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OBM Genetics

Review

Preimplantation Genetic Testing for HLA-matching: An Overview of Clinical Application and Utility

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Academic Editor: Joep Geraedts

Special Issue: Genetic Testing

OBM Genetics
2019, volume 3, issue 3
doi:10.21926/obm.genet.1903084

Received: June 03, 2019
Accepted: July 12, 2019
Published: July 22, 2019

Abstract

Preimplantation Genetic Testing for Human Leucocyte Antigen-matching (PGT-HLA), first reported in 2001, has been one of the most controversial PGT applications. The procedure aims to identify an embryo that is not only healthy but also HLA-matched with a sibling in the family in need of hematopoietic stem cell transplantation (HSCT), considering that sibling HSCT stands the highest chance of success in comparison to alternative approaches of donor selection. HLA-typing can be performed with or without PGT for the exclusion of a monogenic disorder (PGT-M). The diagnostic PGT approach has greatly evolved over the years. HLA haplotyping by linkage analysis has been the most commonly applied generic approach to date but nowadays newer techniques (SNP arrays, NGS) are also being applied. PGT-HLA is a complex procedure that must be very well orchestrated between specialists of many different disciplines to ensure that successful HSCT is completed in time for the maximum benefit of the recipient. This review discusses the procedure and methodology of PGT, clinical application, and utility of PGT-HLA, and underlines how, despite the limitations, it has been a successful and realistic approach for many couples.

Keywords
PGT; PGT-M; preimplantation HLA typing; sibling donor

1. The Interaction of Assisted Reproductive Technology and Preimplantation Genetic Testing

The development and improvement of Assisted Reproductive Technologies (ART) and Preimplantation Genetic Testing (PGT) have been aimed at combating infertility and genetic diseases by facilitating a healthy live birth. Infertility is a condition afflicting nearly 15% of reproductive-age couples and over 186 million people worldwide, while the risk of having a child affected with a recessive disease applies to more than 2% of unrelated couples [1-4]. In accordance with the awareness of responsible reproduction and reproductive choices, the carrier-couples of a genetic disorder may reduce the risk of transmitting the disease by opting for one of the following options: refrain from having children, adopt, undergo prenatal diagnosis with potential termination of an affected pregnancy, use of donor gametes or embryos, and opt for PGT. Nowadays, due to the wider availability and uptake of preconception genetic testing for a large number of recessive and X-linked conditions, carrier-couples are often identified prior to initiating a family. Consequent to this, more than 75% of detected at-risk couples take one of the above actions to avoid having an affected child [5, 6].

Both technologies, ART and PGT, have been progressing in parallel, and the success of one is greatly dependent on the success of the other. During the last 41 years, we have witnessed the incorporation of new techniques in ART for gamete maturation, fertilization, embryo biopsy, embryo culture, and gamete and embryo freezing. Similarly, the technological advancements in PGT over 29 years have enabled ever more accurate and, more recently, concurrent testing for a number of different indications. The applied methodologies include single cell PCR, multiplex PCR, quantitative PCR, fluorescent *in situ* hybridization, comparative genomic hybridization (CGH), array-CGH (aCGH), single nucleotide polymorphism arrays, and next-generation sequencing [2, 7].

2. The PGT Procedure

Preimplantation Genetic Testing can be performed for the detection of monogenic disorders/single gene defects (PGT-M), chromosomal structural rearrangements (PGT-SR) or aneuploidy (PGT-A) in preimplantation embryos [4]. PGT-M and PGT-SR aim to establish a pregnancy unaffected by the familial disorder or by a chromosome imbalance, respectively, through the diagnosis and selection of genetically suitable (unaffected) embryos for transfer to the womb. The aim of PGT-A is to improve the success rate of ART, i.e., to increase implantation, ongoing pregnancy, and live birth rates and to reduce the miscarriage rate. This is attempted by assessing the chromosomal complement in embryos before implantation to exclude the aneuploid embryos from the transfer.

Overall, subsequent to oocyte collection and fertilization, embryos are cultured in the laboratory and cells from either oocytes or embryos are removed (biopsied) and used for genetic analysis. The stage of embryo development, at which biopsy is performed (polar body, blastomere or trophectoderm biopsy), is determined by the indication tested for, the patient's reproductive

potential and history, the requirements of the diagnostic protocol and the embryo-transfer policy of the assisted reproduction center. The advantages and disadvantages of each biopsy strategy have been extensively discussed in the literature [8]. Trophectoderm biopsy has gained popularity as the method of choice for embryo biopsy [9]. This is due to two main reasons; first is the lower detrimental effect on embryo survival and implantation potential in comparison to other biopsy methods. The second reason is that higher credibility and reliability of results are ensured when offering genetic tests based on a few cells rather than a single one [10-13]. Trophectoderm biopsy has also been facilitated by advances in embryo cryopreservation, which is often required to allow sufficient time for completion of genetic analysis. Cryopreservation, followed by the transfer of embryos in a subsequent cycle, has been associated with an increased pregnancy rate. Therefore, many assisted reproduction centers apply a "freeze-all" policy even in the fertility treatment cycles that do not involve genetic testing. Some level of caution should be warranted, as recent reports indicate that, for certain patient groups, the freeze-all strategy may not be the most beneficial approach [14, 15].

