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Preimplantation Genetic Testing: A Review of Past and Current

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ABSTRACT

The field of medical genetics has seen significant and incredible advances in technology for the past several decades. Genetic technologies, particularly in the reproductive medicine discipline, represent a fresh era in medicine that may develop significantly in the coming years. The purpose of Preimplantation Genetic Testing (PGT) in the situation of assisted reproductive technology (ART) treatments with IVF (in vitro fertilization) or ICSI (intracytoplasmic sperm injection) is particularly controversial as it is done before implantation [1]. However, despite the successful application of PGT in the field of IVF in overcoming infertility and genetic defects, the techniques pose various limitations, and concerns that need to be addressed to enhance their success rate [2]. This review will introduce PGT and summarize the molecular techniques used in its application as well as highlight the future advances in the field.

Keywords: Preimplantation genetic testing; karyomapping; in-vitro fertilization; aneuploidy; monogenic disorder.

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1. INTRODUCTION

The discipline of genetics has undergone dramatic transformations over the past few decades leading to incredible advances in the world's medical fields. Recently, sequencing of the human genome was considered the most remarkable medical achievement. However, currently, the entire sequencing of a person's genome is commonly existing and at lower costs. In recent years, PGT's technology has advanced significantly where it has several current applications, procedures, and limitations. A typical IVF cycle comprises controlling injectable gonadotropins to females and bringing about controlled ovarian hyperstimulation. Surgical harvesting of oocytes in the follicles is carried out, and they are artificially inseminated. The process results in embryos that develop for 3 or 5 days in vitro, after which the best single or two embryos are implanted in the uterus and the remaining are usually cryopreserved [3,4,5]. The need to determine the best embryos for transplant is extremely crucial, and morphology traditionally has been applied almost exclusively as a marker for transferable embryos. However, the efforts by researchers to get more accurate diagnostic methods for determining embryo quality and genetically have resulted in promising techniques such as real-time videography, metabolomics, and PGT. This paper focuses on the PGT field precisely in the wake of new technologies. The paper analyzes the evolution of technologies from the use of PCR to the application of next-generation sequencing (NGS). It covers the procedures, limitations, advantages, and implications of each technique applied in the PGT.

2. PREIMPLANTATION GENETIC TESTING (PGT)

PGT is the technology of examining the quality of embryos before being transported to the uterus. The technology is applied to identify a range of genetic disorders in the embryo, such as single-gene disorders (e.g sickle cell anemia), extra or missing chromosome in the embryo (e.g. Down syndrome), and rearrangement of genes [4]. These genetic disorders cause several problems such as miscarriage, implantation failure, and congenital disabilities; hence it is necessary for them to be examined accurately during in vitro fertilization. The group of genetic assays used in examining embryos before transfer to the desired uterus and comprised in PGT includes PGS for abnormal chromosome number (aneuploidy; extra or missing chromosome) (PGT-A), screening for monogenic disorders (PGT-M), and detection of structural rearrangements such as translocation and inversion (PGT-SR). The three forms of PGT are new terms that replace the preceding terms, such as PGD and PGS. PGT-A accomplishes the previous functions of the PGS while the functions of PGD are now indicated by either PGT-SR or PGT-M, where these tests are still carried out similarly [6].

3. PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A)

PGT-A refers to the analysis of the embryo cells to check for the presence of a normal number of chromosomes. This technique aims towards examining the wholeness abnormalities of chromosomes before transfer to the uterus to decree rates of failures of early pregnancies and increase rates of live births. Most individuals have 46 chromosomes; given that they receive 23 chromosomes from each parent. Aneuploidy refers to the condition where a cell or embryo has an extra or a missing chromosome [7]. Turner syndrome is the type of monosomy where the n x chromosome is missing and is the only one that child can survive. Therefore, most failed implantation for pregnancy, various defects in children, and miscarriages are mainly caused by aneuploidy [4]. Preimplantation genetic testing-aneuploidy has developed and includes the use of techniques such as next-generation sequence and comparative genomic hybridization in the assessment of all the chromosomes.

