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Preimplantation genetic testing: A narrative review

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Abstract Preimplantation genetic testing (PGT) is a diagnostic procedure that has become a powerful complement to assisted reproduction techniques. PGT has numerous indications, and there is a wide range of techniques that can be used, each with advantages and limitations that should be considered before choosing the more adequate one. In this article, it is reviewed the indications for PGT, biopsy and diagnostic technologies, along with their evolution, while also broaching new emerging methods.

Key words: PGT-M, PGT-SR, PGT-A, embryo selection, preimplantation genetic testing

Introduction

Preimplantation genetic testing (PGT) is a diagnostic method that has become a powerful complement to assisted reproduction techniques. It is currently widely used to detect hundreds of genetic and chromosomal abnormalities in oocytes and embryos for posterior selection of the ones which are genetically transferable, thus avoiding the transmission of a disease to the offspring. There are three types of PGT: preimplantation genetic testing for monogenic disorders (PGT-M), preimplantation genetic testing for structural rearrangements (PGT-SR), and preimplantation genetic testing for aneuploidy (PGT-A).

The term preimplantation genetic testing was introduced in 2017 as a substitute of the terms preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) after a consensus was reached regarding terminology about infertility and fertility care.[1] The development of PGT started in 1890 with Walter Heape and was then first successfully implemented in 1989 by Alan Handyside as an alternative method to prenatal diagnosis (PND).[2]

PGT is a complex test which includes collection of genetic material by biopsy of an oocyte or embryo, preparation of the material, and genetic analysis. There are various techniques that can be used. In the 1990s, the techniques employed were, initially, based on polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH), and they were applied to polar bodies or cleavage state embryos.[2-4] Since then, PGT has suffered a significant evolution because of the implementation of new technologies, which assure a safer biopsy, collection of material with lower levels of mosaicism, one of the limitations of PGT, and analysis of an increased number of molecular targets, all with reduced analysis time and costs.

Indications for preimplantation genetic testing

Preimplantation genetic testing for monogenic disorders (PGT-M)

PGT-M aims to detect pathogenic variant(s) that cause monogenic diseases, and it is indicated in couples who either have the disease or an elevated risk of transmitting it. The goal here is to select the embryos that do not have the pathogenic variant or are healthy carriers, in the case of an autossomal recessive disease, to originate healthy offspring. Technically, PGT-M can be used for all monogenic diseases whose responsible gene is known, but the affected gene must have a clear family segregation. Specific indications for PGT-M involve X-linked disorders (e.g., Duchenne muscular dystrophy), Y-linked disorders, autosomal dominant disorders (e.g., Huntington disease), autosomal recessive disorders (e.g., cystic fibrosis), mitochondrial disorders, and some severe disorders with high genetic predisposition (e.g., hereditary breast and ovarian cancer). PGT-M can also be used for HLA typing (to identify an HLA-compatible embryo for an affected sibling in need of a transplant), although this generates various ethical concerns.[5-7]

PGT-M presents itself with important challenges. Besides the limitations inherent to the technologies employed, the genetic variability and complexity of monogenic disorders makes it difficult to efficiently identify a specific disease-causing variant and its probable corresponding clinical manifestation. Monogenic diseases can be caused by diverse pathogenic variants on a single-gene or pathogenic variants on different genes that cause similar phenotypes. To overcome this, it is necessary to use improved methods and a collaboration of a multidisciplinary team.[8]

Preimplantation genetic testing for structural rearrangements (PGT-SR)

PGT-SR aims to identify chromosomal rearrangements in embryos, including all types of deletions, duplications, inversions, and translocations, and select the ones that are genetically normal or balanced for the structural rearrangement and will most likely lead to a successful pregnancy.[6]

The major limitation of PGT-SR is based on the variability of structural rearrangements. Even with improved sequencing and analysis methods, the accuracy of the test depends of the size and