According to the Human Fertilization and Embryology Authority in the UK, over 400 monogenic diseases or chromosomal translocations have been approved for testing in preimplantation embryos (https://www.hfea.gov.uk/pgd-conditions/). The European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium, formed in 1997, collects data on the number of PGT cycles performed, pregnancies achieved and children born from its member centers, as well as the biopsy and genetic diagnostic strategies applied. More than 70,000 PGT-M, PGT-SR and PGT-A cycles were reported between 1997 and 2016, of which over 18,000 involved PGT-M for the exclusion of monogenic diseases, and overall, over 10,000 deliveries following PGT have been reported so far (information available up to 2014, unpublished). Although the annual ESHRE data collection does not represent all centers performing PGT, it enables the observation of general trends and technological advances, as the new approaches, such as the increasing use of trophectoderm biopsy and freeze-all cycles, are continuously adopted. The safety of children born following PGT has been supported by a number of studies; however, as new methodologies are applied (such as extended culture, various biopsy stages, cryopreservation, frozen embryo transfer), constant vigilance needs to be applied [16-18].

3. PGT with Human Leucocyte Antigen (HLA) Matching: Clinical Utility

PGT, similar to other advances in the field of reproductive medicine and assisted reproduction has often been the topic of controversies and ethical debates. PGT is not only applied for conditions that present at birth but also for high and low penetrance cancer predisposition, late-onset disorders (e.g., Huntington disease), non-life threatening conditions (e.g., non-syndromic sensorineural hearing loss), and for sex-selection. More recently an attempt to employ PGT for establishing a polygenic risk score of embryos to predict the risk of hypothyroidism or diabetes has also been reported [19]. It must be noted that the legal framework for PGT differs between counties. In the USA, the clinics independently formulate their PGT policies, whereas in Europe, particularly in Italy, Switzerland, France and the UK, the procedure is strictly regulated and national laws determine the approved practices [20]. One of the most controversial PGT applications was first reported in 2001. At that time, PGT was applied not only to initiate a healthy

pregnancy but also achieve treatment of a sibling in the family in need of hematopoietic stem cell transplantation (HSCT), by selecting a healthy and HLA-matched embryo to be used as a donor [21].

The clinical value of PGT with HLA matching has previously been discussed [22]. Allogeneic HSCT can use as a source for hematopoietic stem cells either bone marrow, peripheral blood or umbilical cord blood with the intention of repopulating and replacing the hematopoietic system of the recipient. It was pioneered in 1957 by Donnall Thomas and today can be used to treat a great number of hematological diseases, solid tumors, and immune disorders [23]. The annual activity survey of the European Society of Blood and Marrow Transplantation (EBMT), conducted since 1990, shows a constant increase in the number of transplants performed each year with over 708,000 transplants reported up until 2017. The main indications are myeloid and lymphoid leukemias, solid tumors, and nonmalignant disorders. In 2017, there was a significant increase in allogeneic HSCT for hemoglobinopathies, including the thalassemias and sickle cell disease, the majority of which involved matched sibling donors. Bone marrow was used as the stem cell source in most cases (73%) [24, 25].

Considerable progress has been made toward improving the outcome for both related or unrelated donor transplants, as more accurate high resolution typing for the HLA-A, -B, -C (class I) -DRB1, and -DQB1 (class II) loci is facilitated by the use of next-generation sequencing technology (NGS), thereby improving HLA matching accuracy and reducing rejection rates. The overall outcome is also dependent on patient age and disease status at the time of transplantation. Despite the advances, such as accurate allelic matching, treatment regimens, and improved supportive care treatment, it still holds true that HSCT shows superior outcomes with fewer complications and higher survival rate when a matched sibling donor is available [24, 26, 27].

Finding an HLA matched donor remains the major constraint of the procedure. The probability of an affected child having an HLA-matched sibling is 25% if there is one sibling in the family. With more than one sibling the chances will increase (e.g., 43.7% with two siblings), overall, only 30% of patients are able to find an HLA identical match within their family [28]. Indeed, 75% of beta-thalassemia patients do not have HLA matched siblings [29]. In the absence of a matched sibling, a matched unrelated donor may be identified in national or international registries. Although over 34 million donors are available worldwide (https://www.wmda.info/), finding a suitable donor may still prove very difficult. Alternative resources such as related haploidentical or mismatched unrelated donor may be pursued but cannot guarantee an equivalent success to transplantation with a complete sibling match [30].