The publication of randomized research failed to find improved results of in vitro fertilization tempered with the initial interests in PGT-A through FISH. FISH was the original technique to be applied whose limitation was used in few chromosomes—the effort towards lower rates. Multiple gestations and higher live births in IVF drive the proceeding studies to pursue the emerging techniques that involve cell removal of the multiple cell trophectoderm of the blastocyst. Additionally, there has been the development of platforms that can test all chromosomes where the difference between these platforms is in their capacity to recognize anomalies such as mosaicism, single-gene mutations, structural abnormalities, and mitochondrial copy number. Previous randomized studies that examined the clinical effectiveness of PGT-A found several control trials. The first randomized control trial suggested that there are higher rates of pregnancy in younger patients who have no history of attempted IVF that failed. Secondly, the other suggested that after PGT-A, women aged between 38 and 41 have significantly lower miscarriage rates, a shorter period to pregnancy, and higher rates of live births [8]. Among the limitations of preimplantation genetic testing-aneuploidy is that according to a practice guideline published by ASRM in 2018, the evidence of applying this technique in infertile women is insufficient [9,10,11,12,13]. However, it is necessary to implicate that the traditional diagnostic screening for aneuploidy should be presented in accordance with the commendations for all pregnant patients to all individuals who have had PGT-A.

4. PGT FOR CHROMOSOME STRUCTURAL REARRANGEMENT (PGT-SR)

PGT-SR is a technique that analyses embryos of patients who have a known disorder resulting from chromosomal structural rearrangements such as translocation, deletions, insertions, or inversion. Examples of structural abnormalities examined through the PGT-SR technique include reciprocal and nonreciprocal translocations as well as Robertsonian translocations. Whenever structural rearrangement in a patient is discovered, a discussion of possible preimplantation genetic testing and genetic counseling should be carried out [14]. Currently, it is difficult for PGT-SR to determine the difference between a cell or embryo that carries a balanced form of the familial chromosome rearrangement and the one with a normal karyotype. Having a familial chromosome rearrangement that is balanced and involves imprinted genes is a risk factor for uniparental disomy-linked abnormalities. It cannot be omitted by all techniques of preimplantation genetic testing analysis. Therefore, because this testing method applies just a limited number of trophectoderm cells and the above limitations, it is necessary to confirm PGT-SR results with amniocentesis or CVS in the prenatal stage.

5. PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISORDERS (PGT-M)

PGT-M is a technology that analyses the specific gene mutations known to be carried by one or both parents. In a family context where one or both parents have genetic disorders, there is an increase in a child's chances of being born with a genetic mutation. Such mutations may result in heritable illnesses such as sickle cell anemia and cystic fibrosis or even can cause increased cancer risk (e.g. *BRCA1* and *BRCA2* mutations in breast and ovarian cancer) [15]. The fertility specialist examines specific genetic disorders in the embryo during PGT-M before embryo transfer. Examples to some disorders examined through PGT-M include Tay-Sachs disease, *BRCA1* & *BRCA2* mutations, Sickle cell anemia, Muscular dystrophy, Cystic fibrosis, Fragile-X syndrome, and Huntington's disease [16,15,17].

Additionally, PGT-M can also be used to discover unaffected, human leukocyte antigen-compatible (HLA matching) embryos that can be used to allow unwell family members to obtain compatible bone marrow transplants or cord blood transfusions [16,15,17]. Despite of the high sensitivity and accuracy of PGT-M, CVS or amniocentesis should be used to confirm preimplantation genetic testing-monogenic results.

6. GENETIC TECHNIQUES USED IN THE APPLICATION OF PGT

There are several genetic techniques used with PGT. Historically, PCR was first implemented for PGT-A followed by application of the FISH technique. Such techniques are however being superseded by the advent of chromosomal microarrays and next generation sequencing.