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complexity of the rearrangement, and it can lead to misdiagnoses. While some rearrangements involve a limited number of chromosomes and regions, others extend to numerous chromosomes and segments, therefore requiring a thorough genetic analysis. Detecting the breakpoints in structural rearrangements is vital to assess the extent of the involved segments and its consequences, but this process is made harder by the existence of repetitive or complex segments. It is clearly needed a careful genetic assessment and planning in PGT-SR, by resorting to better techniques and investing in their development, sensibly interpretating test results, and comprehensively counseling couples so they can make informed and conscious decisions.[8]

Preimplantation genetic testing for aneuploidies (PGT-A)

PGT-A aims to detect aneuploidies to select euploid embryos for transfer and, theoretically, achieve higher pregnancy and live birth rates and reduced miscarriage rates.[6] The existing evidence is contradicting, with several studies proving this and many others refuting it.[9,10] A recent retrospective study showed, in fact, no significant evidence of PGT-A improving live birth rates per embryo transfer or lowering miscarriage rates in women with fewer embryos, stating PGT-A in these women could potentially cause harm. It can be concluded that it should to be taken into consideration the number and quality of the embryos available when choosing to implement PGT-A.[11]

The indications for PGT-A include advanced maternal age, recurrent implantation failure, recurrent miscarriages, and infertility due to a severe male factor.[5]

The limitations of PGT-A are mainly related with the techniques used, like impact on the embryo's viability and possibility of misdiagnoses, either false positives or false negatives, and inherent consequences of using PGT-A, like ethical considerations and an additional cost to *in vitro* fertilization (IVF).[8] If there are no euploid embryos available, it is possible to transfer embryos with low to medium mosaicism and segmental aneuploidies, after appropriate genetic counseling.[12]

Biopsy techniques

Biopsy is a fundamental step of PGT, which seeks to provide an adequate amount of genetic material for analysis, and it can be performed on an oocyte or in distinct stages of the embryo. The biopsy must not jeopardize embryo viability, and the genetic material retrieved must reflect the embryo's entire genetic constitution with the highest possible accuracy.[6]

Various methods of extracting the genetic material have been introduced over the years, from chemical methods with acidic Tyrode's solution and mechanical zona pellucida piercing to laser-assisted zona pellucida opening. According to the ESHRE PGT Consortium data collection XVI–XVIII, by 2015, the laser method had almost completely substituted the chemical method, with the former being implemented in 98% of PGT.[13] This is probably due to the laser-assisted approach being less operator-dependent and time-consuming, having a shorter learning curve and causing no alterations on the outcomes.[14]

There are three main types of biopsy, according to the type of sample collected: polar body biopsy from the oocyte or day 1 embryo, blastomere biopsy at the cleavage stage, and trophectoderm biopsy at the blastocyst stage.[6]

Polar body (PB) biopsy consists of the removal of the first and second PB, either individually, the first PB before insemination and the second one about 16 hours after, or simultaneously, 16 hours after insemination. Although it is a minimally invasive technique, PB biopsy comes with significant limitations: it is time-consuming and not cost-effective because both polar bodies must be investigated, and it analyses exclusively maternal genetic material, not providing any information relating to the paternal genetic contribution to the embryo. Moreover, by performing a biopsy on polar bodies, it is not taken into account the postfertilization mitotic errors and meiotic errors which are amended later in the embryo's development process.[14] Despite of its limitations, the PB biopsy is still used nowadays, in few countries where it is not legal to perform embryo biopsy.[8]

The impact of PB biopsy on embryo development has been a controversial topic. One study showed this technique has a negative effect on embryo development, namely on rate of fragmentation and number of blastomeres.[15] A more recent study supported that PB biopsy does not have a negative impact on embryo development and neonatal outcomes while also demonstrating no differences in biochemical pregnancy rates and implantation events, concluding that PB biopsy is a safe approach.[16]

