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PREIMPLANTATION GENETIC DIAGNOSIS APPLICATION INDICATIONS AND TECHNIQUES, A REVIEW

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ABSTRACT

Pre-implantation genetic diagnosis (PGD) is a reproductive technology that is being proposed to improve the effectiveness of in vitro fertilization (IVF) by screening for embryonic aneuploidy using fixed embryo samples prior to implantation. Indications for PGD varies depending on center, but in general they include advanced maternal age, recurrent pregnancy loss, recurrent IVF failure, severe male factor infertility, confirmed chromosomal rearrangement carrier, and bad obstetric history. Several diseases and disorders that can be screened prior to preimplantation are categorized generally as chromosomal abnormalities and monogenic diseases. The genetic analysis is performed by fluorescent in situ hybridization (FISH) for cytogenetic diagnosis, or polymerase chain reaction (PCR) for molecular diagnosis. To improve the accuracy of the diagnosis, new technologies are emerging, with comparative genomic hybridization (CGH) and microarrays.

KEY WORDS: Pre-implantation, in vitro fertilization, aneuploidy, genetic screening.

1. INTRODUCTION

Chromosome abnormalities present in an estimated 10-30% of all fertilized eggs. It has been estimated that approximately 40 to 60% of human embryos are abnormal, and after the age of 40 for females, this number can be as high as 80%.[1-2] Pre-implantation genetic diagnosis (PGD) is a reproductive technology that is being proposed to improve the effectiveness of in vitro fertilization (IVF) by screening for embryonic aneuploidy using fixed embryo samples prior to implantation.[3, 4] Possible impacts or results of embryos that have aneuploidy include poor embryonic development, chromosomal abnormalities, miscarriage, or IVF failure.[2] Preimplantation genetic testing for aneuploidy (PGT-A) which is previously termed preimplantation genetic screening (PGS), was developed in the late 1980s as an alternative to prenatal diagnosis for couples at risk of transmitting a genetic or chromosomal abnormality to their children [4, 5]. In the mid-1990s, several groups used PGD technology as an adjunct to IVF as an embryo selection tool for patients with advanced maternal age or repeated implantation failure.[6, 7]

2. Applications

Pre-implantation genetic diagnosis (PGD) is a well-established application of genetic testing when performing in vitro fertilization cycles as it contributes to diagnosis of many genetic disorders.[8] In this study we reviewed the most common aspects of pre-implantation genetic diagnosis and its impact in the clinical practice. Embryonic aneuploidy is highly prevalent in in vitro fertilization (IVF) cycles and contributes to decreased implantation rates, IVF cycle failure and early pregnancy loss. Preimplantation genetic screening (PGS) selects the most competent (euploid) embryos for transfer, and has been proposed to improve IVF outcomes and then offers couples at risk the chance to have an unaffected child, without facing termination of pregnancy.[9, 10]

3. Indications

Indications for PGD varies depending on center, but in general they include advanced maternal age, recurrent pregnancy loss, recurrent IVF failure, severe male factor infertility, confirmed chromosomal rearrangement carrier, and bad obstetric history. However the most common indication for PGD/PGS was recurrent spontaneous abortion, X-linked diseases or single gene disorders.[11, 12] Age is an important factor that contributes in euploidy, clinical pregnancy and early abortion rate. Table 1 is a result of study carried out by Won et al., and "presents clinical outcome of PGD group according to age. Euploidy rates and clinical pregnancy

rates decrease as age gets older. Reversely, early abortion rate increases as age gets older".[11]

4. Prevalence

To our knowledge, no statistical data are available about the accurate incidence of chromosomal abnormalities worldwide. In Saudi Arabia, 802 embryos were tested by the biopsy method and 290 are found to be abnormal embryos. The structural and numerical abnormalities were found to be 16.8 %.[12] The European Surveillance of Congenital Anomalies database estimated 10 323 cases with a chromosome abnormality, giving a total birth prevalence rate of 43.8/10 000 births.[13]