7. POLYMERASE CHAIN REACTION (PCR)

One of the first published cases of PGT utilized targeted PCR amplification of sequences in the X and Y chromosome in order to check embryos suitable for transfer in a case of X-linked genetic disorder [18]. PCR is relatively simple and cheap to apply, it did not become the main PGT technique due to drawbacks including requirement of relatively large amounts of starting material as well as non-specific amplification and noise that can affect the accuracy of the test. In addition to the Allele drop out (ADO) where only one of the two alleles present in a cell is amplified to a detectable level causing where heterozygous sample appears homozygous or trisomy appears as diploid.

8. FLUORESCENT IN SITU HYBRIDIZATION (FISH)

FISH is based on using fluorescently tagged single-stranded DNA molecules recognizing and binding to its complementary sequence on metaphase chromosomes spread or inside an interphase nucleus. FISH has been used in several investigations to evaluate the viability of preimplantation embryos. FISH is particularly useful in the diagnosis of reciprocal translocations, numerical chromosomal anomalies, or fetal sexing, without the need for cell culture or metaphase preparation. The kind and quantity of FISH probes that are utilized on a sample depend on the rationale of the test. Throughout the case of sex identification, probes for the X and Y chromosomes are utilized together with additional probes as internal controls for one or more of the autosomes. Additional probes can be introduced for aneuploidy identification, especially those which result in premature end to pregnancy, such as a trisomy 21 [19,20,2]. The presence of mosaicism hampers PGS biologically, and the number of examinable chromosomes and the inability to detect unrelated chromosomal rearrangement limits FISH technically. Other limitations that led to misdiagnosis when using FISH in PGS include the need for several cycles of hybridization that can lead to the degeneration of the targeted DNA and influence the accuracy of the outcomes, overlapping indications that result in monosomies misdiagnosis and misdiagnosis of trisomies and hybridization failure that is cussed by signal splitting [21,22].

9. COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

CGH refers to a cytogenetic – molecular technique that is used to analyze the changes of chromosomal copy numbers (deletion/amplification) [23,24]. The technique is based upon the co-hybridization test genomic DNA along with “normal” reference genomic DNA to oligonucleotide probes immobilized on a grass slide. Both sets of genomic DNA are fluorescently labeled, thus allowing for semi-quantitative assessment of the ratio of test to normal DNA which is translated through software analysis to loss or gain in a particular chromosome or area of chromosome. The assessment of all chromosomes and identification of chromosomal breakage is done using the array and metaphase CGH to reduce the rate at which chromosomal abnormalities are transferred to the embryo as they are not detectable by FISH in PGT. A-CGH solves the challenge of the period taken for m-CGH since it can be conducted within 24 hours, its higher sensitivity and precision in automated analysis of copy number aberrations [23,24,25,26]. A recent investigation used array-CGH to diagnose embryos on day three in a 120 patients' clinical program. A rate of 38.4% per cycle of clinical pregnancy, 10.6% miscarriage rate, and 60.3% per embryo transfer was obtained [27]. The sensitivity of a-CGH in detecting the mosaicism was investigated and confirmed its ability in identifying aberrations at different levels [28]. In this study, two groups, blastocyst and TE were used. The aneuploid and euploid cells in each of the TE and blastocyst groups analyzed were combined together in different ratios to form different mosaicism levels. The normal threshold increased with aneuploid cell proportion in the TE group, while in the blastocyst group, the normal threshold shift was utmost when half or more of the cells were aneuploid. Therefore, such studies suggest that a-CGH can identify aneuploid in mosaic embryos and indicate the aneuploid cell ratio [28].

10. NGS (NEXT-GENERATION SEQUENCING)

This is the latest technology and technique applied to carry out embryo biopsy and identify mosaicism in embryos. It uses advanced computing and molecular evaluation to identify chromosomal abnormalities in a highly precise manner. This technology can recognize mosaic embryos that contain different amounts of normal and abnormal cells. Considering an embryo at the blastocyst stage, there are more than 100 cells wherein a mosaic embryo some are normal while others abnormal. The low-level mosaic embryo contains mostly normal cells, while a high-level mosaic embryo contains a few normal ones with predominantly abnormal cells. NGS has enabled geneticists to detect more cases of mosaicism as it offers sensitivity levels not possible with other preimplantation genetic testing techniques [5]. Therefore, NGS-based techniques has led to increases in the probability of successful births and pregnancies.