According to the ESHRE PGT Consortium data collection XXI, in 2018, PB biopsy was used in just 1% of PGT-M, PGT-SR, and PGT-A, similar to the respective 2%, 1%, and 3% used in 2016–2017.[17,18]

Blastomere biopsy at the cleavage stage consists of the extraction of one blastomere from day 3 embryos, when they have 6 to 8 cells.[6] This allows for a fresh transfer, just like PB biopsy, with the advantage of analyzing both maternal and paternal genomes. However, because it is not recommended the extraction of more than 1 cell so as to not affect the embryo's viability, there are important limitations derived from a single-cell analysis.[14]

There can be technical problems if some of the material is lost or degraded during collection, leading to one of four results: allele drop-out, preferential amplification, chimerical DNA molecules formation, or high rate of DNA amplification failure. This can lead to misdiagnoses, creating either false-positive or false-negative results. Furthermore, blastomere biopsy may negatively affect embryo development and reproductive competence. Removal of a single blastomere has been shown to affect cell differentiation processes and the development, hatching, and implantation processes (delayed compaction process and bypassing the period of zona pellucida thinning, which originates a smaller blastocyst with a thicker zona pellucida).[14,19]

According to the ESHRE PGT Consortium data collection XXI, in 2018, cleavage stage biopsy was implemented in 65% of PGT-M and PGT-SR, whereas it was only used in 0.6% of PGT-A.[17] Taking into consideration the ESHRE PGT Consortium data collection XIX–XX, in 2016–2017, cleavage stage biopsy was used in 78% of PGT-M, 67% of PGT-SR, and 8% of PGT-A, so it can be concluded that the use of cleavage stage biopsy is gradually coming to an end.[18]

Trophectoderm (TE) biopsy at the blastocyst stage consists of the extraction of 5–10 cells from day 5/6 embryos.[6] There are three different methods of TE biopsy. The first one involves a laser-assisted zona drilling at day 3 of embryo development, with posterior extended culture until blastocyst, and biopsy on day 5/6 expanded blastocysts.[20] This strategy is believed to make biopsy easier by causing TE cells herniation, which are then better to access. This process brings at least two major problems: the embryo is exposed to suboptimal culture conditions because of leaving the incubator twice, which can affect the embryo's development, and there is a risk of an inner cell mass (ICM)

herniation after zona opening on day 3, which makes the biopsy difficult.[14] The second one involves zona opening and TE cell aspiration on day 5 blastocysts, when ICM is visible and can therefore be avoided.[21] This approach has some advantages over the first one: there is no interference on the hatching process, so it is possible to do a biopsy of any portion of TE and not just the restricted number of herniated cells, and the embryo is kept on optimal culture conditions.[14] The third and most recent one involves zona piercing on day 5 blastocysts, which are then returned to culture to cause a herniation of TE cells which will then be collected. This procedure is not as difficult to perform as the other two, and it does not have the disadvantages of zona opening on day 3 embryos.[22]

Comparing TE biopsy with the other methods, TE biopsy appears to be the best option considering its many advantages: (i) the collection of a larger amount of genetic material for analysis makes it more probable to identify potential mosaicisms and less likely to provide misdiagnoses and it allows multiple analyses for different indications to be implemented on the same sample; (ii) the results are extremely accurate, with an estimated 98%–100% of correct prediction of meiotic errors; (iii) it is less invasive since TE cells are collected and not ICM cells, therefore having less impact on embryo development and no effect on implantation rates; and (iv) it is the most cost-effective technique.[8,14]

TE biopsy is currently the preferred technique for PGT-A, but it is still slowly replacing blastomere biopsy in PGT-M and PGT-SR. According to the ESHRE PGT Consortium data collection XIX–XX and ESHRE PGT Consortium data collection XXI, usage of blastocyst biopsy went from 19% in 2016–2017 to 33% in 2018 in PGT-M and from 30% to 33% in PGT-SR, whereas in PGT-A blastocyst biopsy was already almost exclusively used in 2018, going from 87% to an astounding 98%.[17,18]

Noninvasive biopsy approaches

Considering biopsy is normally an invasive procedure that can affect embryo viability and PGT precision, the scientific community is focusing on noninvasive techniques to create reliable, accurate, and efficient alternatives for PGT. The so called noninvasive PGT (niPGT) is based on the extraction and analysis of cell-free DNA (cfDNA) from blastocoel fluid (BF) sampling (blastocentesis) or spent culture medium (SCM) sampling.