Understanding the importance of finding a matched donor in the past led parents to try natural conception and determine the HLA status of the unborn baby through prenatal diagnosis. The PGT-HLA attempt seemed a natural progression. The first successful pregnancy following PGT-HLA was achieved in 2000, after five ART-PGT cycles and testing of 41 embryos over four years, eventually leading to the birth of a baby boy who was unaffected for Fanconi Anaemia and HLA matched to his affected sister. Allogeneic HSCT was successfully performed with umbilical cord blood hematopoietic stem cells from the sibling donor [21, 31].

HLA typing of preimplantation embryos can be performed as a sole indication when the affected child requires transplantation to treat an acquired disease, or in combination with PGT-M to support parents to concurrently avoid the risk of having another affected child. In some countries, it is only considered acceptable when it is combined with PGT-M. There have been cases, where couples were denied treatment in their country and moved to another country to

complete PGT-HLA [20]. The latest ESHRE PGT consortium data collection records 200 HLA-only cycles and 605 PGT-M/HLA cycles out of 12,712 PGT-M cycles reported in total, throughout the data collections I to XV (years 1997–2012) [32].

There are several ethical considerations regarding the use of PGT-HLA that have been debated for years. These include a) the parental motives in conceiving a child as a means to save an existing one, b) the selection of embryos on the basis of a specific characteristic (HLA type) rather than their disease status, c) the potential health risks imposed to the mother and child to be born via the ART, biopsy, and donation procedures, d) the interests of the donor sibling as opposed to the family interests, and e) the psychological impact on all family members [33-35]. Most of these arguments have been refuted, as it has been experienced that parents truly wish to expand their family and alongside this, the donor child holds a special role in the family [36]. In reality, the PGT-HLA approach has never been used to support a trend toward "designer babies", in contrast to the voiced concerns. Although there is no doubt that it is a physically and emotionally challenging procedure, there are profound benefits for all involved counterparts when it is successful. A significant number of successful cases have been reported in the literature with no serious complications observed among both recipients and donors [37]. In all cases, besides the PGT-HLA context, there are also ethical and legal complexities associated and established criteria that must be met in order for children to serve as donors [38, 39].

4. Overview of PGT-HLA Methodology

The diagnostic approach for HLA typing of preimplantation embryos has greatly evolved over the years. The first reports involved direct genotyping of important HLA specific genes, unique for the family in question, using allele-specific PCR or mini-sequencing [21, 40]. A more generic approach was first reported in 2004 aiming to overcome the need to standardize new protocols for the family-specific informative variations. It involved HLA haplotyping by linkage analysis of polymorphic short tandem repeats (STRs) located throughout the complex HLA region for the identification of the matching haplotypes between the tested embryos and the affected sibling [41]. There are technical difficulties associated with the amplification of the HLA region, such as its large size (3.7Mb), homology with other genomic domains, the high level of polymorphisms, and the high frequency of recombination (near 5%). All these reasons made HLA haplotyping as the preferred methodology. Owing to the high chance of recombination, it is recommended that during the evaluation of a PGT-M protocol, other family members, in addition to the parents and affected child, should also be tested. Numerous STRs have been used in the literature in different PGT protocols; most laboratories employ a panel of STRs for testing and select those that are specifically informative for the family to be included in the protocol. The robustness of the STR linkage approach is strongly correlated with the number of STR markers used for HLA haplotyping. In 2011, the ESHRE PGT Consortium recommended a minimum number of markers to be used, in order to adequately cover the most important regions for HLA matching (HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1). In the published protocols, these markers have been amplified either in multi-step protocols, involving several nested PCRs and analytical steps, or simultaneously in one-step multiplex PCR protocols. When there is a need to concurrently exclude a single gene disorder then the single cell PCR must be standardized for the amplification, additional to the HLA STRs, of the mutated region, and mutation-linked STRs to enable direct and indirect genotyping for

the familial disease. The most frequently requested indication for preimplantation HLA typing involves concurrent testing for beta-hemoglobinopathies. A highly multiplexed PGT-HLA protocol has been reported for this indication that supports detection of any mutation [42, 43].

In the last few years, new technologies have also been applied in PGT for HLA typing, such as the use of whole genome amplification of the biopsied sample prior to STR testing. Furthermore, SNPs, instead of STRs, can be used to define the haplotypes across the genomic regions and such strategies are supported by the use of SNP arrays (for example the commercialized procedure known as karyomapping) or, more recently, NGS [44-46]. The latest technologies can assist in overcoming the limitations of the PCR-based linkage approach for HLA typing, such as the restriction in the number of STRs that can be co-amplified, the time required to characterize new STRs for a family (if the ones already available are not informative), and the need to optimize a new protocol for each couple. In certain settings, these limitations are particularly important, such as in the case of consanguineous couples or genes with low SNP coverage due to the increased probability of having inadequate informative markers (STRs/SNPs), which will impede the ability to establish phase and perform accurate and reliable linkage analysis. Although the use of SNP arrays has simplified the protocol development (as it does not require a family-specific protocol preparation), there are constraints related to the requirement of establishing linkage and phase for the high versus low-risk alleles based on the analysis of first-degree relatives. These constraints can be enlisted as follows: first-degree relatives are not always available; there is an inability to perform linkage analysis and establish the phase for *de novo* mutations; and ambiguous phase results may arise due to recombination. As a consequence of all these restrictions, a center reported an inability to apply SNP haplotyping in over 20% of their PGT-M cases (from a total of 2460 couples) and over 5% of their PGT-HLA cases (from a total 320 couples) [47].

