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Review Article

Molecular techniques used in non-invasive pre-implantation genetic testing: current approaches, applications and challenges

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ABSTRACT

Preimplantation genetic testing (PGT) represents an important diagnostic method in assisted reproductive technology (ART) for the screening of chromosomal and genetic aberrations prior to embryo transfer. Non-invasive PGT (niPGT) is a new technology that analyzes cell-free embryonic DNA (cfDNA) released into spent culture media. This is a safer method than conventional PGT that uses invasive biopsies of embryos that can damage their viability. Advanced molecular techniques support the diagnostic accuracy of NiPGT: array comparative genomic hybridization (aCGH) allows genome-wide detection of whole-chromosome aneuploidies and copy number variations through comparative hybridization. Next-generation sequencing (NGS) allows high-throughput deep-coverage analysis with extended dynamic range and allows detection of whole-chromosome aneuploidies, segmental defects and intermediate mosaic thresholds on all 24 chromosomes. Because embryonic cfDNA is so fragmented and present only in trace picogram quantities, whole genome amplification (WGA) is needed to amplify the genetic material for downstream analysis, and other targeted approaches such as quantitative PCR (qPCR) and high-density single nucleotide polymorphism (SNP) arrays are also important, as they enable copy count calculation and provide vital haplotype-specific information on parental inheritance patterns. niPGT has high embryo concordance rates, ranging from 76% to 91%, when compared to conventional biopsies, but it presents significant technical challenges, including maternal DNA contamination from cumulus cells or maternal shedding, which can mask true embryonic aneuploidies and lead to false-negative results, as well as unequal amplification biases and artifacts generated during WGA. Continued refinement of sequencing and amplification protocols, together with more robust clinical validation, will be needed before niPGT can be widely adopted in routine practice.

Keywords: Preimplantation genetic testing, Non-invasive preimplantation genetic testing, Next-generation sequencing, Whole-genome amplification, Cell-free DNA

INTRODUCTION

Preimplantation genetic testing (PGT) is a sophisticated diagnostic technology used in assisted reproductive technologies for the selection and placement of genetically healthy embryos into the mother's uterus by examining the embryos in a very early stage of development. PGT is most often performed in combination with *in vitro* fertilization (IVF) to detect genetic and chromosomal abnormalities prior to implantation. This enhances the chances of implantation and pregnancy and lessens the risk of miscarriage and transmission of genetic disorders.[1,2] PGT can be used to detect aneuploidies, monogenic diseases and structural chromosomal rearrangements. In the process, embryos grown in a lab dish are biopsied, taking

one or a few cells. Their genes are then analyzed to identify any problems.[2]

PGT has three types, depending on the kind of genetic problem you're screening for: aneuploidies (PGT-A), structural chromosomal rearrangements (PGT-SR) and monogenic diseases (PGT-M). More recently, polygenic embryo screening (PES), also known as PGT-P, has been extended to include conditions with more than one cause.[4] As these analytical methods are more and more integrated, it is possible to analyze more than one genetic disorder at the same time. PGT also needs a multidisciplinary approach as it involves medically assisted reproduction (MAR) and the detection of chromosomal abnormalities, including structural and numerical variants, and hereditary monogenic disorders.[1,3]

Non-invasive PGT for aneuploidy (niPGT-A) has been proposed as a promising alternative to conventional biopsy-based preimplantation genetic testing because it does not require embryo biopsy. This method relies on analysis of the cell-free DNA (cfDNA) released from developing embryos into the blastocyst culture medium during their growth in the lab. However, niPGT-A obtains embryonic genetic material from used culture media rather than directly from the trophectoderm cells of the embryo. This avoids the physical manipulation of the embryo and the associated risks that may occur during the procedure. Recent studies have shown that cfDNA from the spent culture medium can give useful chromosomal information. This suggests that niPGT-A could be a safer and less invasive way to test embryos in assisted reproductive technology.[4]

Some say niPGT is a safer alternative than the more commonly used invasive methods in reproductive medicine. Instead of cells from the embryo itself, use is made of cfDNA. This DNA is released into the culture medium or fluid of the blastocyst. So, you don't need to move the embryo and it reduces the risk of it being damaged and could improve the outcome of the IVF. On the other hand, the accuracy of niPGT is highly affected by the sequencing methods employed. These techniques change the detection ability of chromosomal problems such as aneuploidy. New sequencing methods make it easier to screen embryos more thoroughly, but the results aren't always clear. Some problems still exist that make it difficult to use.