The first identification of DNA in blastocoel fluid dates to 2013 and it presented a revolutionary minimally invasive alternative to standard biopsy.[23] Blastocentesis requires some micromanipulation of the embryo and is therefore not a completely noninvasive procedure. While it has not been proven that blastocentesis single handedly leads to lower live birth rates, it has been shown this procedure causes lower implantation rates.[24] One study showed that cfDNA was detected in 63% of BF samples, with a ploidy concordance rate of only 48% when compared with TE biopsy.[25] However, two others showed that cfDNA was found in 76.5% and 82% of BF samples, respectively, with a ploidy concordance rate of 97.4% and 97.1% when compared with TE biopsy.[26,27] This approach is still considered inferior to TE biopsy because of reported false-positive and false-negative results caused by uneven analysis of a small amount of cfDNA retrieved from BF.[28] There is a significant variation of quantity and quality of DNA retrieved from BF, which in some embryos is not capable of being correctly amplified.

The first identification of DNA in spent culture medium occurred a year later, in 2014,[29] and in 2015, it was demonstrated that SCM samples might contain a larger amount of genetic material in comparison with BF samples.[30] This means SCM is perhaps a better material supply that also relies on an entirely noninvasive approach. SCM sampling shares a limitation with BF sampling: the amount of DNA is still relatively small and possibly of poor quality (degraded). A study published in 2019 showed a concordance between DNA in SCM and whole embryos of 83.3%, which is higher than that of TE biopsy.[31] A study published in 2022 showed evidence of a concordance rate of 75% between cfDNA and TE biopsies in conventional IVF blastocysts while other publications from 2019 to 2020 showed a similar concordance rate in ICSI blastocysts, so it seems the implementation of niPGT is a promising option for the future.[32] In contradiction, a meta-analysis from 2023 estimated that the overall concordance between SCM analysis and standard techniques was not enough to lead to its wide implementation.[33]

Another main problem that surges with SCM sampling is genetic contamination, with a study from 2018 estimating a median percentage of embryonic DNA in SCM of only 8%.[34] Genetic contamination can have five different sources: (i) maternal-derived material, (ii) paternal-derived material, (iii) exterior sources, (iv) resulting from manufacturing procedures, or (v) resultant of microbial contamination during IVF.[34,35]

Overall, collection and analysis techniques must be improved and standardized for both BF and SCM sampling. Moreover, the nature of the quantity and quality differences in BF samples must be investigated and, regarding SCM, culture conditions must be optimized.

Diagnostic strategies

Direct multiplex polymerase chain reaction (PCR) assays were the first ever analysis methods to be widely implemented in PGT. Initially, the goal was direct amplification of target DNA regions of interest[36] while later it evolved to analyzing polymorphic sequences, copy number variations, and aneuploidies with improved amplification approaches like whole genome amplification (WGA).[37] There were identified various limitations with direct PCR, such as contamination, which may cause false-positive results, amplification bias, introduction of DNA sequence errors, and allele dropout during amplification, all of which can lead to misdiagnoses. There are ways to try and surpass them, for example simultaneously amplify and test for polymorphisms closely linked to the target DNA region and test parental haplotypes.[38] Multiplex PCR for linkage markers alone (preimplantation genetic haplotyping) can be applied no matter what genetic alteration the couple has, and its costs are reduced. This approach has been commonly used in PGT-M and HLA typing.[14]