NGS has the advantage of potentially providing nucleotide-resolution data for genetic analysis and overcoming the difficulties associated with the lack of STR informativity, *de novo* mutations, recombination and requirement of a workup based on relatives. Assuming that the disease-causing variant(s) in a family can be directly detected in the embryo-biopsy samples, an affected embryo can be used for the haplotyping. Different approaches involving individual NGS-custom protocols (targeted and semi-targeted sequencing) have been attempted for PGT-M [48-50]. A recent study reported NGS-based HLA sequencing on single blastomeres (amplification of HLA locus-specific sequences and library preparation) and achieved low-resolution typing in 92.2% of all alleles of embryos and conclusive high-resolution typing in 88.9% of the alleles. Considering the high chance of recombination in the HLA region, the authors highlight that with this methodology, NGS can determine where the recombination event has taken place and detect a matched sample more reliably compared to linkage analysis [44].

Both SNP arrays and NGS maximize the genetic information available from each embryo with also reference to chromosome copy number in the context of PGT-A [51]. In earlier applications, the testing of two different biopsies was performed to enable PGT-M with aneuploidy screening but, more recently, this has been achieved from the same biopsy and whole genome amplification product (WGA) in family-specific or universal protocols, improving pregnancy rates [52]. The combination of PGT-M with HLA typing and aneuploidy screening was performed in a study using WGA that was followed by aCGH and multiplex PCR from different portions of the same WGA product. This provided a very high success rate after embryo transfer, increased pregnancy rate per embryo transfer by 20% (for both PGT-M and PGT-M/HLA cases), and significantly reduced

spontaneous abortion rate [47]. A possible explanation for this impact may be that the majority of patients requesting PGT-HLA are of advanced reproductive age, as it is well-known that aneuploidy, common in human oocytes, increases with maternal age [53].

5. The PGT-HLA Procedure: Clinical Application

The PGT-HLA procedure is complex and requires close collaboration between specialists of many different disciplines, hematology, oncology, gynecology, embryology, genetics, genetic counseling, nursing, and radiotherapy. Prior to initiating the procedure, parents should receive confirmation for the adequacy of this approach, relevant to the specific disease in the family, as well as counseling from all specialists involved, on the safety, accuracy, technical and genetic limitations, risks, complications, and success rate at all stages, as indicated in Figure 1. This includes the likelihood of overall success of PGT (including failure of diagnosis or misdiagnosis), the fate of unaffected non-matched embryos, and all aspects and options of HSCT (stem cell source, timing, and success rate). Careful coordination among teams is essential to ensure that successful HSCT is completed on time, while psychological evaluation and support for all involved (parents, affected child, and sibling donor) must be considered throughout. When pregnancy is achieved, confirmatory prenatal testing by chorionic villus sampling or amniocentesis for both HLA type and disease status is recommended [22].

