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Original Article

Exploring The Role of Preimplantation Genetic Testing in Monogenic Disorder

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ABSTRACT

Preimplantation genetic testing or PGT, has proven itself as a reliable substitute for invasive prenatal diagnosis, despite the relative difficulty of testing for genes of one or fewer cells. Theoretically, Pre-implantation genetic testing for monogenic disorders - PGT-M can be used to treat any monogenic disorder for which the gene responsible for the illness has been definitively discovered. In actuality, depending on PGT regulations, the set shows symptoms that PGT is used to permitted could differ significantly between nations. In a technical sense, the transition from resilient generic processes using multiplex polymerase chain reaction and replication of the entire gene following Single nucleotide polymorphisms scanning. The Next-generation sequencing- NGS is a significant advancement over a previous ten years: the couples wait times have been significantly decreased because the specific preclinical workup can be skipped and the amount of work for the labs has dropped. Another development is the ability to analyze PGT-M and Preimplantation genetic testing for aneuploidy - PGT-A simultaneously thanks to generic approaches. With this development of novel algorithms and the ongoing decrease in sequencing costs, PGT is moving toward an everything at once sequencing-based solution for PGT-M, PGT-SR, and PGT-A. This will produce an enormous Quality of intricate DNA data, posing significant difficulties to genetic guidance. Here we provide an overview of PGT-M's current state of the art and offer some future predictions in this review.

Keywords: Preimplantation, PGT-M, PGT-A, PGT-SR, Monogenic Disorder.

INTRODUCTION

Preimplantation genetic testing (PGT) is a comprehensive summary of the evolution. The shift towards PGT for aneuploidies (PGT-A), PGT for monogenic/single-gene defects (PGT-M), and PGT for chromosomal structural rearrangements (PGT-SR) reflects a more precise categorization of the various applications within scientific field. It's interesting to see how terminology evolves to better align with the advancements and nuances in technology and clinical practice (Zegers et al.,2017). PGT, or preimplantation genetic testing, encompasses PGT-A, or anemia screen; PGT-SR, or structural rearrangement; and PGT-M, or monogenic/single gene illnesses, were all of the major types. Each type serves a different purpose in assessing embryos for genetic abnormalities before implantation during assisted reproductive procedures (Carvalho et al.,2020 a). PGT has indeed become a crucial tool in managing genetic conditions and ensuring healthier outcomes in assisted reproductive procedures like Intracytoplasmic sperm injection-ICSI. Genetic counseling's evolution alongside PGT highlights the comprehensive approach in addressing genetic factors in reproductive medicine. How do we see this integration shaping future advancements in fertility treatments (Parikh et al.,2021). Absolutely, preimplantation genetic testing for monogenic disorders (PGT-M) has indeed acquired significant traction in recent years. It's a powerful tool that couples can use in conjunction with in vitro fertilization- IVF to check eggs with specific molecular disorders earlier implantation. This allows couples to make informed decisions about which embryos to transfer, thus reducing the possibility of inheritance through genetic conditions for children (Sulivan-Pyke.,2018). Genetic counseling indeed has a vital part to guiding couples through a complexity of PGT within the context of IVF. It not only helps them understand the potential benefits and limitations but also provides support in making informed decisions aligned with their values and preferences. This approach ensures that couples are empowered to navigate the process with clarity and empathy (Resta et al.,2006). Genomic reference materials (RMs) have vital part to the validation and quality control of

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preimplantation genetic testing for monogenic disorders (PGT-M). They are essential to ensuring of accuracy and reliability using genetic testing within public health and medical facilities. The continuous development and standardization of PGT-M techniques have led to increased detection accuracy, especially for complex recessive diseases. Using DNA RMs in clinical genetic testing laboratories is recommended to support the ongoing evolution and standardization of PGT-M (Carvalho et al.,2020). Preimplantation Genetic Testing (PGT) has indeed undergone significant evolution since its experimental beginnings in the early 1990s. Initially focused on gender determination and detection of X-linked diseases, it now offers a comprehensive approach to identifying genetic aberrations in embryos before implantation, thereby reducing the need for invasive Evaluation of parents or possible termination of pregnancy (Handyside et al., 1990). Biopsy at the blastocyst stage followed by genome-wide technologies has indeed emerged as a cutting-edge approach, gradually replacing older methods. These genome-wide techniques offer comprehensive data on genotyping and chromosome copy numbers, facilitating studying simultaneously with both PGT-M and PGT-A, marking a significant advancement in assisted reproductive technology (Treff et al., 2013a; Natesan et al., 2014; Esteki et al., 2015; Backenroth et al.,2019; Masset et al., 2019). That is a comprehensive definition of genetic counseling.

It is a vital process for individuals and families dealing with genetic conditions, providing them with knowledge and assistance in making defensible choice regarding the health (Resta et al.,2006 b). It is great to hear that there are comprehensive guidelines from various international societies like Preimplantation Genetics Diagnosis International Society -PGDIS, American Society for Reproductive Medicine -ASRM, and European Society of Human Reproduction and Embryology- ESHRE PGT Consortium. These guidelines play a crucial role in ensuring high standards and best practices in PGT services, covering aspects from organization to technical procedures like embryo biopsy and genetic testing. Keeping up with the latest updates from these societies is essential for practitioners in the field (Carvalho et al.,2020 b; Kakkali et al., 2020; Carvalho et al., 2020; Coonen et al., 2020). The transition from single cell simplex PCR to multiplex PCR in PGT-M marked a significant advancement, allowing for the co-amplification with informative brief tandem repetition which are very similar Short tandem repeat -STR markers. This method, often coupled with single cell biopsy at day 3, became the preferred approach for detecting monogenic disorders (Laurie et al., 2010). Transport PGT indeed offers a solution for leveraging the expertise of specialized genetic laboratories while overcoming geographical barriers. It is a testament to the importance of collaboration in advancing reproductive technologies (Carvalho et al., 2020).t sounds like you're summarizing a review paper on preimplantation genetic testing for monogenic disorders (PGT-M). It's essential to cover patient inclusion criteria, the testing process, and follow-up procedures for babies born through this method. Reflecting on future developments can help anticipate advancements in the field.