Fluorescent in situ hybridization (FISH) is one of the oldest analysis techniques used in preimplantation testing of biopsied blastomeres, dating back to 1993.[39] The use of fluorescent molecular probes allows the identification of only a limited number of chromosomes or their fragments in interphasic nuclei because only 5–9 probes during 2–3 cycles of hybridization are used. This means that FISH cannot analyze all 24 chromosomes and is limited to diagnosis of the most common aneuploidies, encompassing chromosomes 13, 15, 16, 18, 21, 22, X, and Y.[40] Major limitations are difficulties regarding hybridization failure, nonspecific hybridization, and misinterpretation due to overlapping signals.[39] FISH has since fallen into disuse and been substituted by better procedures such as array CGH (aCGH), single-nucleotide polymorphism (SNP) array, qPCR, and next-generation sequencing (NGS), all thanks to the introduction of WGA.

WGA has been introduced as a preamplification technique capable of amplifying an entire single-cell genome, originating sufficient DNA (a few micrograms) for numerous downstream applications. Other important requirements of WGA for PGT include maximum possible coverage of the genome, homogeneously amplified material, and preserved original nucleotide sequence (no loss of material).[41] Therefore, it is crucial to use enzymes with high processivity and high fidelity, improve cycling conditions, and reduce the complexity of the DNA before amplification by fragmenting it.[42] WGA combined with downstream techniques can be used for haplotyping and analysis of single-gene pathogenic variants, and for detection of chromosomal imbalances. These approaches have been shown to be quite accurate in detecting and diagnosing multiple diseases, despite its difficulties to achieve quantitatively representative amplification.[14,38]

Conventional comparative genomic hybridization (CGH) determines gains or losses of chromosome fragments that were amplified by WGA by comparing fluorescent dyed normal DNA with fluorochrome marked blastomere DNA, which were hybridized to a metaphase spread. Conventional CGH has a low resolution, so it has been replaced by aCGH, which is accurate and highly specific. In aCGH, the DNA is hybridized to a microarray, and the intensity of hybridization signals is estimated by log2Ratio. A limitation of array CGH is it cannot be used for haploid or polyploid embryos, balanced rearrangements, low-grade mosaicism, and single mutations, meaning it can only be used to identify whole chromosome aneuploidy or segmental aneuploidies (greater than 10 Mb).[43,44]

Single-nucleotide polymorphism microarray identifies highly variable pairs of single nucleotides in a DNA sample and compares them with a reference genome, usually maternal and paternal. There are three essential steps in a SNP microarray: DNA hybridization, fluorescence microscopy, and solid surface DNA capture. SNP microarray has the ability to find up to 300,000 of SNPs in a single genetic analysis and genotype the entire DNA sample, being thus able to detect whole chromosome aneuploidy, chromosome structural alterations, uniparental disomy, and duplications and deletions (by studying the intensity ratio of two alleles at heterozygous loci), with high resolution. The limitations of this analysis method include an increased analysis time (up to 72 hours), restricted capability to detect small structural aberrations (smaller than 5 Mb) and genetic alterations in a consanguineous couple (SNPs can be homozygous at every loci), and being time consuming, costly, and complex.[43,45]

Quantitative polymerase chain reaction (qPCR) is a PCR-based approach which can determine the number of copies of each chromosome and subsequently whole chromosome aneuploidy. This technique incorporates amplification by multiplex PCR of a minimal of 2 sequences on each arm of each chromosome and compares three or four locus-specific amplicons along each chromosome to a reference gene from the same chromosome. On one hand, qPCR is extremely precise and is the fastest of all diagnostic methods, taking a mere 4 hours to complete a genetic analysis and therefore permitting an embryo transfer in the same IVF cycle. On the other hand, qPCR can only process a small number of samples simultaneously and is unable to identify segmental aneuploidy and uniparental disomy.[43,46]