NGS-based techniques are more than 90% accurate in analyzing the 23 pairs of chromosomes of an embryo, examining the chromosomal aneuploidies of whole chromosomes, and identifying the losses or gains of genetic material [5]. However, depending on the chromosomal alteration site resulting from the WGA (whole genome amplification), NGS may not detect micro chromosomal transformations.

11. COMPARISON OF NGS AND STR ANALYSIS (ADVANTAGES AND DISADVANTAGES)

NGS-based techniques offer a superior sensitivity and specificity over the classical STR-based analysis. The former has significantly lower limit of detection, comprehensive genome converges, and higher sensitivity that allows low-frequency variant detection [5]. The disadvantages include time consuming and expensive for low sequencing numbers of targets.

12. KARYOMAPPING

The concept of karyomapping, as described by Alan Handyside's group, is a parental haplotyping that is genome-wide applying the high-density SNP analysis. This technology eliminates the necessity for developing customized tests just by knowing the genotyping of the close relative or parent of a patient with a recognized illness. Karyomapping defines the embryo-carrying normal chromosome copies by identifying the informative loci for the four parental haplotypes within each chromosome and mapping the crossovers in the proband with these haplotypes' inheritance preimplantation embryos [29]. As a component of an IVF cycle, embryo biopsy is performed, and the cells are analysed for the DNA unique haplotypes, uncovering those incipient embryos that have acquired the genetic fingerprint of the disease. Karyomapping additionally gives data across the whole genome, which means additional aberrations can be identified thus increases the chance of preventing other unexpected genetic diseases in the embryo leading to a higher chance of successful pregnancy with a healthy fetus.

13. COMPARISON BETWEEN PCR AND KARYOMAPPING

The DNA sequences amplified by PCR involves primers targeting specific genomic loci, albeit limited in number. Karyomapping, on the other hand, is able to produce genome-wide targeting within few hours. Karyomapping enhances high and accurate analysis of the DNA and inheritance of any single-gene defect. This includes the combination of single-cell level and loci which greatly expands a range of conditions for the purpose of preimplantation genetic diagnosis.

According to an investigation conducted in 55 clinical cases using karyommaping and PCR testing in combination, Karyommapping can provide a higher complete assessment of the required region compared to conventional PCR. Karyommapping and PCR test were applied in combination to detect direct mutation alone in 139 embryos where both tests agreed in 135 of them. Firstly, the discrepancy resulted from monosomy influencing the target chromosome where karyomapping readily detected it but had been misinterpreted by PCR mutation detection. The results were visible because Karyomapping provided comprehensive diagnosis of the embryo that facilitated the detection of a mutation site undetectable by traditional PCR.

Moreover, as per the results of 23 assessed embryos, 21 of the 22 embryos confirmed the agreement of the two methods. Karyommapping provided comprehensive results while traditional STR-based technique failed to identify the abnormality which was a single point mutation segregating with a chromosomal abnormality. Generally, karyommapping presented a conclusive diagnosis in 99.6% of the embryos compared to 96.8% conclusive diagnosis provided by conventional PCR testing [30]. On the other hand, PCR testing facilitated the results as the diagnosis of embryos was influenced by the single gene disorder.

Identification of single-nucleotide polymorphism in embryos by karyomapping has been shown to be successful in 213 out of 218 cases corresponding to 97.7% of samples [31]. The advantages of karyomapping was further highlighted in identifying haplotypes (97.7% success rate) with a limitation caused by regions of homozygosity [31]. Such studies and many others [32,33,29,34] demonstrate that genome-wide Karyomapping is accurate and most of the times it facilitates analysis of inheritance in single-gene defect. The major drawback with this approach is the significantly high cost of analysis as it requires dedicated chips and advanced array scanners.