For example, the quantity of cfDNA is often very low and may not amplify. Another concern is that contamination, especially from maternal DNA, could make the results inaccurate. There also remains much debate about how to interpret mosaicism. Also, different labs don't always follow the same steps, so the results can be different. So niPGT sounds promising, but it's not quite ready to replace what's happening now. Sequencing needs to improve, cfDNA needs to be worked with better and more solid clinical evidence needs to be generated before it can be used widely.[5]

DNA sequencing technologies have improved dramatically since the sanger sequencing method was developed in the 1970s.[6] This first generation approach relies on incorporation of chain terminating dideoxynucleotides during DNA synthesis, which were first radioactively labelled and then fluorescently labelled.[6,7] The fragments generated are separated by gel or capillary electrophoresis for nucleotide sequencing.[6,7] Sanger sequencing is highly accurate, and it is used to sequence genomes from bacteria to humans, but it has a low throughput because it processes only one DNA fragment at a time.[8] Furthermore, sequencing of diploid genomes frequently requires subcloning to distinguish individual haplotypes.[8] The advent of next-generation sequencing (NGS) technologies between 2004 and 2006 revolutionized the field of genomics research allowing for the massively parallel sequencing of millions of DNA fragments simultaneously.[8,9]

Molecular techniques such as whole-genome amplification (WGA), array comparative genome hybridisation (aCGH), single nucleotide polymorphism (SNP) array, quantitative polymerase chain reaction (qPCR) and NGS are important in preimplantation genetic testing and have contributed to advances in embryo genetic assessment.

WGA is primarily used to increase the very small amount of embryonic DNA, creating sufficient material for subsequent genetic analysis. aCGH enables genome-wide chromosomal screening for whole-chromosome aneuploidies and copy number variations by comparing embryo DNA to a reference DNA sample. The SNP array technology tests thousands of genetic markers all over the genome to compare them with parental profiles to determine the chromosomal status and patterns of inheritance. WGA is primarily used to increase the very small amount of embryonic DNA, creating sufficient material for subsequent genetic analysis. aCGH enables genome-wide chromosomal screening for whole-chromosome aneuploidies and copy number variations by comparing embryo DNA to a reference DNA sample. The SNP array technology tests thousands of genetic markers all over the genome to compare them with parental profiles to determine the chromosomal status and patterns of inheritance. qPCR is a rapid and sensitive technique for quantifying the copy number of chromosomes by amplification of specific genomic regions relative to reference sequences, and is useful for screening for aneuploidy. NGS is a high-throughput sequencing platform that can analyze multiple DNA fragments simultaneously and detect chromosomal gains, losses, copy number changes and segmental abnormalities in a comprehensive manner and with high analytical resolution.

Collectively, these methods have contributed to improve the accuracy and efficiency of embryo screening and continue to support the advancement of reproductive genetics.[10]

EMBRYO BIOPSY IN PGT

Embryo biopsy has been the gold standard for PGT for a long time and is still the best way to obtain genetic material from an embryo for genetic analysis. The first successful embryo biopsy procedures became possible with advances in IVF and molecular genetics in the early 1990s. During an embryo biopsy, one or more cells are carefully removed from a developing embryo. If the embryologists are experienced, this does not much affect the embryo's ability to develop. After the cells are separated, molecular genetic techniques are used to identify chromosomal abnormalities or inherited genetic diseases before embryo transfer. A biopsy can be a polar body biopsy, a cleavage stage (blastomere) biopsy or a trophectoderm biopsy, depending on the developmental stage of the embryo, each having their own advantages and disadvantages.[1,28,32,33]