Next-generation sequencing is the most recent method of genetic analysis, and it significantly revolutionized the whole analysis process. NGS encompasses WGA, after which the sample DNA is fragmented into small pieces, marked with a specific sequence, and compared with a reference genome.[43] One advantage of NGS is that it allows for numerous fragments from different samples to be analyzed at the same time. NGS can detect whole chromosome aneuploidies, segmental aneuploidies (of 14 Mb or larger), mosaicism, mitochondrial copy number, deletions and duplications, translocations, and single-gene disorders. NGS is consequently a better alternative to aCGH because of reduced costs of a single analysis, better automation, better detection of segmental aneuploidies, and detection of mosaicism. However, as any technique, it has its limitations, like failure to identify balanced chromosome translocations and problems with interpretation of segmental aneuploidies secondary to artifacts related to WGA.[47-49]

According to the ESHRE PGT Consortium data collection XXI, in 2018, PCR was still the most used DNA amplification and analysis method in PGT-M (85%), whereas in PGT-SR, it was FISH (52%) and in PGT-A, it was NGS with WGA (95%). The second preferred method was SNP with WGA in PGT-M (8%) and aCGH with WGA in PGT-SR (16%) and PGT-A (3%).[17]

Karyomapping is a genome-wide linkage analysis method based on SNP array that is used to detect parental haplotypes in embryo samples, and it targets all available platform SNPs. First, it is a determined set of informative SNP markers for each of the four parental chromosomes and the phase of the alleles for each informative SNP locus. Subsequently, it is an established linkage between the risk alleles with the parental chromosomes by reference to someone with a known disease status, like an affected child or fetus from a previous pregnancy. The parental origin of each chromosome in the embryo is then established by comparison with the reference genotype.[50,51]

One advantage of this approach is it can be applied to either single-cell or multiple-cell biopsy. Another one is the unique use multiple displacement amplification (MDA), one of many WGA technologies, which can originate large fragments and keep high sequencing quality. It should also be mentioned that karyomapping does not require preclinical workup, therefore reducing waiting times for couples, and it can also identify chromosome CNV, so it allows for simultaneous PGT-M and PGT-A. Some of its disadvantages are the costly equipment needed, the limitation to inherited genetic pathogenic variants only, the need for familial samples, and the absence of direct pathogenic variants analysis, which would enable for a more accurate diagnosis in consanguineous families.[8,50,51]

Prenatal screening after preimplantation genetic testing

The utility of prenatal screening in a pregnancy conceived after IVF with PGT-A is still debated and an object of studies, with only the Society of Obstetricians and Gynecologists of Canada (SOGC) having established recommendations regarding this subject. The SOGC does not recommend the traditional screening for trisomy 21 and 18 for pregnancies conceived after IVF with PGT-A and states noninvasive prenatal testing (NIPT) can be considered in these situations.[52,53]

The American College of Obstetricians and Gynecologists believes that undergoing prenatal screening should be a "process of shared decision making with a focus on the patient's individual risk, reproductive goals, and preferences." Considering that PGT cannot detect all genetic defects and a negative result does not always translate into genetically normal offspring, they consider prenatal screening should be offered in every pregnancy conceived after IVF with PGT-A and every pregnancy conceived

after IVF with PGT-M or PGT-SR which did not undergo PGT-A afterward. However, it should be made known the limitations of doing a prenatal screening after PGT and the probability of a false-positive result.[54]

In a retrospective study carried out between January 2013 and June 2022, the performance of a first-trimester screening, a combination of maternal serum markers (free β-hCG and PAPP-A), ultrasound markers (nuchal translucency and ductus venosus pulsatility index) and maternal age, in pregnancies achieved after IVF with PGT-A was analyzed, in comparison with pregnancies achieved after IVF without PGT-A and spontaneous pregnancies. It was concluded that the specificity of the combined screening is lower in pregnancies achieved after IVF with PGT-A, owing to an increased a priori risk related to maternal age. Although it was not possible to establish the positive predictive value of the screening in the studied population, it is assumed that the positive predictive value in pregnancies achieved after IVF with PGT-A is also lower. Seeing that an elevated number of false-positive results leads to additional tests and consultations, which then lead emotional distress and increased risks and costs, it is suggested a new screening protocol, using noninvasive prenatal testing (NIPT) and first-trimester ultrasound markers.[52]

