Polymorphism

RESEARCH

Birth of a savior sibling after preimplantation genetic testing for β-thalassemia, HLA haplotyping and aneuploidy screening

Arundhati Athalye, Dattatray Naik, Rupesh Sanap, Prochi Madon, Dhanashree Warang, Firuza Parikh*

Department of Assisted Reproduction and Genetics, Jaslok-FertilTree International Fertility Centre, Jaslok Hospital and Research Centre, Mumbai, India.

*Corresponding author e-mail: frparikh@gmail.com

ABSTRACT

Aim: To undertake preimplantation genetic testing (PGT) for a couple where both partners were carriers of the c.126_129delCTTT variation in the HBB gene and have a daughter affected with beta-thalassemia major. This is with the hope of selecting thalassemia unaffected, human leukocyte antigen (HLA) matched, chromosomally normal embryos for transfer to get a saviour sibling to help cure their daughter with related, HLA matched hematopoietic stem cell transplantation in the near future.

Methods: Pre-test genetic counselling and pre-PGT for monogenic disorders (PGT-M) study was done on the family by combined direct and indirect approaches on the HBB gene and indirect genetic study of the HLA region by fluorescent polymerase chain reaction (PCR). Couple karyotyping was done to rule out balanced chromosomal rearrangements. The couple underwent 3 in vitro fertilization (IVF) cycles from June 2019 to November 2019 to collect adequate number of embryos, which were biopsied at the day 5/6 of blastocyst stage prior to vitrification. The biopsied cells were tubed and frozen. Genetic analysis was carried out on 14 embryo biopsies starting with the whole genome amplification. Direct detection of c.126_129delCTTT polymorphism was carried out by PCR amplification and genotyping. Indirect studies included PCR amplification of the polymorphic markers linked to the HBB gene, and HLA region. Fragment analysis of the PCR products was done by capillary electrophoresis. The unaffected embryos were further subjected to 24 chromosomes aneuploidy screening by next generation sequencing.

Results: Of the 14 embryo biopsies tested, one was uninformative, while 10 were unaffected with beta-thalassemia. Of these, six were chromosomally normal or euploid, though only one was HLA matched with the affected child. Though this embryo was unaffected, it was heterozygous for the c.126_129delCTTT polymorphism. A healthy baby was born and the umbillical cord blood stem cells have been stored for later use with bone marrow stem cells after the child is two years old.

Conclusion: This HLA matched saviour sib will help to cure the couple's elder child suffering from beta-thalassemia major, with tailored hematopoietic stem cells. This is the first such report from Mumbai, and 2nd from India. Awareness needs to be spread for the benefit of others.

KEYWORDS: Preimplantation genetic testing; HLA-matched sibling; Thalassemia cure, savior sibling.

Citation: Athalye A, et al. Birth of a Savior Sibling after Preimplantation Genetic Testing for β-thalassemia, HLA haplotyping and aneuploidy screening. Polymorphism 2022; 7: 64-72.

Editorial history: Received: October 31, 2021; Revised: December 13, 2021; Accepted: December 16, 2021

INTRODUCTION

Hemoglobinopathies encompass all genetic diseases of hemoglobin formation and utilization. They are caused by inherent mutations in genes coding for globin synthesis. Symptomatic hemoglobinopathy is the most important monogenic disease in the world (Ghosh et al., 2020). Hemoglobinopathies result in substantial mortality and morbidity. They commonly occur in the populations of Africa, the Mediterranean area and Southeast Asia regions.

In India, β-thalassemia is the most commonly encountered single gene disorder. About 3-4% of the Indian population carries the β-thalassemia gene mutation. Its incidence is much higher in northeastern India and in the Lohana, Marwadi, Sindhi, Aggarwal and many more communities. Certain individuals of the Islamic and Sikh religions are more susceptible. About 10,000-20,000 babies with Thalassemia Major are born every year in India (Chandy et al., 2008, National Health Mission Guidelines on Hemoglobinopathies in India, 2016, Roy 2019). Individuals with β-thalassemia major need blood transfusions, chelations and multiple hospitalization cycles, putting tremendous pressure on their parents, siblings, family, medical services and society at large.

Currently, the only definitive cure for β-thalassemia major is hematopoietic stem cell transplantation (HSCT) (Angelucci et al., 2014). The probability of finding an allele match for the Indian population in multinational Human Leukocyte Antigen (HLA) registries is 16% and only about 0.008 % in Indian registries (Tiwari et al., 2015). HSCT with HLA unmatched related donors or HLA matched unrelated donors carries a high risk of transplant-related complications such as rejection, graft failure, graft vs host disease or mortality and becomes more expensive as compared to the use of HSCT from a matched sibling. As per the Indian Pre-Conception and Pre-Natal Diagnostic Techniques (PCPNDT) Act, one cannot terminate a pregnancy in which the fetus is unaffected (either carrier or normal) but HLA unmatched (The PC-PNDT bare act with short comments 2017). It is not practical to undergo multiple pregnancies to get one HLA matched unaffected child; hence, the concept of utilizing Preimplantation Genetic Testing (PGT) for monogenic disorders (PGT-M) and HLA matching to get a child as a saviour sibling becomes a feasible option.