5. PGD for genetic abnormalities

Several diseases and disorders that can be screened prior to preimplantation were summarized in table (2) and categorized generally as chromosomal abnormalities and monogenic diseases. PGD has been shown to be an effective strategy for couples who had pregnancies with Down syndrome previously.[14] Moreover, it should be considered in the infertility treatment of the patient with mosaic Turner syndrome and in cases with Klinefelter's syndrome, and this information should be discussed with the couple when genetic counseling is given.[15, 16] Additionally, this approach is accurate and applicable to a larger number of patients at risk of transmitting fragile X to their offspring.[17] Considering autosomal recessive disorders, PGD is an alternative that allows identification of embryos affected by cystic fibrosis.[18] Furthermore, the established PGD protocol was successfully applied in embryos with α- and β-double thalassemia and Tay-Sachs disease where it resulted in successful pregnancy and birth.[19, 20] PGD is also adopted in six families with endocrine diseases: persistent hyperinsulinemic hypoglycemia of infancy (PHHI), congenital adrenal hyperplasia (CAH) salt-wasting form, Sanjat-Sakati syndrome and multiple endocrine neoplasia 2A (MEN 2A), and showed effectiveness in preventing birth of affected children with endocrine disorders.[21] Additionally, it is recommended as an emerging therapeutic tool for couples who are at risk of passing a genetic disease on to their offspring of single gene disorders as stated in a case report documented a successful PGD involving a couple at-risk for MEN1 syndrome, with a birth of a healthy infant.[22]

6. Laboratory techniques

The genetic analysis is performed by fluorescent in situ hybridization (FISH) for cytogenetic diagnosis, or polymerase chain reaction (PCR) for molecular diagnosis. To improve the accuracy of the diagnosis, new technologies are emerging, with comparative genomic hybridization (CGH) and microarrays.[10] These technologies with their advantages and disadvantages were illustrated in table (3).

Table 1: Clinical outcome of preimplantation genetic diagnosis group according to age.

Variables	≤30 yr	31–35 yr	≥36 yr
No. of cycles	9	57	50
Euploidy rates (%)	40.6±24.5	30.0±19.9	24.0±23.2
Implantation rates (%)	20.0 (3/15)	20.7 (19/92)	19.6 (10/51)
Ongoing pregnancy rates (%)	33.3 (3/9)	17.5 (10/57)	12.0 (6/50)
Early abortion rates (%)	0 (0/4)	20.0 (3/15)	37.5 (3/8)
Clinical pregnancy rates (%)	44.4 (4/9)	26.3 (15/57)	16.0 (8/50)

Table 2: Common genetic disorders for which PGD has been more commonly applied.

Type		Disease	Technique used
Chromosomal abnormalities		Down syndrome[14]	FISH
		Turner Syndrome[15]	FISH
		Klinefelter's syndrome[16]	FISH
		Fragile X syndrome[17]	Multiplex PCR
Monogenic diseases	Autosomal recessive	Cystic fibrosis[18]	NA
		β thalassaemia (β globin gene)[19]	Multiple displacement amplification (MDA)
		Sickle cell anaemia (β globin gene)[23]	Non-labelled PCR and later fluorescent PCR
		Spinal muscular atrophy (type I)[24]	Single cell nested PCR-RFLP
		Tay-Sachs disease[20]	Nested PCR
		Endocrine disorders[21]	Multiplex fluorescent markers
	Autosomal dominant	Multiple endocrine neoplasia type 1 (MEN1)[22]	NA
	X linked	Hemophilia[25]	FISH

FISH fluorescent in situ hybridization, PCR: polymerase chain reaction, RFLP: restriction fragment length polymerization, NA: not available

Table 3: advantages and disadvantages of techniques used to screen chromosomal abnormalities.

Technique	Advantage	Disadvantage
FISH	High sensitivity and specificity in recognizing targeted DNA or RNA sequences Direct application to both metaphase chromosomes and interphase nuclei Visualization of hybridization signals at the single-cell level.[26]	Unacceptably low in most people's hands Not recommended for routine clinical use.[27, 28]
Quantititative PCR (qPCR)	Fast, effective methods enabling the quantification of gene and/or transcript numbers within environmental samples. Providing unparalleled specificity and sensitivity to target sequences present within a mixed community background.[29]	Limited number of samples extends the time taken to analyze all of the samples.[27, 28]
Next-Generation Sequencing (NGS)	Reduced the read depth necessary for accurate sequencing of the mutation site, which reduces the time required and cost. qPCR of the multiplex PCR products provided rapid analysis of chromosome copy number.[27, 28]	Because the number of fragments from a particular chromosome should be proportional to the copy number, trisomy or monosomy will result in greater or less read depth, respectively.[27, 28]
Array CGH	Reliable, accurate, and relatively fast Allows 24-chromosome copy number analysis.[27, 28]	Relatively high cost when testing multiple samples.[27, 28]

CONCLUSION

PGD is used prior to implantation to help identify genetic defects within embryos whom are usually created during the process of IVF, a procedure which serves to prevent certain genetic diseases or disorders from being passed on to the child. It can screen monogenic disorders along with chromosomal abnormalities. The most important indication is to improve the outcome of IVF in patients with translocations, advanced reproductive age, recurrent IVF failure or recurrent pregnancy loss. PGD can be carried out using several techniques, depending on the nature of the studied condition. PCR-based methods are used for monogenic disorders and FISH for chromosomal abnormalities.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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