INDICATION FOR PGT-M

That regulation of preimplantation genetic testing (PGT) varies widely from country to country, influenced by cultural, ethical, and legal considerations. Some nations permit it for specific medical reasons, while others have stricter regulations or outright bans (Ginoza et al., 2020). This regulation of PGT varies significantly from country to country. Some nations, like the UK and France, have specific regulatory bodies overseeing its use, while others, like the USA, have fewer restrictions, allowing for a broader range of applications, including nonmedical ones like social sexing (Bayefsky et al., 2016). Preimplantation Genetic Testing (PGT) regulations can vary widely across different countries. It seems like Malta and Bosnia & Herzegovina have more stringent regulations in place regarding PGT compared to other European countries (Calhaz-Jorge et al., 2020a). Concerns about eugenics have indeed been a focal point After the introduction for preimplantation genetic testing (PGT). Legislation in many countries reflects this concern by banning selection for non-pathological characteristics. PGT-A's exclusion in some European countries underscores the ongoing debate over where to draw the line between medical necessity and ethical boundaries (Calhaz-Jorge et al., 2020 b). The advancements in embryo selection and preconception carrier screening raise important ethical and legal considerations. Balancing the need for thorough medical assessment with ethical reflections is crucial

to ensuring responsible implementation and decision-making in this field. Continuous debate and amendments based on evolving knowledge and societal values are essential to navigate these complexities responsibly (Dondorp and De Wert, 2019). PGT-M (Preimplantation Genetic Testing for Monogenic Disorders) has the potential to exist offered to a wide range of genetic conditions, both nuclear and mitochondrial, if the disease-causing genetic variants are identified. This could encompass various inheritance patterns such as X-linked, autosomal dominant or recessive, and maternally inherited mitochondrial disorders (Richards et al., 2015). Preimplantation genetic testing (PGT) of various genetic disarray, including autosomal recessive, autosomal dominant, and X-linked disorders. PGT offers advantages such as identifying healthy embryos and allowing for selective transferred according to doctors' preferences and center policies. However, it's important to note that PGT using sex selection for purpose other than medicine similar to familial balance is forbidden in many nations. The ethical considerations surrounding Human Leucocyte Antigen (HLA) matching of preimplantation embryos are indeed complex. While it may not be addressing a pathological condition directly, it raises questions about the purpose and implications of such interventions, particularly in terms of selective breeding and the potential for creating "designer babies" (Kakourou et al., 2018 a). PGT can be used to identify embryos that are not only free of genetic disorders but also comfortable to the sibling who is impacted in require of a bone marrow transplant. This way, families can have a child who can potentially serve as a donor for their sibling, providing a chance for life-saving treatment into the years to come. Blood-forming stem cells can be sourced in either at the childbirth or thereafter, the link plasma from the bone marrow of a child born through PGT. These stem cells serve as the basis for bone marrow transplantation to treat the affected siblings. To pairs who have kids affected by an inherited hematologic cancer, HLA typing alone is often conducted to identify potential donors. However, when dealing with monogenic disorders like immunodeficiencies and hemoglobinopathies, HLA typing is typically combined with PGT to ensure both genetic health and compatibility for transplantation, maximizing the chances of a successful outcome for an impacted cousin (Kakourou et al., 2018).

These ethical considerations surrounding the choice for eggs with matching Antigen are indeed complex and varied. While many countries permit preimplantation genetic testing (PGT) in order to stop the spread of specific illnesses, regulations concerning HLA typing are more restrictive. It's a delicate balance between medical necessity and concerns about potential instrumentalization of the child. Preimplantation Genetic Testing (PGT) with exclusion testing allows individuals at probability for transferring on inherited diseases such as Huntington's disease to have biological children who are unaffected by the condition. It involves screening embryos generated in vitro fertilization (IVF) to select people who lack the illness-causing mutation before implantation in the uterus. This can provide peace of mind for couples who wish to order to prevent transferring along the state for their children (Van et al., 2012). By allowing parents to have children who are not impacted by the disease, exclusion testing honours their decision to remain ignorant of their own genetic status. Through the provision of thorough information on the genetic implications, dangers, and accessible options, genetic advice plays a critical part in this process, enabling people to come to educated decisions concerning their reproductive choices. It ensures that they understand the implications of exclusion testing and the potential outcomes for their future children (Carvalho et al., 2020) The ethical considerations surrounding exclusion testing in PGT are indeed significant. The potential for discarding embryos with a 50% chance of being healthy, coupled with the risks and side effects of IVF/PGT treatment, raises concerns about the balance between benefit and harm in the use of these techniques. The prohibition of PGT with exclusion testing in some countries reflects the ethical concerns associated with this approach. However, the alternative of direct testing with non-disclosure poses its own challenges, such as the need for extreme confidentiality and the potential for unethical behavior among practitioners. These complexities highlight the importance of careful consideration and regulation in the use of genetic testing techniques in reproductive medicine (Shenfield et al., 2003). PGT (Preimplantation Genetic Testing) for mitochondrial DNA pathogenic variants is indeed limited in availability, mainly due

to the complexity of analysing mitochondrial DNA and its hetero plasmic nature. The variable pathogenic variant load in different cell types and over time adds another layer of complexity to diagnosis and treatment. The inheritance pattern of pathogenic variant mt DNA can vary widely due to the genetic bottleneck during oogenesis, further complicating the prediction of disease manifestation in subsequent generation. PGT can be used to select embryos with a mitochondrial DNA pathogenic variant load below the threshold for clinical expression, potentially reducing the risk of having an affected child. However, it's an ethically challenging area because it doesn't entirely eliminate the risk; it only decreases it. This necessitates thorough case-by-case counselling to ensure families understand the limitations and implications of the procedure (Smeets et al., 2015).