Preimplantation genetic diagnosis (PGD), now known as preimplantation genetic testing (PGT) utilizing in vitro fertilization (IVF) technology, was developed in 1990 and aneuploidy screening was introduced in 1993 (Handyside et al., 1990, Griffin et al., 1993, Munne et al., 1993). The world's first few cases of PGT for β-thalassemia were reported in 1998 (Kuliev et al., 1998), while in 2001, the same group reported the use of PGT for obtaining HLA-matched embryos in a case of Fanconi Anemia (Verlinnsky et al., 2001). In 2002, Kuliev and Verlinsky also reported the use of PGT with HLA matching for β-thalassemia and a few other conditions (Kuliev et al., 2002). Since then, this technology has been utilized globally for having an unaffected HLA-matched savior sib for several disorders. PGT becomes a viable preventive modality that can deselect HLA unmatched embryos with monogenic disorders, thereby preventing the need for pregnancy termination. Though the couple is fertile, to test embryos before implantation, this form of treatment requires IVF with PGT looking at gene variations and HLA haplotyping which are all done from the 5 to 8 trophectoderm cells biopsied from a day 5 or day 6 blastocyst embryo. PGT is subclassified as PGT-M for monogenic disorders, PGT-A for 24 chromosome aneuploidy testing and PGT-SR for structural variations in cases of a balanced rearrangement in one of the partners.

Because of the limited availability of HLA matched donors, PGT with HLA haplotype matching to select a saviour sibling becomes a very feasible option for couples who have a Thalassemia Major affected child. The use of Umbilical Cord Stem Cell Transplantation (USCT) from the cryopreserved umbilical cord stem cells for repopulating the affected child's haematopoietic system/or HSCT from the bone marrow at a later date can be

curative. With recent advances, it may be possible to consider umbilical cord stem cells for grafting rather than using bone marrow transplantation.

Case report

We report the successful live birth of a male baby, free of the β-thalassemia gene mutation c.126_129delCTTT with a 100% HLA match to his older sibling. The umbilical cord blood has been cryopreserved for future USCT together with HSCT from bone marrow.

This couple consulted us in July 2018 as they were keen to have a thalassemia-free and HLA matched second baby to cure their 2-year-old β-thalassemia major daughter using USCT or HSCT in the future. Both the parents were HLA incompatible with their daughter and were heterozygous for the common Indian variation c.126_129delCTTT in the HBB gene. Pre-PGT genetic counseling was offered and all the steps of the procedure were explained to the couple. These included ICSI, performing trophectoderm biopsy for mutation testing, HLA haplotype-matching using PGT-M using PCR technology. Importance of use of PGT for aneuploidy screening (PGT-A) was also discussed. The possibility of encountering failure of HLA matching as well as the need for multiple IVF cycles for pooling of embryos for testing, so as to obtain a minimum of one β-thalassemia unaffected embryo were explained. Couple karyotyping was done to rule out a balanced chromosomal rearrangement.

MATERIALS and METHODS

Informed written voluntary consents based on PCPNDT act India, were obtained for all the procedures. The steps followed in the PGT are summarized in Figure 1.

Pre-PGT work up

A case specific protocol with pre-PGT work up was designed to ensure that PGT-M was effective by testing the peripheral blood samples of the couple and the affected daughter. Laboratory Developed Test (LDT) combining direct and indirect genetic studies of the HBB gene and indirect genetic studies of the HLA region using fluorescence PCR was carried out. Fragment analysis of PCR products was done using capillary electrophoresis using AB3130 sequencer. Direct detection of c.126_129delCTTT mutation was carried out by PCR amplification of the HBB gene sequence. Indirect analysis was carried out by PCR amplification of several HBB gene-linked polymorphic markers (D11S988, D11S4181, D11S2351, D11S1871, D11S4891, D11S1760, D11S1338, D11S4957) to check for heterozygosity / informativity. Several HLA regions linked markers (D6S1683, MOG3, TNFa, D6S2924, D6S1560, D6S1583, D6S1629) were analyzed for heterozygosity / informativity. Once the pre-PGT work up was ready, IVF cycles were initiated.

IVF stimulation cycles

The couple underwent 3 IVF cycles from June 2019 to November 2019. Controlled ovarian stimulation was carried out using the antagonist protocol with Day 2 stimulation with rFSH 225 IU and hMG 225 IU followed by hCG trigger. Oocytes were retrieved under mild anesthesia. The semen sample was obtained and prepared for ICSI. After fertilization, the embryos were cultured till day 5-6 to reach the blastocyst stage. At this stage, there is differentiation into the fetal cells (inner cell mass) and outer placental cells (trophectoderm) allowing a trophectoderm biopsy.

Embryo Biopsy

A total of 14 blastocyst stage embryos were formed over 3 cycles. Laser assisted hatching was performed. A small opening was made in the zona pellucida of each embryo using the LIKOS Diode LASER system attached to an inverted microscope of the micromanipulation system. Around 5-8 trophectoderm cells were biopsied and transferred into the 0.2 ml PCR tubes containing PBS for each embryo individually. Post biopsy, each embryo was vitrified separately. The genetic analysis of all 14 embryo biopsies was carried out.