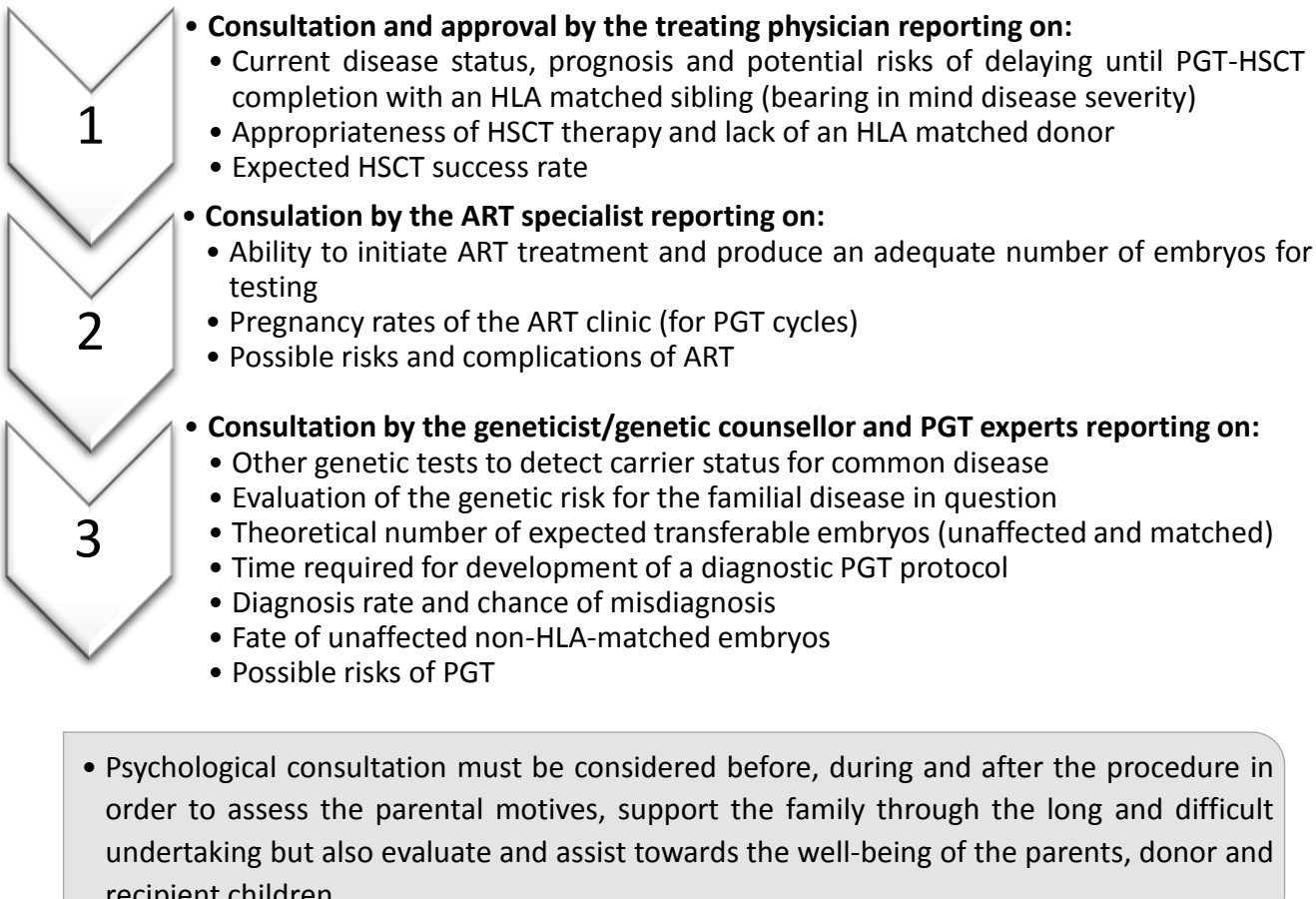

Figure 1 Steps to undertake prior to embarking on the PGT-HLA procedure.

Overall, the success of the procedure is closely associated with the number of oocytes collected and fertilized, the number and quality of embryos cultured in the ART lab, the number of embryos biopsied, the genetic chance of detecting a matched unaffected embryo, and the chance of achieving pregnancy. The expected number of transferable embryos varies according to the "genetic chance", which is as follows: 25% of embryos when testing for HLA only, 18.8% when applying PGT for an autosomal recessive condition with HLA typing, and 12.5% for an autosomal dominant condition with HLA typing. After all, a PGT laboratory should follow the guidelines of best practice, for example, in Europe published by the ESHRE PGT Consortium, with regards to the ART and genetic workup before and during the PGT cycle. The first guidelines were published in 2005 and four revised recommendation documents were published in 2011. The 2011 documents are currently being updated (PGT lab organization, embryo biopsy, PGT-M/SR and PGT-A) and are expected to be published soon.

The PGT centers that follow the amplification-based PGT recommendations demonstrate a high (>90%) diagnostic accuracy in PGT-M, although, for PGT-M/HLA, a lower diagnostic accuracy of 78.5% has been reported. The number of transferable embryos may be reduced due to the phenomena specific to single cell PCR, such as allele dropout or preferential amplification, but also other issues such as recombination across the HLA locus, aneuploidy or contamination. Therefore, it can never be predicted if a cycle will lead to embryo transfer. The data from the ESHRE PGT Consortium indicate that near 21% of all PGT cycles (44.5% of all PGT-M/HLA and HLA-only cycles) do not lead to embryo transfer [32]. If an embryo transfer is achieved, the chance to achieve pregnancy is 40% for PGT-HLA only and 24% for PGT-M with HLA, based on the latest Data Collection XIV-XV of the PGT Consortium. Overall, throughout all the data collections, 30% (36/120) of the HLA-only cycles and 35.5% (116/327) of the PGT-M/HLA cycles with embryo transfer have led to a positive heartbeat, though practice generally varies and different success rates have been reported in the literature [32, 54].

The chance of success in a PGT cycle is defined by the percentage of initiated cycles leading to live birth, including all initiated cycles, whether they led to an embryo transfer or not. This is termed as the 'take-home baby rate'. The success of the PGT-HLA HSCT procedure is extended to include the cure of a child by the HSCT, facilitated following the successful identification of a healthy matched embryo, its implantation and birth. The information about the overall outcome of HSCT following a PGT-HLA procedure has been reported only in a limited number of large studies and a few individual-center reported cases. A recent multi-center study, organized by the ESHRE PGT Consortium attempting to clarify the clinical utility of PGT-HLA, identified that maternal age, the number of oocytes and the genetic chance are the major limitations to the PGT-HLA procedure. It also confirmed that a center's experience is crucial in selecting patients, acting quickly between the initial diagnosis of the affected child to when the couple decides to undergo this procedure, and optimizing the ART treatment for the particular indication. In this study, 19.3% of all initiated cycles led to live birth and HSCT was reported in 50% of the cases with at least one baby born. The observation that most data are missing highlights the need for proper evaluation and follow-up of the entire procedure, especially following the PGT-HLA stage [55]. The HSCT procedures performed after PGT-M/HLA have a high success rate based on the data from published studies [37].