PRE-IMPLANTATION GENETIC TESTING FOR MONOGENIC DISORDER (PGT-M)

PGT-M and PGT-SR focus on genetic disorders, when changes are discovered among parental hands, while PGT-A deals with de novo conditions, which are not inherited. Each serves a distinct purpose in ensuring healthy pregnancies'-M, or Preimplantation Genetic Testing for Monogenic Disorders, aims to stop the transmission of cells impacted by particular genetic diseases. It is done by choosing embryos which both do not carry the mutation or are healthy carriers. It is customized for each pair and involves studying relatives. It is applicable for known monogenic diseases with identified genes, available index cases, and a feasible diagnostic protocol. However, it isn't suitable for instances of significant gene elimination duplicates or increases of quads de novo. An exceptional use is HLA typing, where embryos are selected to be compatible donors for siblings needing stem cell transplants (Carvalho et al.,2020; Zeng et al., 2018; McArthur et al., 2005; Orvieto et al.,2020; Greco et al., 2020; Rubino et al., 2020). Preimplantation genetic testing for monogenic disorders allows prospective parents to screen embryos for specific genetic mutations associated with known hereditary conditions or predispositions within the family. It helps to identify embryos without the mutation before implantation during IVF procedures, lowering the possibility for handing with genetic disorders to the next era (De Rycke et al., 2017 a). PGT-M, or Preimplantation Genetic Testing for Monogenic Disorders, is indeed a powerful tool used to identify disease-causing genetic variants in embryos. It allows for the selection of embryos without these harmful variants, helping families to order to prevent on genetic diseases to their children (De Rycke and Berckmoes;2017). Preimplantation genetic testing-monogenic (PGT-M) indeed focuses on specific single-gene disorders and hereditary cancer syndromes, providing an option for prospective parents to screen embryos for these conditions before implantation. It is an important tool for families with a history of genetic disorders (De Rycke et al., 2017). Handyside's group indeed made significant advancements in reproductive technology by reporting the first live births using IVF followed by preimplantation genetic diagnosis (PGD) for an X-linked disorder in 1990. It was a milestone moment within the context of genetic technology support (Handyside et al.,1990). Haplotyping is one of the methodologies that emerged for diagnosing genetic disorders post-2005. It has been particularly useful in Preimplantation Genetic Testing (PGT), allowing for more precise identification and screening of genetic disorders (Fiorentino et al., 2005). Preimplantation genetic testing-monogenic indeed offers a promising avenue for identifying human leukocyte antigen-compatible, unaffected embryos, facilitating treatments like bone marrow implant or stem blood transplantation for ill family members. While a slight chance of misinterpretation exits, modern techniques have minimized such occurrences, with no reported cases in data from the European Society of Human Reproduction and Embryology Consortium (De Rycke et al., 2017) Embryo selection based on HLA typing and PGT for late-onset diseases like Huntington's have indeed sparked ethical debates. In cases where the at-risk partner prefers not to know their genetic status but still wants to avoid transmitting the mutation to their child, exclusion testing or non-disclosure testing during PGT can be considered. These options aim to address concerns about genetic transmission while respecting the preferences of the at-risk partner. Confirming preimplantation genetic testing-monogenic results with CVS or amniocentesis is a crucial step to ensure accuracy and provide comprehensive information for informed decision-making. It is essential for ensuring the health and well-being of both the parents and the unborn child.

I. **Analyze Strategies**:

Preimplantation genetic testing for monogenic disorders (PGT-M). Starting during an examination from the trophectoderm (TE) cells, 5 to10 cells are typically Gathered. After that, A method may branch either Whole-Genome amplified or targeted amplified depending on a specific testing protocol and the genetic information needed (Harton et al.,2011). various Method of genomic analysis, for example multiplex PCR, substitution arrays, also NGS, all aimed at haplotyping to identify risk-associated haplotypes linked to mutations. These methods are crucial for determining whether embryos have inherited risk alleles. It is essential to conduct preclinical genotyping of SNP markers near the gene of interest in DNA samples from the couple and family members to ensure accuracy (Aj J;1985). The sophisticated process of Preimplantation Genetic Testing for Monogenic Disorders (PGT-M) and Aneuploidies (PGT-A). Integrating SNP genotyping with real-time PCR allows for efficient mutation and SNP marker identification. Adding CNV assays to TaqMan assays allows a simultaneous identification for aneurisms, expanding scope of PGT-M and PGT-A. Advancements in genetic testing techniques, particularly regarding the target protocol and whole genome amplification (WGA). The low allele dropout rate and quicker diagnoses through real-time PCR are significant advantages. The development of WGA, especially with multiple displacement amplification (MDA), has opened new possibilities for amplifying the entire genome. Advancements in genetic testing techniques, particularly regarding the target protocol and whole genome amplification (WGA). The low allele dropout rate and quicker diagnoses through real-time PCR are significant advantages. The development of WGA, especially with multiple displacement amplification (MDA), has opened new possibilities for amplifying the entire genome (Treff et al., 2013b). PGT-M (Preimplantation Genetic Testing for Monogenic disorders) involves a thorough process to ensure accuracy and reliability. The focus on family study and screening for various factors seems crucial for couples undertaking this path (Huang et al.,2015; Harton et al.,2011a)