POLAR BODY BIOPSY

Polar body (PB) biopsy is the removal of the first and/or second polar bodies. These bodies are produced during meiotic division and have no effect on the growth of the embryo. The procedure can be performed either after fertilization as a one-step biopsy or as a two-step biopsy with removal prior to and after intracytoplasmic sperm injection (ICSI). A PB biopsy will only tell you about the genetic contribution from the mother. It can't find any problems with the father's genes or with the chromosomes after conception. Even though it has not shown a large increase in live birth rates compared to regular IVF, PB biopsy is useful in places where embryo biopsy or embryo cryopreservation is illegal because genetic testing can be done before the embryo is transferred without touching the embryo.[28,32,34]

CLEAVAGE-STAGE (BLASTOMERE) BIOPSY

The cleavage stage biopsy is usually performed on the day 3 embryos at the 6-8 cell stage. The zona pellucida is opened by laser-assisted hatching, mechanical methods or acidic Tyrode's solution, and one or two blastomeres are removed for genetic analysis. Removing two blastomeres improves the diagnostic accuracy, but also removes a substantial proportion of the embryo and may have a negative impact on implantation potential. On the other hand, biopsy of a single blastomere is less invasive but with lower diagnostic accuracy due to allele dropout and embryonic mosaicism. Consequently, in current PGT practice, the cleavage-stage biopsy has been progressively substituted by the blastocyst-stage trophectoderm biopsy.[1,28]

TROPHECTODERM BIOPSY

Currently, trophectoderm (TE) biopsy is the preferred method to obtain a biopsy of an embryo in clinical PGT. There are two types of cells in an embryo at the blastocyst stage. The ICM gives rise to the fetus and the trophectoderm gives rise to the placenta. TE cells are not required for embryo development because they do not directly contribute to fetal tissues. Also, at the blastocyst stage, the embryonic genome is fully active, allowing more accurate genetic analysis. A blastocyst contains roughly 100 to 150 cells, and 5 to 8 TE cells are normally harvested from it. This provides sufficient DNA and causes little damage to the embryo. TE biopsy is usually performed using laser-assisted hatching; it is associated with improved embryo survival after vitrification, more accurate diagnoses and supports the choice of single-embryo transfer. Although day 5 blastocysts generally show higher euploid rates than day 6 embryos, day 5 and day 6 euploid blastocysts have similar live birth rates. Day 7 blastocysts have lower rates of euploidy and implantation, but can still lead to healthy live births. This provides the possibility to evaluate all appropriate blastocysts regardless of the time of reaching this stage of development.[1,28,32,33]

TYPES OF PREIMPLANTATION GENETIC TESTING

PGT is a means of determining the genetic status of embryos prior to implantation. It is performed during IVF. There are three types of PGT depending on what they are used for in medicine. PGT-M is used for testing for monogenic disorders before conception. PGT-SR is used for testing for structural rearrangements. PGT-A is used for testing for aneuploidies. Different types are designed to look for different genetic problems. This helps doctors select the embryos that have the best chance to lead to a healthy pregnancy.[30]

PGT-M detects pathogenic variants responsible for single-gene disorders in couples already affected by a single-gene disorder or at increased risk of passing it on to their child. In the case of autosomal recessive conditions, the aim is to select embryos that either do not have the disease causing variant or are healthy carriers. PGT-M can be used for many single-gene disorders with a clear family history and a known causative gene, including X-linked, Y-linked, autosomal dominant, autosomal recessive, mitochondrial disorders and some hereditary cancer syndromes. It can also be used to type HLA. However, the genetic complexity and variability inherent in monogenic disorders make the precise identification of pathogenic variants and the prediction of clinical outcomes challenging, requiring advanced testing methods and interdisciplinary collaboration.[30]