6. Future Prospects

The continuous progress in both the fields of ART and PGT promises an improved overall success rate in the future. This includes prospects of the personalization of hormonal stimulation

protocols, improved gamete and embryo manipulation conditions and improved criteria for the selection of the embryo with the best implantation potential. At the same time, patient or donor selection, HSCT treatment protocols, and supportive care treatment are being constantly optimized and are expected to lead to improved outcomes of HSCT even without the availability of sibling HLA-matched donors. Other therapeutic approaches, such as gene therapy or even gene editing, may set an alternative path for a future cure [56-58]. The safety and efficacy of gene therapy have already been investigated for different pathologies such as β-thalassemia, sickle-cell disease, severe combined immunodeficiency, Wiskott-Aldrich syndrome, muscular dystrophy, and chronic granulomatous disease. However, a recent cost-efficacy analysis of gene therapy versus HSCT for β-thalassemia concluded that the preparation and procedure costs are currently higher for gene therapy [29]. Additionally, a longer follow-up is required so that, as anticipated, the long-term health benefits of gene therapy become evident. For the time being PGT-HLA has been a useful and realistic approach for many couples. Despite the complexity of the procedure and the lower chance of success with respect to PGT-M cycles, each healthy child born and each ill child cured is an extraordinary outcome worth every effort.

Author Contributions

GK drafted the article and all authors (GK, TM, CV, JTS) contributed to decision on the manuscript content, revised it critically and approved the final version to be published.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Inhorn MC, Patrizio P. Infertility around the globe: New thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015; 21: 411-426.
2. Niederberger C, Pellicer A, Cohen J, Gardner DK, Palermo GD, O'Neill CL, et al. Forty years of IVF. Fertil Steril. 2018; 110: 185-324.
3. Ropers HH. On the future of genetic risk assessment. J Community Genet. 2012; 3: 229-236.
4. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The international glossary on infertility and fertility care, 2017. Hum Reprod. 2017; 32: 1786-1801.
5. Kraft SA, Duenas D, Wilfond BS, Goddard KAB. The evolving landscape of expanded carrier screening: Challenges and opportunities. Genet Med. 2019; 21: 790-797.
6. Johansen Taber KA, Beauchamp KA, Lazarin GA, Muzzey D, Arjunan A, Goldberg JD. Clinical utility of expanded carrier screening: Results-guided actionability and outcomes. Genet Med. 2019; 21: 1041-1048.
7. Treff NR, Zimmerman RS. Advances in preimplantation genetic testing for monogenic disease and aneuploidy. Annu Rev Genomics Hum Genet. 2017; 18: 189-200.
8. Cimadomo D, Capalbo A, Ubaldi FM, Scarica C, Palagiano A, Canipari R, et al. The impact of biopsy on human embryo developmental potential during preimplantation genetic diagnosis. Biomed Res Int. 2016; 2016: 7193075.