II. Conditions For PGT-M:

The intricacy and unpredictability of genetic of monogenic diseases pose significant challenges to preimplantation genetic testing for monogenic disorders (PGT-M). Since these diseases is caused by mutations in a single gene, the genetic landscape can be intricate, with various mutations and genetic variations complicating the testing process. This complexity can make it difficult to accurately detect and diagnose the specific mutation responsible for the disorder, leading to limitations in the effectiveness of PGT-M. The complexity of hereditary genetic diseases is fascinating yet challenging. With multiple alteration in a single or distinct genetic causing comparable outcomes, pinpointing the exact culprit can be like finding a needle in a haystack. It requires meticulous genetic analysis and understanding to unravel the mysteries behind these conditions. Addressing genetic variability in monogenic disorders is indeed complex due to the influence of environmental and epigenetic factors. Preimplantation diagnostic tests face challenges in accurately identifying mutations, especially with Alternative that may not be very important, which may make it more difficult to evaluate results and risk assessment for developing the disease. Managing the intricacies of genetic variability and monogenic disorders necessitates a multifaceted approach. Integration of advanced molecular techniques, diagnostic tools, and bioinformatics is essential for pinpointing disease-causing genetic variants. Moreover, collaborative efforts among specialists such as clinical geneticists, molecular biologists, and bioinformaticians are vital for accurate interpretation of genetic data and identifying pertinent mutations for Preimplantation Genetic Testing for Monogenic Disorders (PGT-M).Preimplantation Genetic Testing for Monogenic Disorders (PGT-M) often requires a significant amount of genomic DNA for accurate analysis, necessitating whole genome amplificated (WGA) to produce sufficient supplies from a limited input samples obtained during the procedure. This step ensures that there's ample DNA available for molecular analyses, including sequencing or PCR-based assays (Harton et al.,2011; Vanneste et al., 2011). The complexities of preimplantation genetic testing for monogenic disorders (PGT-M). The challenges of allele drop out, amplification failure, and contamination in embryo analysis indeed highlight the need for meticulous indirect analysis methods. Additionally, the reliance on first-degree relatives for haplotype determination can pose obstacles when unavailable. While targeted protocols offer a solution, they do require more time compared

to next-generation sequencing (NGS), which offers more comprehensive data in a shorter timeframe. Whole-genome amplification (WGA) has indeed opened new possibilities in genetic screening by allowing genome-wide analysis from even single or few cells. This advancement has been crucial in detecting mutations that were previously undetectable with conventional methods (Handyside et al., 2004; Hellani et al., 2004). Karyomapping is a fascinating technique leveraging SNP array technology. Despite SNPs being individually less informative than STRs, the combination of multiple SNPs can accurately determine genotypes. With just four SNP markers, it's possible to determine genotypes for parents and the index case, showcasing the efficiency of this method. Karyomapping depends on linking research; it identifies if the allele containing the variant is present or not through contrasting the short tandem repeats (SNPs) linked with the disease-causing mutation in the index case and parental chromosomes with those found in embryo cells. Thus, karyomapping represents a change from a diagnostic strategy focused on a family or a disease to a "genome-wide" strategy that can potentially be applied to any monogenic mutation including informative SNPs. Furthermore, this technique addresses the issue of failed allele amplification in single-cell situations by separating critical SNPs in the embryo sample from non-key SNPs. While genotyping errors are not completely eliminated, their frequency is much decreased by this method, which typically yields a consistent set of important SNP markers.

GENETIC AND REPRODUCTIVE - (PRECLINICALWORKUP)

A comprehensive approach to ensure prospective parents are well-informed and supported before starting a clinical cycle. It is crucial to address both the genetic and psychological aspects of the process. Practice for parents to sign informed consent before any medical procedure, ensuring they understand the risks and benefits involved. Collecting blood samples for preclinical workup allows for thorough genetic testing and analysis, helping to inform decisions and potential outcomes. Preimplantation Genetic Testing for Monogenic Disorders (The medication PGT-) requires the inclusion of blood samples and genetic data from relevant first-degree members of the family. This comprehensive method helps for identify and understand potential genetic risks within the family lineage, providing valuable information for the clinical cycle. Patients receiving Preimplantation Genetic Testing (PGT) go through the same preliminary fertility investigation and stimulation of ovaries process as those receiving traditional IVF (In Vitro Fertilization). This ensures that the necessary steps are taken to optimize the chances of successful embryo development and implantation. This helps for determining whether chromosomal anomalies which may affect ovulation or raise the possibility of genetic disorders in offspring. It is an important step in ensuring a thorough assessment of genetic health before proceeding with assisted reproductive techniques. Complementing conventional karyotyping with screening tests Regarding the possession of frequently occurring variants in genes such as cystic fibrosis, spinomuscular atrophy, or hemoglobinopathies provides a more comprehensive understanding of potential genetic risks. In the future, extended carrier screening is likely to replace individual tests, offering broader coverage of genetic variants associated with inherited disorders, thus enhancing the accuracy and efficiency of genetic screening in reproductive medicine. Having genetic reports accessible with the ingestion of a Preimplantation Genetic Testing (PGT) request is crucial for ensuring that the testing process can be efficiently initiated and tailored to the specific genetic concerns of the prospective parents. This allows for timely decision-making and planning for the clinical cycle based on the information provided in reports. while approach to the preclinical genetic workup for monogenic disorders based on various factors, including the test methodology and the specific strategy employed. Targeted testing focuses on specific genes or regions of interest, while genome-wide testing examines the entire genome for potential variants. Additionally, the strategy can involve indirect tests based on genetic markers or direct tests that detect the pathogenic variant itself. Each approach has its advantages and may be selected based on factors such as the known genetic mutation, the availability of family member genetic information, and the specific disorder being screened for.