PGT-SR is used to screen for chromosomal structural problems such as deletions, duplications, inversions, and translocations. The main aim of this is to find embryos with normal or balanced chromosomes for the structural rearrangement. This will increase the chances of a successful pregnancy. The accuracy of PGT-SR is also influenced by the size and complexity of the chromosomal rearrangement. Moreover, the presence of repetitive or complex regions within the genome can make it difficult to detect the breakpoint and increase the risk of

misdiagnosis. Therefore, a comprehensive genetic test, correct interpretation of the results and complete genetic counselling are important.[30]

PGT-A can identify chromosomal aneuploidies and allow for the selection of euploid embryos for transfer. It is usually given to women who are very old, have had multiple failed attempts at implantation or miscarriages or have severe male factor infertility. PGT-A is expected to increase the number of pregnancies and live births and decrease the number of miscarriages. But there's not much evidence that it works, and its benefits may depend on how many and what type of embryos are available. Some disadvantages of this method include that it can affect the viability of the embryo, give false positive or negative results, present ethical concerns and be more costly than traditional IVF. If no euploid embryos are found, you may be able to transfer embryos with low to medium levels of mosaicism or segmental aneuploidies, after receiving the right genetic counselling.[30]

NEXT GENERATION SEQUENCING

NGS is an important tool for preimplantation genetic testing as it is capable of analysing the genomes of embryos at a very high level of detail.[11] NGS is more accurate and reliable than aCGH in detecting whole-chromosome aneuploidies, segmental deletions/ duplications and embryonic mosaicism.[11] NGS has a higher dynamic range and better sensitivity. Moreover, NGS is less expensive and less laborious than older technologies.[11] The standard NGS method includes the whole-genome amplification, barcoding, library preparation, sequencing, the alignment of sequenced reads to the human reference genome, and bioinformatic analysis of the data.[11] With NGS, it is now possible to carry out a genomic analysis with a higher resolution. This has made it possible to search for embryonic mosaicism and help with clarification.[12]

The only way to work with low input highly fragmented nucleic acids is NGS. It is useful in niPGT-A both in the clinic and in the lab. SBCM contain small amounts of embryonic cfDNA, mainly due to cell death and active shedding.[13] To overcome these structural problems, massively parallel sequencing using NGS provides the very high sensitivity to amplify and accurately read these fragmented templates after WGA. NGS is important as it can accurately detect CNV on all 24 chromosomes by counting the frequency of sequence reads over certain genomic regions and analyzing them using standard bioinformatics methods.[13] NGS also has a much wider dynamic range and deeper coverage compared to traditional cytogenetic methods. This feature allows one to accurately identify intermediate signal thresholds, which is important to find embryonic chromosomal mosaicism, a mixed cellular state that can make a localized, single-site trophectoderm biopsy much less accurate for diagnosis.[14,15]

NGS as the main platform for niPGT is much better than traditional methods from a clinical and technical point of view. The advantage of NGS is that it can be used on cfDNA collected directly from the used culture medium, so that an invasive trophectoderm biopsy is not needed. This prevents the blastocyst from being damaged by lasers or tools.[16,17] And this is the greatest advantage. NGS technologies also have the highest resolution and throughput, thus all 24 chromosomes can be screened simultaneously and with high accuracy.[18,19] This higher resolution enables clear visualization of sub-chromosomal errors, segmental deletions, and low level chromosomal mosaicism, thereby increasing the dynamic range of detection.[20,21] In practice, this level of diagnostic accuracy translates to high concordance rates, generally between 76% and 91%, with trophectoderm biopsies and whole blastocysts. This in turn allows the best embryo to be selected, reduces the risk of miscarriage and shortens the time to a healthy live birth.[13,18]

