclusion

PGD provides new hope of having unaffected children and helps to avoid the possibility of terminating an affected spontaneous pregnancy when genetic parents carry an affected gene or have balanced chromosomal status. With the help of PCR-based molecular techniques, the indication for PGD has been expanded. In the past, FISH has been widely applied to detect chromosome abnormalities, and this has been applied to PGS in the situations of AMA, a history of recurrent miscarriage, a history of RIF, or a partner with very low sperm quality. Unfortunately, RCTs have not shown any benefit of PGS when used with cleavage stage biopsy, which is probably attributable to the high levels of mosaicism at cleavage stage and to the limitations of FISH.

Two main types of array-based technology combined with WGA are being developed for use with PGD and have been shown to have promising results. Further validation of these array platforms is needed, and it remains to be decided which is the best embryo stage to biopsy. If RCTs are able to show that array-based testing using an optimal embryo biopsy stage increases delivery rates, this will be a major step forward and help assisted reproductive technology patients around the world [74].

Finally, as Handyside [75] has noted, PGD should not be an option available to only a few couples at risk of a serious genetic condition who can afford the cost of the technique. With the increasing emphasis in medicine on early diagnosis and prevention of disease, together with the increasing availability of new molecular genetic diagnostic tools, a national IVF-PGD program would seem to be the next step in modern health care.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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