IVF, EMBRYO BIOPSY, TRANSFER, AND CRYOPRESERVATION

I. IVF and Current Embryo Biopsy Methods

Figure 1. Following the cycle of assisted fertilization, a biopsy is conducted, and the sample of retrieved cells is analyzed genetically to establish the suitability of the embryo for transfer (Giuliano et al.,2023).

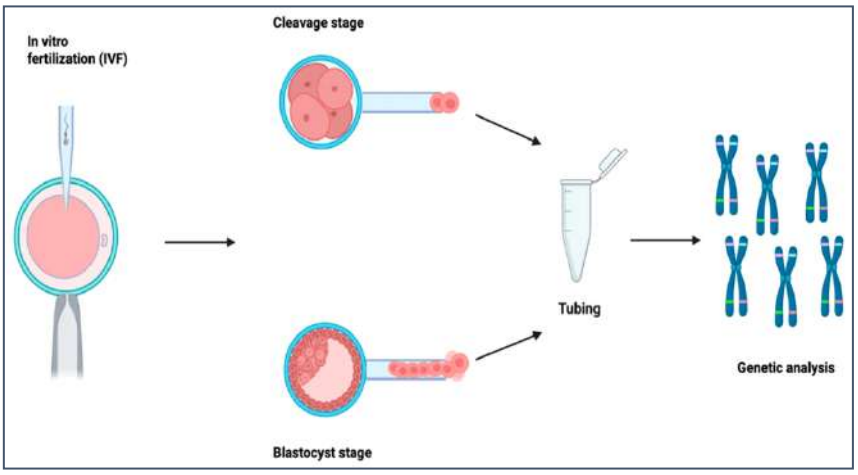

Figure 1: *In-Vitro* Fertilization (IVF)

ICSI (Intracytoplasmic Sperm Injection) is indeed recommended over regular IVF (In Vitro Fertilization) for PGT (Preimplantation Genetic Testing) treatment to minimize the risk of contamination from remaining cumulus cells or residual sperm cells attached to the zona pellucida. This targeted approach ensures a higher degree of accuracy in genetic testing and reduces the chances of false results due to contamination.Ccertain indications for Preimplantation Genetic Testing (PGT) may indeed be linked to reduced spermatogenesis, which can result in lower fertilization rates during assisted reproductive procedures like IVF (In Vitro Fertilization) or ICSI (Intracythe male partner's ADPKD status might have a greater impact on fertilization rates and live birth delivery rates compared to when the female partner is affected, although the age of the female partner could also be a contributing factor. This underscores the importance of considering both partners' health conditions and ages when evaluating fertility outcomes in couples undergoing assisted reproductive procedures. Plasmic Sperm Injection. In such cases, additional measures may be necessary to optimize fertilization and improve the chances of successful embryo development. Autosomal dominant polycystic kidney disease (ADPKD), caused by mutations in the PKD1 and PKD2 genes, can indeed affect the male reproductive system. Men with ADPKD may experience lower sperm motility and reduced sperm concentration, which can impact fertility. When undergoing assisted reproductive techniques like IVF or ICSI, these factors may contribute to lower fertilization rates and need to be taken into consideration during treatment planning (Berckmoes et al., 2019). The approach to oocyte or embryo vitrification before biopsy can vary among fertility centers. Some centers adopt a systematic approach where vitrification is routinely performed before biopsy to accumulate a larger number of embryos for testing. On the other hand, in other centers, vitrification before biopsy may be reserved as a rescue strategy, typically in a minority of cycles, particularly in cases where rapid intervention is needed to preserve fertility, such as in individuals undergoing cancer treatment. This tailored approach depends on various factors including the center's protocols, patient preferences, and specific medical circumstances (Ubaldi et al., 2016; Chamayou et al., 2017; Hu et al., 2017). Biopsy can be performed at different developmental stages. All present methods are invasive. Biopsying the first and second polar bodies, which are both necessary for accurate diagnosis, is indeed not widely practiced and is currently applied in only a minority of fertility centers. This information is based on unpublished data from the ESHRE PGT consortium of 2016 and 2017. The choice of biopsy method can vary among centers based on factors such as expertise, equipment availability, and patient preferences. One advantage of Biopsying polar bodies is that their removal doesn't typically impact embryonic development. However, a significant limitation is that only the maternal genetic contribution can be evaluated since polar bodies contain genetic material from the egg. This means that any potential genetic abnormalities from the sperm are not assessed, providing an incomplete picture of the embryo's genetic makeup. Cleavage-stage embryo