14. LIMITATIONS OF PGT

PGT means that the embryo should be biopsied to carry out specialized analysis of genetic diseases. Carrying out a biopsy within 5 days of development rather than 3 days has been facilitated to ensure that the embryo biopsy does not come along with negative impacts on the viability of the embryo. The most significant limitations revolve around the FISH- PGS evaluation and the application of biopsy acquired from the three days-old embryos. The use of SNP and CGH arrays is hampered by technical limitations that, when not well executed, cause spurious results. Additionally, a highly-trained geneticist needs to interprets the raw data. Variations in interpretation quality leaves a significant space for human error adding to the imprecise automated outcomes.

Using FISH, geneticists could evaluate a maximum of 10 chromosomes which allowed up to 70% of diagnostic accuracy [35,2,36]. However, NGS screening methods allows the evaluation of the 23 chromosomes pair simultaneously [5]. A disadvantage of PGS is that when a sample size is small, only four to 10 cells are amenable to be removed safely at an early stage embryo without damaging it. Additionally, if there is one cell which is biopsied to be abnormal, the embryo will not be selected for transfer although some healthy babies are frequently born from the mosaic embryos.

The presence of cellular discordance around the developing embryo is the most significant cause of errors originating from PGT-A using blastocyst biopsy and 23 chromosome pair evaluation. Trophectoderm (TE) and an inner cell mass (ICM) are the components of the blastocyst, and the ICM contains cells that are refocused on forming fetal tissues, while TE contains cells that create the placenta [37]. The blastocyst biopsy utilizes the cells from the TE to reduce the effects that are potentially harmful and may result from ICM biopsy or cells focused on fetus formation. However, data proposes that aneuploidy may be present in up to 10% of developing blastocysts in the TE but not in ICM. Hence, the TE engaged from a biological standpoint at the blastocyst stage may not be collectively extrapolative of the chromosomal position of the emerging embryo even without practical error during the genetic analysis performance. Also, there may be the existence of mosaicism around a specified cell population of TE. However, the array technology can detect deferent levels of all mosaicism around the analyzed samples of TE provided. Therefore, the patients need to be adequately guided and counseled on the limitations and risks of PGS, by a genetic counselor, geneticist, or a specialized physician.

15. RISKS AND FUTURE OF PGT

The risks associated with PGT revolve around inaccurate test findings. The testing is highly prone to errors, and thus the patients are advised to undertake prenatal testing such as amniocentesis when they are pregnant. PGT testing proves to be safe for children's health, but there are other health risks to the mother and child associated with the IVF. There is a small risk of damage associated with handling embryos, freezing, biopsy, and thawing. Typically, the number of lost embryos due to such damage when evaluated by PGT is around 5%. One of the most outstanding features of PGT that is undoubtedly futuristic is its use in the prospective selection of specific traits to pass to the child . This can be achieved through embryo selection and was initially applied to increase the number of IVF births born healthy. Indeed, the future of PGT technology is its application by parents to select the traits they want to pass to their children and select the ones they do not want to pass. With the advances in gene editing and the potential treatment of genetic disorders, PGT will become a front-line test in pregnancies where positive family history of genetic diseases is reported.

16. CONCLUSION

PGT is a significant tool to diagnose and prevent transmission of genetic syndromes and reduce the rates of early miscarriages. Preimplantation Genetic Testing for Monogenic Disorders (PGT-M) is essential to protect children from inheriting monogenic disorders. Using the idea of savior sibling, hematological disorders must heal the affected child. Therefore, Preimplantation Genetic Testing (PGT) is undoubtedly an incredible technology that needs to be integrated into ART to provide the finest results to patients. Finally, more investigations and awareness should be carried out to enhance understanding of PGT pitfalls and ensure that the technology is not used judiciously.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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