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RECENT ADVANCES IN PREIMPLANTATION GENETIC DIAGNOSIS - A REVIEW

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Abstract Preimplantation genetic diagnosis (PGD) is an important method for the identification of chromosomal abnormalities and genes responsible for genetic defects in embryos that are created through *in vitro* fertilization before pregnancy. As the list of conditions and indications for PGD testing is continuing to extend enormously, novel *in vitro* fertilization techniques and newly established genetic analysis techniques have been implemented in clinical settings in the recent years. Blastocyst-stage biopsy, verification techniques, time-lapse imaging, whole-genome amplification, array-based diagnostic techniques, and next-generation sequencing techniques are promising techniques for the accurate diagnosis of diverse genetic conditions and also for the selection of the best embryo that has the highest implantation capacity. The timing and technique used for biopsy, the amplification techniques, the genetic diagnosis techniques, and appropriate genetic counseling play important roles in establishing a successful PGD. In this review, those key points of PGD will be reviewed in detail.

Keywords: *PGD, chromosomal, genetic, in vitro, diagnostic*

1. INTRODUCTION

PGD involves the genetic testing of biopsy material from embryos that are generated through *in vitro* fertilization (IVF) techniques and involves the transfer of chromosomally normal and disease-free embryos to the uterus. PGD allows couples who have an increased risk of transmitting genetic disorders not only to have healthy children, but also to prevent complications such as health problems and the psychological and financial burdens that may result from the termination of a pregnancy. PGD was first performed by Handyside *et al* (1990) using the polymerase chain reaction (PCR) technique in order to prevent transmission of a sex-linked disorder. Since then, PGD has been applied to many different conditions, and the list of indications for PGD testing has extended enormously. Over those years, it is estimated that PGD has been performed for over 300 monogenic conditions; this treatment has resulted in the births of thousands of healthy children (Harton *et al*., 2011, Harper *et al*., 2008).

2. POLAR BODY BIOPSY

Polar body diagnosis (PBD) is a diagnostic method for the indirect genetic analysis of oocytes. Polar bodies are by-products of the meiotic cell cycle, which have no influence on further embryo development. The biopsy of polar bodies can be accomplished either by zona drilling, laser drilling within a very short time period. However, the paternal contribution to the genetic constitution of the developing embryo cannot be diagnosed by PBD. The major application of PBD is the detection of maternally derived chromosomal aneuploidies and translocations in oocytes. For these indications, PBD may offer a viable alternative to blastomere biopsy as the embryo's integrity remains unaffected, in contrast to preimplantation genetic diagnosis (PGD) by blastomere biopsy (PGDIS, 2008).

3. BLASTOMERE BIOPSY

Blastomere biopsy is accomplished at the cleavage stage, which is typically on day 3 of in vitro development. This technique is the oldest and most widely used method for PGD. In this method, one or two blastomeres are retrieved from embryos with more than six cells. With cleavage-stage (Capalbo *et al*., 2013) biopsy, both maternal and paternal meiotic errors can be detected. In addition, blastomere biopsy allows enough time for genetic analysis to be performed before the embryo reaches the blastocyst stage.

Modern *in vitro* fertilization practices involve transfer of embryos as blastocysts, when anabolic metabolism is well established and pregnancy rates can be maintained while transferring (Christopikou *et al*., 2013 and Geraedts *et al*., 2011) embryos singly to avoid multiple pregnancies. Embryo biopsy for preimplantation genetic diagnosis (PGD) (Xu and Montag, 2012), however, is generally performed on day 3, when the embryo comprises just 6 to 8 cells, one or two of which are removed for testing. For exclusion of genetic disease, day 5-6 blastocyst-stage biopsies are more likely to be followed by implantation and singleton births than is the case after PGD performed on day 3.