biopsy, as the gold standard, typically involves opening the zona pellucida (the outer layer of the embryo) using mechanical, chemical, or laser methods. Then, one or more blastomeres (cells) are removed from the embryo, usually by aspiration, on day 3 of preimplantation development when the embryo has reached the 6-8 cell stage. This procedure allows for genetic testing of the embryo's cells while still at an early developmental stage. Major disadvantages associated with day 3 biopsy. One is the limited amount of DNA available for testing due to the small number of cells in the embryo at this stage. Additionally, the removal of embryonic cells during biopsy can negatively impact embryonic development and implantation potential. Research has shown that removing two cells at the cleavage stage can harm embryonic development and implantation potential more than removing just one cell, highlighting the importance of minimizing any potential damage to the embryo during the biopsy process (De Vos et al., 2009). Based on the concerns you mentioned, the recommendation of removing only a single cell at the cleavage stage has been advocated. This approach helps mitigate potential harm to the embryo while still allowing for genetic analysis. Cleavage-stage biopsy also provides sufficient time for genetic analysis to be completed before fresh embryo transfer, typically scheduled for day 5 or later in the embryo development process. This timing ensures that the results of genetic testing are available before the embryo is transferred into the uterus, optimizing the chances of a successful pregnancy. Cryopreserving supernumerary genetically transferable embryos for later use is a common practice in assisted reproductive technology. Blastocyst or trophectoderm (TE) biopsy is currently the most widely used technique for Preimplantation Genetic Testing (PGT). This method involves removing a small number of cells from the trophectoderm, which eventually forms the placenta, of a blastocyst-stage embryo (around day 5-7 of development). Laser energy is commonly used to open the zona pellucida, either on day 3/4 or on day 5, depending on the clinic's protocols and the stage of embryo development. TE cells are then aspirated and excised with a laser from herniating blastocysts, or aspirated in combination with mechanical dissection from blastocysts, typically on day 5/6 (Cimadomo et al., 2020). The blastocyst stage is considered less sensitive to possible embryo damage during biopsy compared to earlier stages, such as cleavage-stage biopsy, because the inner cell mass (ICM), from which the fetus originates, is left intact. This means that the cells biopsied from the trophectoderm (TE) have less potential to harm the future fetus (Scott et al., 2013). Another advantage of trophectoderm (TE) biopsy is the lower level of chromosomal mosaicism observed at the blastocyst stage compared to the cleavage stage. Mosaicism refers to the presence of cells with different genetic compositions within the same embryo, which can complicate genetic testing results.

II. Present Advancements and upcoming sample Techniques

To facilitate the biopsy at this stage, the embryo is artificially decompacted using a Ca/Mg-free medium (Zakharova et al., 2014). Morula-stage biopsy has its appeal due to several factors. Firstly, it typically allows for the retrieval of more cells compared to cleavage-stage biopsy, potentially providing a larger sample for genetic analysis. Additionally, since the biopsy is performed before the embryo reaches the blastocyst stage, the cells are generally intact, reducing the risk of damage that can occur during trophectoderm (TE) biopsy. That's an important observation from Iranian and associates. Their reliance on morula biopsy at day 6 for slowly developing embryos aimed for increase a number for embryos testable now. However, their outcomes revealed lower implantation and live birth rates, as well as higher rates of complex aneuploidy associated with this approach (Irani et al., 2018). Blastogenesis is involves the desire for be less intrusive than trophectoderm (TE) biopsy because it involves the aspiration of blastocoel flowing (BF) from the blastocoel cavities rather than the embryos cells being removed itself. In addition, blastocentesis causes embryo distegration, which is a typical modification used in standard vitrifying procedures to increases the chance of survival of embryos after vitrifying (Van Landuyt et al., 2015). Several studies have indeed shown that the karyotype concordance between blastocoel fluid samples obtained through blastocentesis and inner cell mass (ICM) and/or trophectoderm (TE) cells can vary usually. This variability suggested the accuracy of genetic analysis after blastocentesis may be inadequate for clinical preimplantation genetic testing for structured

modification (PGT-SR) or Aneuploidy (PGT-A) (Capalbo et al., 2018 a). Preimplantation genetic testing (PGT) considers the analysis of cell-free DNA (cfDNA) from spent embryo culture medium obtained at the blastocyst stage to be a promising alternative sampling technique. The technique involves analyzing the genetic material released by the embryo into the culture medium during its development (Capalbo et al., 2018). Substituting laser-based biopsy methods with non-invasive sampling techniques, such as the analysis of cell-free DNA (cfDNA) from spent culture medium, blastocoel fluid, or other sources, could potentially revolutionize the field of Preimplantation Genetic Testing (PGT). This shift would indeed represent a safer option for the embryo, as it eliminates the need for invasive procedures that carry some risk of harm.

III. Embryo Transfer and Cryopreservation

The trend toward single embryo transfer (SET) in Preimplantation Genetic Testing (PGT) centers is influenced by various factors including reimbursement mechanisms, legislation, and the recognition this SET is linked to more secure medical outcomes of pregnancies. By transferring only one embryo, the possibility of many pregnancy stages and the problem they can cause significantly reduced, leading to better maternal and fetal health outcomes (Loutradi et al.,2008). The evidence suggests Diagnostic finding following a voluntary freezing embryo transfer (FET) is comparable to or even better than those afterwards the implantation of a new cell (ET) When the broader populace, particularly normo-ovulatory patients. Additionally, FET has been shown to offer advantages for particular group such as individuals who are more susceptible to ovarian hyperstimulation syndrome (OHSS) (Bosch et al., 2020).

DIAGNOSTIC METHODS

I. Early Methods of PGT-M

Figure 2. summarizes the history, current state, and future of PGT-M techniques. Early in the 1990s, single-cell simplex PCR amplification was used to launch PGT- M From these initial methods, contamination and allele drop out (ADO) emerged as significant problems that could result in an incorrect diagnosis (Rechitsky et al., 1999) While so many amplification cycles are needed to increase the minuscule amount of DNA, contamination with extraneous DNA or carry-over from prior amplification reactions was and remains a significant issue. By implementing stringent preventative measures, it can be reduced. ADO occurs when alleles in a heterozygous sample are amplified unequally (preferentially), resulting in undetectable alleles. Sensitive techniques for allele detection and refined procedures for cell lysis and amplification are essential components of ADO control. Adding closely related informative short tandem repeat (STR) markers to the PCR process was by far the most crucial step in controlling and detecting ADO as well as contamination. A more accurate test can be obtained by co-amplifying STR markers with or without the pathogenic variant amplicon(s) at the level of one or a small number of cells. This technique, known as haplotyping, has been the industry standard for more than 20 years (De Rycke et al.,2017; Cimadomo et al., 2020; Spits et al., 2006).