The use of NGS has the potential to revolutionize the approach to niPGT, but cannot be undertaken immediately due to significant biological and technical hurdles. The primary sources of maternal DNA contamination include maternal cells released into the spent embryo culture medium and residual cumulus cells remaining after oocyte retrieval.[16,18] Maternal DNA is often present in much higher quantities than the limited amounts of embryonic cfDNA, increasing the risk of inaccurate genetic analysis.[18,22] This means that there is a high chance of dilution and false-negative or false-euploid calls that hide real embryonic aneuploidies. If maternal contamination is above a threshold of ~60%, accurate ploidy profiling is not possible without high sequencing depth or specialized deep learning deconvolution techniques.[22] This is because the accuracy of NGS copy number variation (CNV) estimation decreases substantially. The WGA process needed to amplify the first few picograms of fragmented cfDNA also generates a lot of background noise and artifacts for NGS operations.[18] Standard NGS methods have blind spots from a technological point of view. For example, they cannot detect cases of uniparental disomy (UPD) without additional, deep genotyping steps.[22]

WHOLE GENOME AMPLIFICATION

WGA is a molecular technique that amplifies lots of DNA from very little DNA. This creates enough genetic material for sequencing and genomic analysis. The WGA method has transformed the field of genomics enabling the analysis of tiny quantities of DNA, including DNA from a single cell or DNA not associated with a cell which are often encountered in reproductive genetics and preimplantation genetic testing. Significant WGA techniques include DOP-PCR, MDA, MALBAC, PicoPLEX and PTA. These methods vary in terms of DNA amplification, coverage of the genome, accuracy, and bias. WGA is required in modern genomic workflows to extract genome-wide DNA information from very small amounts of material.

In niPGT, the cfDNA released into the spent embryo culture medium is typically present in small quantities and is highly fragmented, making direct genomic analysis challenging. WGA solves this problem by increasing the amount of DNA available for NGS and chromosomal analysis.[26] This amplification step allows the analysis of the embryonic DNA on a genome-wide level and the identification of chromosomal abnormalities such as aneuploidies and copy number variations which are relevant predictors for the selection of embryos for use in ART procedures.[26] With the help of WGA and advanced sequencing technologies, NIPGT has become a useful alternative to embryo biopsy-based genetic testing requiring less invasive procedures.[26]

WGA has been widely used in many areas of biomedical research outside reproductive medicine, including single-cell genomics, cancer biology, circulating tumor cell analysis, microbial genomics, forensic investigations, and environmental DNA studies.[23,24] The ability to amplify DNA from very small samples makes possible the study of genetic heterogeneity, rare genomic variations, and cellular subpopulations that would not otherwise be identifiable by traditional molecular techniques.[24] Thus, WGA has become a powerful tool to extend the use of genomic analysis to a wide range of scientific disciplines.[23,24]

However, whole genome amplification has several technical limitations that can affect the accuracy and reliability of downstream genomic analyses despite its many advantages. Amplification bias is a major problem causing uneven representation of genomic regions and incomplete coverage of the genome. Moreover, allele dropout, non-uniform amplification, sequence-dependent errors and amplification artifacts also impede the detection of genetic variants and chromosomal abnormalities. Moreover, the quality of DNA, chemistry of amplification, properties of enzymes and reaction conditions influence the performance of WGA and there are variabilities between different WGA methods.[25] Nevertheless, ongoing improvements in amplification technologies and enzyme engineering continue to enhance coverage uniformity, reduce error rates, and improve the overall reliability of WGA-based analyses, thereby strengthening its role in NIPGT and other genomic applications.[23,25]

QUANTITATIVE POLYMERASE CHAIN REACTION

One of the first molecular methods employed for NIPD was qPCR, which is also referred to as real-time PCR. This approach allows the amplification and simultaneous quantification of specific fetal DNA sequences in maternal plasma, which enables targeted analysis of selected genetic markers. In clinical practice, qPCR has been extensively used for fetal sex determination based on Y chromosome-specific sequence detection and for fetal RHD genotyping in RhD-negative pregnancies.[27]