4. BF SAMPLING

BF is the liquid substance within the blastocyst cavity sealed in by the TE epithelium. Palini et al. showed, for the first time, the presence of genomic DNA in the BF by whole-genome amplification (WGA), quantitative PCR (qPCR), and analyzed multicopy genes such as TSPY1 and TBC1D3. This implies the possibility of using this method in sex-linked disorders screening. (Palani *et al*., 2013; Cohen *et al*., 2013). Gianaroli et al. (2014) used aCGH to evaluate BF biopsies for ploidy prediction in comparison with the conventional biopsy methods, i.e., blastocyst, blastomere, and PB biopsy, and concluded that BF biopsy was comparable to conventional biopsy materials for chromosomal analysis (Gianaroli *et al*., 2014). Nevertheless, discordance of ploidy prediction was observed between BF and blastocyst biopsy possibly due to the embryo quality (Kj *et al*., 2014), suggesting that more experiments and clinical trials should be performed before BF sampling is ready for PGD/PGS (Lu *et al*., 2016)

5. NONINVASIVE PRENATAL DIAGNOSIS

Not only PGD and PGS, but also the noninvasive prenatal diagnosis (NIPD) and noninvasive prenatal testing (NIPT) will offer some new options in prenatal diagnosis for carriers of single gene disorders and chromosomal constitution in fetuses. This will involve fertile patients who reject PGD, patients after PGD for result confirmation, those who reject amniocentesis (AC) or chorionic villus sampling (CVS), patients with previous loss of pregnancy because of the listed procedures, and so forth.

These carriers or patients at high risk for chromosomal or monogenic disorder are target groups for the health professionals working in the area of prenatal care. The cell-free DNA from the fetus has been found in the plasma of pregnant women, and this has been used successfully for noninvasive determination of the fetal gender and fetal RhD genotype in RhD negative women. The basis of these tests is the detection of fetal-specific DNA sequences in maternal plasma. The same approach of searching for fetal-specific nucleic acids, such as DNA methylation and mRNA markers in maternal plasma, has been proposed for noninvasive detection of fetal aneuploidies instead of performing invasive sampling of fetal genetic material through the AC or CVS.

As source for testing is the circulating in maternal blood 4-6% cell-free fetal DNA/RNA fraction in the 1st trimester of the pregnancy (PGDIS, 2008).

6. RECENT PERSPECTIVES ON SAMPLING APPROACHES

In PGD/PGS, there are three major biopsy methods: blastocyst biopsy, blastomere biopsy, and polar body (PB) biopsy. Blastocyst biopsy has been more widely used than PB biopsy and blastomere biopsy, especially in the past 5 years, due to its low misdiagnosis rate (Munne *et al*., 1994 and Novik *et al*., 2014) and cost-effectiveness (Greco *et al*., 2015 and Craft *et al*., 2010). Compared to the conventional biopsy methods, the newly developed non-invasive sampling methods have many advantages with regard to the ethical, legal, and economic issues.

7. MINIMIZING GENETIC RISKS FOR ART GENERATIONS

Epimutation is also a hot topic, since many PGD laboratories already provide diagnosis for some syndromes and many recent articles search for correlation between ART and some imprinting disorders. Therefore, the field of epigenetic inheritance seems to be a quite interesting area, especially because ART can induce epigenetic variation that might be transmitted to the next generation. The Angelman syndrome is a serious neurodevelopmental disorder although there are no estimates of its absolute risk after ART would be small (1 in 3000). Therefore, it seems unlikely that this would result in many couples requesting ART to decline treatment. Epimutations causing Beckwith-Wiedemann syndrome (BWS) are more frequent than those causing Angelman syndrome but, not in comparison to the risk of serious complications such as exomphalos and embryonal tumours, BWS is usually compatible with normal living. In order to provide prospective parents with accurate risk information, there is a pressing need to define the absolute risk of imprinting disorders after ART by prospectively following a cohort of ART children. It is acknowledged that many couples will still choose trying for pregnancy despite the known and unknown risks for the child. Suboptimal conditions during oocyte and embryo development may also lead to persistent changes in the epigenome influencing diseases susceptibility later in life. In order to minimize the risk it is clear that the prolongation in vitro culturing to blastocyst stage should be very well optimized. The oocytes with big smooth endoplasmic reticulum (SER) aggregation might be followed by increased frequency of imprinting disorders. Therefore, their use for fertility treatment must be limited. It is also not known how the embryo biopsy affects the embryo quality. However, apparently the highest risk for rare imprinting disorders in children born following ART remains higher-order multiple pregnancies. Today a successful pregnancy is mainly defined by the outcome at birth; however, the consideration for the consequences of ART conditions for later life remains. The fetuses adaptations to under nutrition are associated with changes in the concentrations of fetal and placental hormones. Maternal reproductive health is a reflection of events over generations. It is multifactorial, environmentally sensitive and involves genes undergoing reprogramming during the critical period of gametogenesis. It is now widely accepted that the adverse preconceptional and intrauterine environment is associated with epigenetic malpro-gramming of the fetal metabolism and predisposition to chronic, in particular metabolic disorders, later in life—or the so-called "Barker hypothesis" regardless if the child is born following assisted or natural conception. There are also some new forthcoming horizons of the meaning of miRNA, siRNA, and piRNA that may play an important role in many biological processes including differentiation of male reproductive cells, and they all may have control over the gene expression and need to be elucidated further (Geraedts *et al.,* 2011).