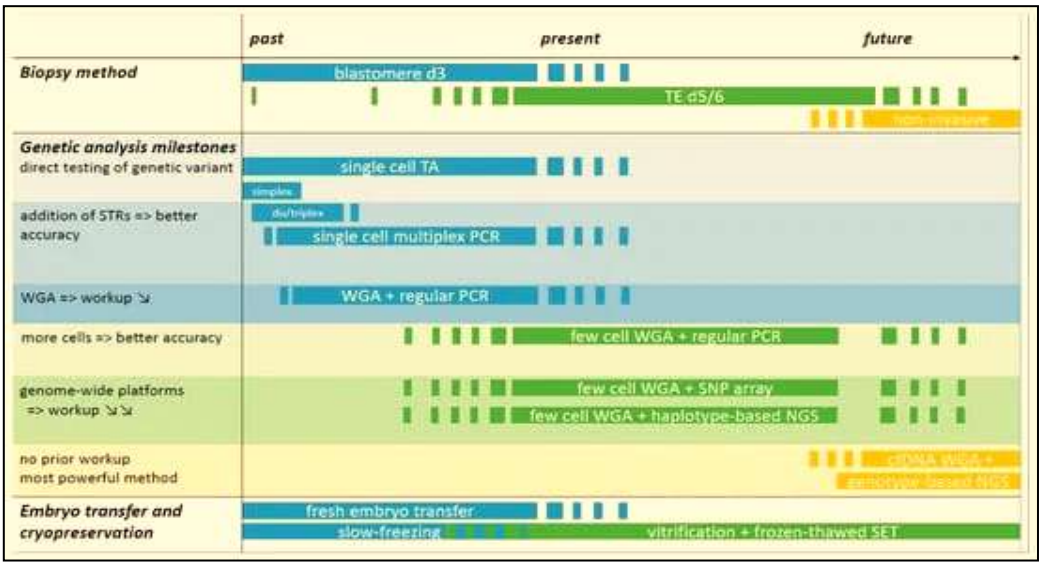

Figure 2: Biopsy Method

II. Whole Genome Amplified methods

Technology advancements such as one- or few-cell entire genome amplified (WGA) led to the development of more widely used techniques. The initial Whole Genome Analysis techniques relied on PCR and were hampered by partial genomic cover along with amplified biases. The application of Taq DNA polymerase resulted in numerous DNA sequence mistakes and 400–500 bp annually for fragmentation width (maximum size 3 kb) (Coskun et al.,2007).A single-cell numerous dislocation amplified (MDA) method based upon thermal displacement of strands amplified originated more than ten years ago(Coskun et al., 2007).Random exonuclease-resistant starters converge to the damaged target DNA in an MDA process, causing an a thermodynamic reaction at 30 ◦C. A polymerase enzyme with strand-displacement activity, such as Phi29, then elongates those primers. Every displaced strand may experience additional priming processes, creating a system with branching DNA strands spanning more than 10 kb. While the problem that percentage for MDA based WGA is significantly lesser than that of Taq DNA polymerase-based techniques due to the proofreading activity of Phi29 polymerase, non-linear amplification results in either an over- or under-representation of genomic areas (Spits et al., 2006). Afterwards, WGA techniques that combined PCR amplification with MDA were presented. During fragmentation of DNA and a combination gene pre-amplification MDA response, PCR is the next step in both the Rubicon Pico PLEX and MALBAC (A few annealing and Using loops Base Amplified Phases) methods. (Zong et al., 2012)

III. Genetic sequence targeting PGT-M

Using SNP arrangement, which are Extremely dense oligo arrangement with A few billion probe or more, hundreds as thousands as chosen SNPs can be genotyped for each chromosome with an individual response. The SNP arrays that are sold commercially employ several techniques for SNP genotyping. It is common practice to use single base extension reactions or hybridization to SNP allele-specific probes (LaFramboise,2010). The arrays of DNA are scanned according to the amount of brightness then the proportion for hybridized intensity for A and B (the allele frequency), as well as SNP genotype are assigned (for example, AB if the intensities are similar at an intermediate level). The linkage-based testing approach for PGT-M is shared by targeted multiplex PCR and SNP array; however, the SNP array procedure is considerably more uniform and standardized and does not require a locus-specific preclinical workup. As a result, the couples' wait times and the workload in the lab are greatly decreased. One disadvantage of SNP arrays appears to be the expensive cost of consumables and equipment, which appears to be impeding their wider clinical application Specifically effective for double indications (two monogenic illnesses, for example (Handyside et al., 2010). It works with both NGS and SNP arrays. The commercially accessible karyomapping algorithm relies on a near relative for phasing and employs Irregular bipolar substitution responses (assumes AA, BB, AB, or No call as potential status for every single Genotype) along with fundamental Mendelian rules.

IV. PGT-M for De Novo Pathogenic Variants

This is essential to incorporate Genomic variety discovery into the study method in the event that there are de novo pathogenic variation(s) and if parent members' pertinent DNA samples cauld be collected. Both targeted and genome-wide approaches are conceivable for this, while the latter's validation process is considerably simpler. PCR may identify a wide range of genetic variations, and if required, it can be augmented with a post-PCR reaction. By using fragment length difference, Remove and replace variation with particular nucleotides may be instantly identified. Several allele discrimination techniques, such as Mini sequencing, has undergone development for single nucleotide substitutions (Bermudez et al., 2003), Since the precise breakpoints of gene rearrangements are often unclear, it has been challenging to directly detect complicated and/or bigger rearrangements. Long-read nanopore sequencing has made it possible to characterize breakpoints outside of highly repetitive regions with great resolution (Chow et al., 2020).