The clinical utility of qPCR is the ability to provide important prenatal information that can support the management of a number of pregnancy-related conditions. Fetal sex determination is especially valuable for pregnancies at risk of X-linked diseases and congenital adrenal hyperplasia, and fetal RHD testing facilitates the prevention and management of RhD incompatiblity. Determination of fetal sex is particularly useful in pregnancies at risk for X-linked disorders and congenital adrenal hyperplasia, while fetal RHD testing is useful in the prevention and management of RhD incompatibility. Such applications have led to the integration of qPCR-based testing in routine prenatal care for specific indications.[27] Despite its clinical utility qPCR has a number of practical limitations. The technique requires generating standard curves for quantification, and using an additional assay for the determination of fetal fraction, making the workflow relatively labor intensive. Accordingly, newer molecular techniques have been investigated to improve the testing protocols and to enhance analytical efficiency. qPCR continues to be a proven and reliable approach for targeted prenatal uses.[27]

SINGLE NUCLEOTIDE POLYMORPHISM

The SNP array is a microarray-based molecular approach that allows the analysis of hundreds of thousands of SNP markers distributed across the human genome. In niPGT, cell-free embryonic DNA in spent embryo culture medium is amplified by WGA and then hybridized to SNP-specific probes immobilized on a microarray platform. The resulting SNP profiles are compared to parental genotypes to assess chromosomal copy number variation and genome-wide inheritance patterns, which allows for the determination of embryonic ploidy status.[28,29]

SNP-array technology has great potential for detection of chromosomal abnormalities and assessment of parental genetic contribution. In addition to whole-chromosome aneuploidies, SNP arrays can also detect segmental chromosomal imbalances, uniparental disomy, loss of heterozygosity, and some microdeletions or microduplications. Haplotype-based analysis further improves the interpretation of chromosomal abnormalities and inheritance patterns in embryos, in addition to parental genotype data.[29,30] Although non-invasive, SNP-array analysis in niPGT has some drawbacks. The main principle of genetic testing is the amount and quality of cell free embryonic DNA in used culture medium which often is very limited and is very variable.[27] Moreover, maternal DNA contamination is still a major issue and may also explain differences in results between niPGT and conventional trophectoderm biopsy-based tests. Other lab procedures, conditions to grow embryos and ways to amplify the whole genome also play a role in the success of a diagnostic test. SNP-array technology provides us with useful genomic data, but it needs to be standardized and tested on a large scale before it can be used regularly in clinical settings.[31]

Table 1: Comparison of molecular techniques used in niPGT based on their principles, advantages, and limitations.

Techniques	Principle	Advantages	Limitations
WGA	Amplifies minute quantities of embryonic cfDNA to generate sufficient material for downstream analysis	Enables analysis of very low-input DNA; essential step before NGS and SNP array; supports genome-wide analysis; allows use of non-invasive cfDNA from spent culture medium	Amplification bias and non-uniform coverage; allele dropout; amplification artifacts and chimeric reads; sequence dependent errors; performance varies between methods and reaction conditions
aCGH	Compares embryo DNA with reference DNA on a microarray to detect chromosomal copy number gains or losses across the genome	Genome-wide detection of aneuploidies and copy number variations; well-established and widely used; does not require prior knowledge of the genome	Lower sensitivity for mosaicism compared to NGS; cannot detect balanced chromosomal rearrangements; lower resolution for small segmental changes; limited dynamic range
NGS	Massively parallel sequencing of amplified embryonic DNA followed by bioinformatic analysis to detect chromosomal abnormalities	High throughput and high resolution; detects whole-chromosome aneuploidies, segmental abnormalities, CNVs and mosaicism; wide dynamic range and high sensitivity; lower cost per sample with multiplexing	Requires WGA, which may introduce bias; affected by maternal DNA contamination; complex bioinformatics and interpretation; cannot detect uniparental disomy (UPD) without additional genotyping
qPCR	Amplifies and quantifies specific genomic regions in real time to estimate chromosome copy number	Rapid and simple workflow; high sensitivity and specificity for targeted regions; relatively inexpensive; short turnaround time	Limited genomic coverage (targeted analysis only); cannot detect segmental abnormalities; lower resolution compared to NGS and aCGH; requires optimization for each target
SNP	Uses SNP markers distributed across the genome to assess chromosomal copy number and parental inheritance patterns	Detects aneuploidy, copy number changes, UPD and loss of heterozygosity; provides information on parental origin and inheritance patterns; genome-wide analysis with high density	Requires high-quality DNA and parental samples; susceptible to maternal DNA contamination; lower sensitivity for low-level mosaicism compared to NGS; interpretation can be complex