9. Kokkali G, Vrettou C, Traeger-Synodinos J, Jones GM, Cram DS, Stavrou D, et al. Birth of a healthy infant following trophectoderm biopsy from blastocysts for PGD of beta-thalassaemia major. Hum Reprod. 2005; 20: 1855-1859.
10. Kokkali G, Traeger-Synodinos J, Vrettou C, Stavrou D, Jones GM, Cram DS, et al. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of beta-thalassaemia: A pilot study. Hum Reprod. 2007; 22: 1443-1449.
11. Scott RT, Jr., Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: A randomized and paired clinical trial. Fertil Steril. 2013; 100: 624-630.
12. Cimadomo D, Capalbo A, Levi-Setti PE, Soscia D, Orlando G, Albani E, et al. Associations of blastocyst features, trophectoderm biopsy and other laboratory practice with post-warming behavior and implantation. Hum Reprod. 2018; 33: 1992-2001.
13. Xu K, Montag M. New perspectives on embryo biopsy: Not how, but when and why? Semin Reprod Med. 2012; 30: 259-266.
14. Li Z, Wang AY, Bowman M, Hammarberg K, Farquhar C, Johnson L, et al. Cumulative live birth rates following a 'freeze-all' strategy: A population-based study. Hum Reprod Open. 2019; 2019: hoz004.
15. Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: A multicentre, randomised controlled trial. Lancet. 2019; 393: 1310-1318.
16. Heijligers M, Peeters A, van Montfoort A, Nijsten J, Janssen E, Gunnewiek FK, et al. Growth, health, and motor development of 5-year-old children born after preimplantation genetic diagnosis. Fertil Steril. 2019; 111: 1151-1158.
17. Greco E, Greco A, Minasi MG. Reassuring data concerning follow-up data of children born after preimplantation genetic diagnosis. Fertil Steril. 2019; 111: 1111-1112.
18. Desmyttere S, De Rycke M, Staessen C, Liebaers I, De Schrijver F, Verpoest W, et al. Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD. Hum Reprod. 2012; 27: 288-293.
19. Treff NR, Zimmerman R, Bechor E, Hsu J, Rana B, Jensen J, et al. Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform. Eur J Med Genet. 2019: 103647.
20. Bayefsky MJ. Comparative preimplantation genetic diagnosis policy in Europe and the USA and its implications for reproductive tourism. Reprod Biomed Soc Online. 2016; 3: 41-47.
21. Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA. 2001; 285: 3130-3133.
22. Kakourou G, Vrettou C, Moutafi M, Traeger-Synodinos J. Pre-implantation HLA matching: The production of a Saviour Child. Best Pract Res Clin Obstet Gynaecol. 2017; 44: 76-89.
23. Duarte RF, Labopin M, Bader P, Basak GW, Bonini C, Chabannon C, et al. Indications for haematopoietic stem cell transplantation for haematological diseases, solid tumours and immune disorders: Current practice in Europe, 2019. Bone Marrow Transplant. 2019. doi: 10.1038/s41409-019-0516-2.

24. Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, et al. Hematopoietic stem cell transplantation in Europe 2014: More than 40000 transplants annually. Bone Marrow Transplant. 2016; 51: 786-792.
25. Passweg JR, Baldomero H, Basak GW, Chabannon C, Corbacioglu S, Duarte R, et al. The EBMT activity survey report 2017: A focus on allogeneic HCT for nonmalignant indications and on the use of non-HCT cell therapies. Bone Marrow Transplant. 2019. doi: 10.1038/s41409-019-0465-9.
26. Schofl G, Lang K, Quenzel P, Bohme I, Sauter J, Hofmann JA, et al. 2.7 million samples genotyped for HLA by next generation sequencing: Lessons learned. BMC Genomics. 2017; 18: 161.
27. Buhler S, Baldomero H, Ferrari-Lacraz S, Nunes JM, Sanchez-Mazas A, Massouridi-Levrat S, et al. High-resolution HLA phased haplotype frequencies to predict the success of unrelated donor searches and clinical outcome following hematopoietic stem cell transplantation. Bone Marrow Transplant. 2019. doi: 10.1038/s41409-019-0520-6.
28. Besse K, Maiers M, Confer D, Albrecht M. On modeling human leukocyte antigen-identical sibling match probability for allogeneic hematopoietic cell transplantation: Estimating the need for an unrelated donor source. Biol Blood Marrow Transplant. 2016; 22: 410-417.
29. Coquerelle S, Ghardallou M, Rais S, Taupin P, Touzot F, Boquet L, et al. Innovative curative treatment of beta thalassemia: Cost-efficacy analysis of gene therapy versus allogenic hematopoietic stem-cell transplantation. Hum Gene Ther. 2019; 30: 753-761.
30. Gluckman E, Cappelli B, Bernaudin F, Labopin M, Volt F, Carreras J, et al. Sickle cell disease: An international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood. 2017; 129: 1548-1556.
31. Grewal SS, Kahn JP, MacMillan ML, Ramsay NK, Wagner JE. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. Blood. 2004; 103: 1147-1151.
32. De Rycke M, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C. ESHRE PGD Consortium data collection XIV-XV: Cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. Hum Reprod. 2017; 32: 1974-1994.
33. Pennings G, Schots R, Liebaers I. Ethical considerations on preimplantation genetic diagnosis for HLA typing to match a future child as a donor of haematopoietic stem cells to a sibling. Hum Reprod. 2002; 17: 534-538.
34. Devolder K. Preimplantation HLA typing: Having children to save our loved ones. J Med Ethics. 2005; 31: 582-586.
35. Edwards RG. Ethics of PGD: Thoughts on the consequences of typing HLA in embryos. Reprod Biomed Online. 2004; 9: 222-224.
36. Sheldon S, Wilkinson S. Should selecting saviour siblings be banned? J Med Ethics. 2004; 30: 533-537.
37. Kurekci E, Kupesiz A, Anak S, Ozturk G, Gursel O, Aksoylar S, et al. Hematopoietic stem cell transplantation using preimplantation genetic diagnosis and human leukocyte antigen typing for human leukocyte antigen-matched sibling donor: A turkish multicenter study. Biol Blood Marrow Transplant. 2017; 23: 790-794.
38. Then SN, Kerridge IH, Marks M. Children as haematopoietic stem cell donors: Ethically challenging and legally complex. Med J Aust. 2018; 208: 334-337.