The future of PGT

Several new approaches have been presented in later years that enable the simultaneous offer of PGT-M, PGT-SR, and PGT-A, in an attempt to make the testing process more cost-effective and thorough than standard individual procedures. Although comprehensive PGTs have considerable benefits, they have significant limitations and must be further developed.

New noninvasive methods revolving around embryo assessment have been introduced as complementary to PGT. These methods include embryo morphology grading, various *in vitro* embryo monitoring methods such as time-lapse video, and metabolic measurements of embryo culture medium. Morphological assessments have been used to improve the chances of choosing high-quality embryos for transfer, but they have significant limitations. Artificial intelligence (AI) has been shown to improve objectivity and reproducibility of morphological assessments.[55] There is also evidence of a higher accuracy after combination of AI with time-lapse video, of static images with time-lapse video data, and of AI with other data.[56] These approaches appear to be associated with euploidy, yet still must be improved and further tested, along with oxygen consumption measurements. Oxygen consumption measurements combined with morphological imaging can also be used in selection of competent and high-quality embryos for transfer.[57]

In 2019, a PGT was successfully applied for risk reduction of type 1 diabetes, giving way to the emergence of a PGT specific for polygenic diseases (PGT-P). PGT-P has been shown to be useful to screen for other conditions like breast, testicular, and prostate cancers, atrial fibrillation, or coronary artery disease.[58] PGT-P is based on a polygenic risk score (PRS), estimated by running statistical tests based on machine learning on large data sets of genomes, and it establishes embryo risk scores for different polygenic disorders.[59] PGT-P appears to be advantageous since it can test multiple disorders at once, and it does not appear to need family history and subjective experience of a condition to give an accurate result. By using PGT-P, embryos do not have to be excluded and instead an embryo ranking can be created to help with embryo selection.[60]

Although PGT-P has been offered commercially, it has not been applied in health care, probably because of its limitations and the concerns it raises. First, indications of PGT-P are still debated, seeing that polygenic disorders have a large spectrum of severity and phenotypes. Second, PGT-P can determine nonmedical traits, such as intelligence and height, so there are ethical concerns regarding the possibility of creating designer babies with selected traits. Third, considering PGT-P screens for the risk of several conditions simultaneously, compared with the general population, counseling the couple and making an informed decision can be more difficult. A high relative risk does not necessarily translate into a high absolute risk of developing a condition because the latter is influenced by environmental and other nongenetic factors. Finally, the databases used are mainly based in data from people of European ancestry, so PGT-P might be less accurate for people with non-European ancestry. It should also be noted there is still a lack of guidelines and standardized protocols for PGT-P.[60] Considering the high prevalence of polygenic disorders, further development and eventual wide use of PGT-P can be discussed, but the ethical concerns regarding this method must be seriously addressed.

Third-generation sequencing (TGS) allows for reading of long fragments of single-DNA molecules and direct sequencing of linked genetic markers with less need for reconstructing molecules by analyzing overlapping reads. All evidence up until now has shown that TGS may revolutionize sequencing methods in the future, being a faster and less costly technology, which provides flexibility in terms of read lengths and ability to also sequence RNA, and being evidently accurate.[38]

Conclusion

Preimplantation genetic testing presents itself as a revolutionary step in reproductive and genetic medicine. It has suffered a significant evolution since its development, currently constituting an accurate procedure to offer.

Nonetheless, the techniques most commonly used still show considerable challenges and are invasive, meaning the scientific community must continue to invest in better methods, such as the ones previously mentioned.

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