8. RECENT ADVANCES IN ANEUPLOIDY TESTING

Although PGD first emerged as a diagnostic tool for the elimination of SGDs, it has been more widely used for the purpose of eliminating chromosomal abnormalities in pre implantation embryos and for increasing the take-home baby rates for IVF treatments. Aneuploidy is defined as a numerical chromosomal abnormality that results in a deviation of the number of copies of any of the 23 pairs of chromosomes. Aneuploidy can originate from either an excess number of chromosomes (eg, trisomy) or from missing chromosomes (eg, monosomy). Haploidy (one set of chromosomes [n]) and triploidy (3n) are associated with the abnormalities of whole chromosome sets. Aneuploidy significantly contributes to IVF failures and is the major cause of the first trimester miscarriages (Forman *et al*., 2013).

A diagnostic method combining the principles of FISH and the fixation of a single blastomere from cleavage-stage human embryos in an attempt to provide an alternative to the PCR technique for the determination of the sex of the embryos. After that, a short FISH procedure was developed in order to screen aneuploidy; this procedure uses fluorochrome- and digoxigenin-labeled DNA probes that are specific for the chromosomes most involved in miscarriages (X, Y, 18, 13, and 21) (Munne *et*

al., 1993) . The FISH technique was also used to test polar bodies (Verlinsky *et al*., 1996 and kuliev *et al*., 2003). This procedure involved either the simultaneous or sequential removal of two polar bodies from a fertilized egg, and it was done with the aim of diagnosing chromosomal nondisjunctions and premature separation of sister chromatids during maternal meiotic divisions. After an improvement of culture media to better support blastocyst-stage embryos (McArthur *et al*, 2005).

The FISH technique was coupled with a trophectoderm tissue biopsy for the purpose of testing for aneuploidy in blastocysts. The FISH technique has also been used successfully in the diagnosis of unbalanced products and embryos that belong to balanced rearrangement carriers. Because cases for translocation and inversion carriers are different from aneuploidy cases, there is a need for a preclinical workup study in order to both confirm the rearrangement breakpoint regions and to test the efficiency of the probes that will be used for PGD. For this preclinical study, at least ten metaphase cells and 100 interphase cells should be analyzed with the same probe combination to assess signal specificities and any possibility of polymorphism in the probe-specific regions that may cause difficulties in signal interpretation during PGD. Regardless of the embryonic stage during which the biopsy is performed, the FISH technique has many limitations. The number of chromosomes that are screened is limited, and the subjectivity of the method itself leads to inaccurate results when the technique is used suboptimally. The FISH method is only available to screen a maximum of 9–12 chromosomes and it is able to detect 60%–80% of all aneuploid embryos. Given the fact that aneuploidy could affect any of the chromosomes, FISH still cannot detect a significant proportion of the aneuploidies and segmental abnormalities. The second limitation is suboptimal fixation, since the quality of the FISH results is highly dependent on the quality of the nucleus, which is limited by the experience of the laboratory personnel who performs micromanipulations, such as fixation and biopsy procedures. These issues are considered to be the major reasons that previous randomized controlled trials have failed to show any benefit of PGD in improving live birth rates (Munne *et al*., 2012).