39. American Academy of Pediatrics. Committee on B. Children as hematopoietic stem cell donors. Pediatrics. 2010; 125: 392-404.
40. Fiorentino F, Biricik A, Karadayi H, Berkil H, Karlikaya G, Sertyel S, et al. Development and clinical application of a strategy for preimplantation genetic diagnosis of single gene disorders combined with HLA matching. Mol Hum Reprod. 2004; 10: 445-460.
41. Van de Velde H, Georgiou I, De Rycke M, Schots R, Sermon K, Lissens W, et al. Novel universal approach for preimplantation genetic diagnosis of beta-thalassaemia in combination with HLA matching of embryos. Hum Reprod. 2004; 19: 700-708.
42. Kakourou G, Destouni A, Vrettou C, Traeger-Synodinos J, Kanavakis E. A generic, flexible protocol for preimplantation human leukocyte antigen typing alone or in combination with a monogenic disease, for rapid case work-up and application. Hemoglobin. 2014; 38: 49-55.
43. Kakourou G, Vrettou C, Kattamis A, Destouni A, Poulou M, Moutafi M, et al. Complex preimplantation genetic diagnosis for beta-thalassaemia, sideroblastic anaemia, and human leukocyte antigen (HLA)-typing. Syst Biol Reprod Med. 2016; 62: 69-76.
44. Rafati M, Akhondi MM, Sadeghi MR, Tara SZ, Ghaffari SR. Preimplantation high-resolution HLA sequencing using next generation sequencing. Biol Blood Marrow Transplant. 2018; 24: 1575-1580.
45. Volozonoka L, Perminov D, Kornejeva L, Alksere B, Novikova N, Pimane EJ, et al. Performance comparison of two whole genome amplification techniques in frame of multifactor preimplantation genetic testing. J Assist Reprod Genet. 2018; 35: 1457-1472.
46. Natesan SA, Bladon AJ, Coskun S, Qubbaj W, Prates R, Munne S, et al. Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. Genet Med. 2014; 16: 838-845.
47. Rechitsky S, Pakhalchuk T, San Ramos G, Goodman A, Zlatopolsky Z, Kuliev A. First systematic experience of preimplantation genetic diagnosis for single-gene disorders, and/or preimplantation human leukocyte antigen typing, combined with 24-chromosome aneuploidy testing. Fertil Steril. 2015; 103: 503-512.
48. Hao Y, Chen D, Zhang Z, Zhou P, Cao Y, Wei Z, et al. Successful preimplantation genetic diagnosis by targeted next-generation sequencing on an ion torrent personal genome machine platform. Oncol Lett. 2018; 15: 4296-4302.
49. Treff NR, Fedick A, Tao X, Devkota B, Taylor D, Scott RT, Jr. Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease. Fertil Steril. 2013; 99: 1377-1384.
50. Wells D, Kaur K, Grifo J, Glassner M, Taylor JC, Fragouli E, et al. Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation. J Med Genet. 2014; 51: 553-562.
51. Sermon K. Novel technologies emerging for preimplantation genetic diagnosis and preimplantation genetic testing for aneuploidy. Expert Rev Mol Diagn. 2017; 17: 71-82.
52. Backenroth D, Zahdeh F, Kling Y, Peretz A, Rosen T, Kort D, et al. Haploseek: A 24-hour all-in-one method for preimplantation genetic diagnosis (PGD) of monogenic disease and aneuploidy. Genet Med. 2018; 21: 1390-1399.
53. Hassold T, Hall H, Hunt P. The origin of human aneuploidy: Where we have been, where we are going. Hum Mol Genet. 2007; 16: R203-R208.

54. Kahraman S, Beyazyurek C, Yesilipek MA, Ozturk G, Ertem M, Anak S, et al. Successful haematopoietic stem cell transplantation in 44 children from healthy siblings conceived after preimplantation HLA matching. Reprod Biomed Online. 2014; 29: 340-351.
55. Kakourou G, Kahraman S, Ekmekci GC, Tac HA, Kourlaba G, Kourkouni E, et al. The clinical utility of PGD with HLA matching: A collaborative multi-centre ESHRE study. Hum Reprod. 2018; 33: 520-530.
56. Cai L, Bai H, Mahairaki V, Gao Y, He C, Wen Y, et al. A universal approach to correct various hbb gene mutations in human stem cells for gene therapy of beta-thalassemia and sickle cell disease. Stem Cells Transl Med. 2018; 7: 87-97.
57. Daniel-Moreno A, Lamsfus-Calle A, Raju J, Antony JS, Handgretinger R, Mezger M. CRISPR/Cas9-modified hematopoietic stem cells-present and future perspectives for stem cell transplantation. Bone Marrow Transplant. 2019. doi: 10.1038/s41409-019-0510-8.
58. Ghiaccio V, Chappell M, Rivella S, Breda L. Gene Therapy for beta-hemoglobinopathies: Milestones, new therapies and challenges. Mol Diagn Ther. 2019; 23: 173-186.