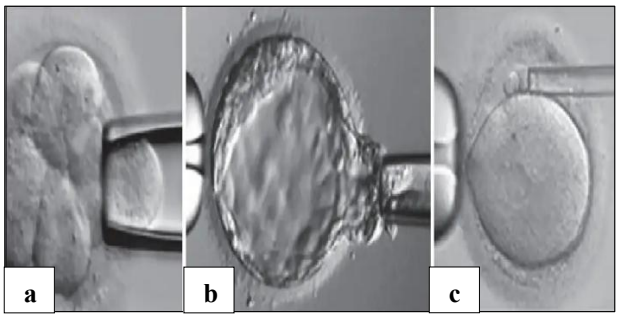

Figure 1: Biopsy techniques: (a) blastomere biopsy, (b) trophectoderm biopsy, and (c) polar body biopsy.[35]

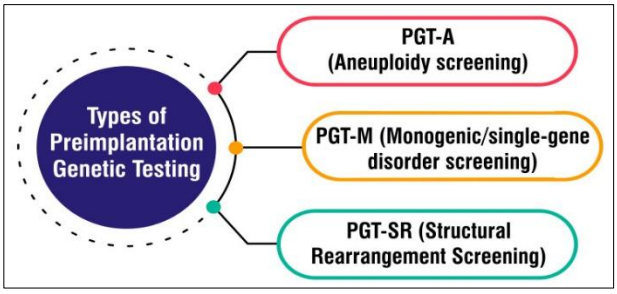

Figure 2: Classification of PGT.[36]

CONCLUSION

PGT is an important diagnostic tool in ART to detect chromosomal and genetic abnormalities in embryos prior to transfer to the uterus. NiPGT, which analyzes cfDNA released into the spent blastocyst culture medium, is now considered a safer alternative to conventional PGT, which relies on invasive embryo biopsy that may compromise embryo viability. This is where the advanced molecular techniques, particularly aCGH and the NGS are essential for the diagnostic accuracy of niPGT. While aCGH enables genome-wide detection of whole-chromosome aneuploidies and copy number variations by comparative hybridization, NGS allows high-throughput, deep coverage and an extended dynamic range for detection of whole-chromosome aneuploidies, segmental defects and intermediate mosaic thresholds for all 24 chromosomes. Embryonic cfDNA is highly fragmented and present at trace picogram levels, making WGA necessary to scale the genetic material for downstream analysis. Apart from aCGH and NGS, other focused methods like qPCR and high-density SNP arrays, also help by computing copy counts and giving vital haplotype-based information on parental inheritance patterns. While niPGT has high embryo concordance rates ranging from 76% to 91% when

compared to conventional biopsies, it has significant technical challenges. These include significant maternal DNA contamination from cumulus cells or maternal shedding, which can mask true embryonic aneuploidies and lead to false negative results, and unequal amplification biases and artifacts generated during WGA. Embryonic cfDNA is highly fragmented and present in trace picogram quantities requiring WGA to scale the genetic material for downstream analysis. Beyond aCGH and NGS, other focused methods such as qPCR and high-density SNP arrays also help in computing copy counts and providing essential haplotype-based information on parental inheritance patterns. niPGT has high concordance rates (76% to 91%) with embryo biopsies but it has important technical challenges. These include significant maternal DNA contamination from cumulus cells or maternal shedding that can mask true embryonic aneuploidies and result in false negative results and unequal amplification biases and artifacts created during WGA.

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