9. PGD BY MULTIPLEX PCR METHOD

The most common method of PGD of SGD involves the use of a single-cell multiplex PCR for the amplification of short tandem repeat (STR) polymorphic markers that are located in close proximity to the mutation site. Those polymorphic STR markers are repeats of DNA that are mostly heterozygous and whose sizes vary greatly among individuals. STR length values that are linked to the mutated allele can be determined via fragment analysis using paternal and maternal genomic DNA prior to PGD, and the genotypes of the generated embryos can be diagnosed by linkage analysis during PGD (Harper *et al*., 2008).

10. SNP ARRAYS

An SNP is a variation at a single position (single DNA base pair) in a DNA sequence. An SNP microarray contains immobilized DNA sequences. This detection enables karyomapping, a process in which the SNP genotype of a person is determined. For SGDs, the SNP genotypes of the parents and a reference (an affected child or another affected relative) can be analyzed at a gene of interest, and linkage information can be used to select unaffected embryos during PGD. (Natesan *et al.,* 2014 and Handyside *et al.,* 2010). The main advantage of karyomapping over the traditional, targeted approach is that karyomapping is applicable to any inherited SGD within the informative SNP loci without the development of costly, time-consuming, and laborious patient- or disease-specific designs. In addition, SNP data enable the detection of chromosomal abnormalities (meiotic trisomies, monosomies, and deletions) the elimination of those embryos may improve implantation rates and lower miscarriage rates. Karyomapping data can also be used for HLA typing via the use of SNP data over the HLA region located on chromosome. However, the major disadvantage in karyomapping is that it does not include a mutation detection method; therefore, a reference (an affected child or another affected relative) is always needed to establish linkage information, which is not always available for every couple. Similarly, it is not possible to apply karyomapping to

de novo mutation cases without additional work with direct mutation testing. Since array technology is used in the karyomapping technique, the cost of the test per embryo is currently higher than that of the traditional multiplex PCR technique. But it should be noted that the preclinical workup cost, which is necessary for the existing STR method, is not required in karyomapping; this saving may result in the reduction of costs overall (Forman *et al*., 2011).

11. NGS

Although recent advances in NGS have created opportunities for possible PGD applications. The experience with NGS on single cells is limited. NGS has been performed successfully on single tumor cells to quantify genomic copy number (Navin *et al*., 2011) and on sperm cells to determine recombination hot spots (Wang, 2012). The sensitivity and specificity of this method depends on the sequencing depth and the coverage of the regions of interest. Complex bioinformatic analyses are necessary to provide a sequence analysis since there is a large quantity of data obtained through massive parallel sequencing. First applications of the NGS technique on PGD for monogenic disorders (Treff *et al*., 2013) involved a targeted, semiconductor technology-based NGS method, which was used with a bar-coding protocol that gave results in less than 24 hours. Genotype results from the NGS method were consistent with the results of the conventional STR method. However, further studies are necessary to establish the accuracy and reliability of this technique, as well as its applicability for single blastomeres. NGS will strengthen the place of PGD in ART and increase the demand for PGD. Currently, the choice of technique depends mostly on the indication (whether the purpose is either mutation testing or chromosomal analysis) and on the cost, the availability, and the applicability of the technique. As the list of conditions and indications for PGD testing is continuing to extend enormously, the techniques have been evolving toward a universal method for the simultaneous diagnosis of multiple types of genetic conditions they are: Monogenic disorders, HLA typing, Aneuploidy screening, Selection of the best embryo that has the highest implantation capacity. The development of such a method will be possible in the near future (Forman *et al*., 2013).

12. CONCLUSION

It can be concluded that the preimplantation and prenatal genetic diagnosis and screening are of enormous value for providing healthy baby to couples with genetic disorders or for preventing the repeated spontaneous miscarriages. Nevertheless, there are some concerns about the aggression of the embryo biopsy by itself and potential epigenetic disturbance; therefore, there are some new noninvasive approaches for evaluation of the genetic status of human embryos and fetuses by a nondirect manner. Some of these approaches are interesting and seem to be quite promising, but further research is needed to elucidate if some of them could replace the existing procedures in the future or can only have additive value in diagnosis.

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