V. SNP Array for Concurrent PGT-M and PGT-A

Given that the raw data set contains information on both Combining Variable genotyping & genetic number of copies., SNP arrays can facilitate the simultaneous investigation of PGT-M and PGT-A. Therefore, the presence of aneuploidies, polyploidies, and uniparental disomy can be detected using SNP arrays (Kubicek et al., 2019). Combining Variable genotyping & genetic number of copies. A typical

copy number (n = 2) is represented by BAF values of 0, 0.5, and 1, however deviations will result in changes to the allele frequencies and overall intensity. Because of WGA problems, SNP arrays at the single or few cell level produce a plenty of sounds, necessitating the adoption of especially sophisticated algorithms for data interpretation. Errors will be produced in regions with copy number changes by genotyping techniques that use Requests of distinct haploid mutation, such as karyomapping (true). To get around this restriction, several PGT centers use newly created or extended algorithms that are developed internally. For example, the computational pipeline known as haplarithmisis, which mainly uses continuous BAFs, enables the detection of haplotypes and copy numbers as well as the identification of the chromosomal anomaly's parental origin.[81]

VI. NGS for Concurrent PGT-M and PGT-A

Using adapters with barcodes, NGS breaks down DNA and creates a library of templates for a less expensive analysis that can analyze several samples in a single run. Then, either directly (third-generation) or after previous clonal amplification (second-generation), the single molecule templates are sequenced in parallel from one end or from both ends. The sequence reads are then mapped to a reference genome. The read depth, also known as genome coverage, is a critical metric that indicates how many reads are present at a specific genomic location. It has been shown that a comparatively low average coverage is adequate for precise numerical chromosomal analysis (Yin et al., 2013). Since sequencing at high coverage is necessary for monogenic disorders and is now too costly for normal clinical applications at the whole-genome scale, a few techniques are being researched with the goal of developing quick and inexpensive protocols. These methods can be divided into two categories based on whether sequencing data for the monogenic locus are obtained using a targeted or a genome-wide approach. Typically, they offer a tandem solution that combines PGT-A with PGT-M. Similar steps are involved in the MARSALA method (mutated allele revealed by sequencing with aneuploidy and linkage analyses). First, an aliquot of MALBAC-based WGA products is subjected to targeted amplification, and the mixture of WGA and targeted enriched templates is then sequenced at low depth (0.1–2×). This yields genome-wide PGT-A data as well as targeted SNP haplotyping results for PGT-M (Yan et al., 2015). Chamayou and colleagues devised a universal approach for simultaneous PGT-A and PGT-M in cystic fibrosis (Chamayou et al., 2020). In a different NGS method, MDA-based WGA products were treated to a target enrichment gene panel (TruSight One sequencing panel) that contains nearly 5000 Mendelian disease-associated genes. This allowed for the direct testing of any pathogenic variants found in the family as well as indirect pathogenic variant detection via SNP haplotyping and chromosome copy number detection via the log ratio of signal intensities, i.e., PGT-M and PGT-A together in a single workflow (Del Rey et al., 2018).It is evident that using PGT-M and PGT-A concurrently opens up new possibilities, but it may also result in unintentional discoveries and more intricate genetic data than we now know. As a result of our inability to evaluate data with sufficient expertise, genetic counseling faces numerous ethical dilemmas and discussions.

CLINICAL OUTCOME

Comparing clinical results across different institutions is challenging due to a variety of factors, including variations in genetic testing and IVF methods, indication types, and mother age. It is also difficult to properly benchmark against consortium data because reports of significant data collections have been delayed. The most recent publication pertains to the ESHRE European IVF-Monitoring Consortium's summary PGT data for 2015. Across all PGT indications, this data set, which was gathered from 23 nations, revealed a pregnancy rate of 39.7% per fresh embryo transfer cycle and a rate of 41.0% each frozen embryo transfer (De Geyter et al., 2020). Any laboratory that wants to do PGT must have a high diagnostic efficiency because it can improve the clinical outcome. This is especially true for indications with low success rates presumptively, as PGT-M with HLA typing, where it has been demonstrated that there is only a 16% probability of producing a genetically suitable embryo (De Geyter et al., 2020). This resulted in a live birth delivery rate of 30.3% per transfer, demonstrating the value of PGT with HLA typing. The procedure's high complexity and low delivery rate are counterbalanced by the successful outcome of transplantation and the favorable effects on families (De Rycke et al., 2017). Since many embryos transfers result in neither pregnancy nor delivery and only a small percentage of

children receive prenatal or postnatal testing, it is challenging to determine the actual misdiagnosis rate. To offer an internal estimate of the misdiagnosis rate, PGT clinics are advised to reanalyze a portion of the non-transferred embryos

CONCLUSIONS AND FUTURE PERSPECTIVE

Over the years, significant developments have been made in the fields of assisted reproduction and PGT, solidifying PGT's status as a reliable, accurate, and secure clinical treatment. The adoption of genome-wide approaches has made it possible for the genetic laboratories to become more consistent and standardized. But other genetic discoveries beyond the desired genetic condition—like chromosomal mosaicism in embryos examined for a monogenic disorder—have created new challenges for genetic counseling and the formulation of embryo transfer policies. PGT is theoretically moving toward an all-in-one, sequencing-based solution for PGT-M, PGT-SR, and PGT-A as sequencing costs continue to drop. Since the number of diseases with known genetic causes is increasing and individuals are becoming more conscious of the hazards of passing on genetic problems, the total number of therapies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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