PGT testing

(a) The genetic material of biopsied cells was subjected to the whole genome amplification (WGA) using Ion SingleSeq™ kit (ThermoFisher Scientific, MA, USA).

(b) The WGA product was further analyzed first for β-thalassemia c.126_129delCTTT mutation by PCR amplification and genotyping. The indirect analysis was carried out by PCR amplification of polymorphic markers D11S988, D11S2351, D11S4891 and D11S1760 linked to the HBB gene.

(c) The unaffected embryos were then subjected to HLA matching by PCR amplification of polymorphic markers MOG3, TNFa, D6S2924, D6S1560 and D6S1583 linked to the HLA region.

(d) Fragment analysis of PCR products was carried out by capillary electrophoresis (CE) using ABI 3130 genetic analyzer (Applied Biosystems, USA).

(e) All the β-thalassemia unaffected embryos were further subjected to PGT-A using the Ion ReproSeq™ PGS Kit (Next Generation Sequencing) for 24 chromosomes aneuploidy screening (Thermo Fisher Scientific, USA). The kit/assay was performed on the Ion Chef™ and Ion S5 System instruments (Thermo Fisher Scientific, Inc, MA, USA). Data analysis was performed using Ion Reporter software, which aligned the reads using the last human genome build (hg19) (Thermo Fisher Scientific, USA).

Embryo Transfer

The single euploid HLA haplotype-matched embryo with β-thalassemia carrier status was thawed and transferred after preparing the endometrium in a natural cycle in January 2020. No other embryo passed the test for all the three parameters.

Pregnancy test

A serum bhCG test was carried out 14 days after embryo transfer.

Prenatal diagnosis

The chorion villi biopsy (CVS) at 12 weeks of pregnancy was carried out to re-confirm the β-thalassemia status of the fetus.

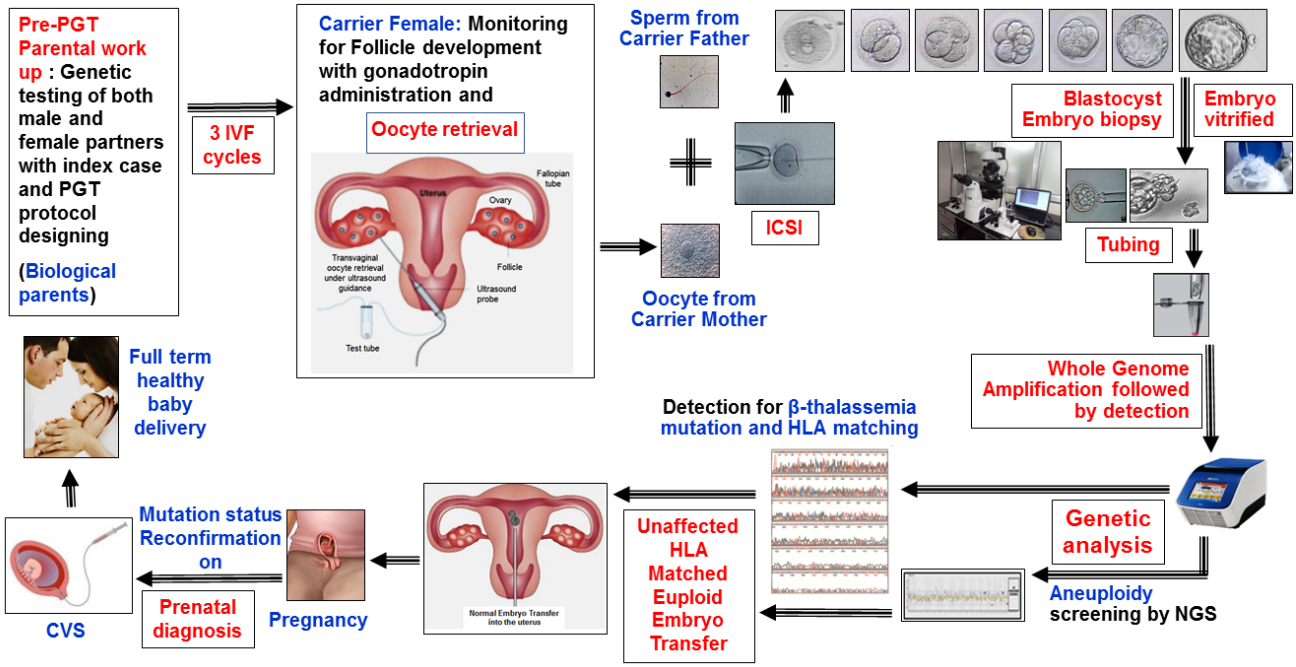

Figure 1: Steps involved in PGT.

RESULTS

In the pre-PGT workup for β-thalassemia, the HBB gene-linked polymorphic marker D11S1338 was uninformative as the wild type and mutant alleles had the same polymorphic region in both the heterozygous partners. The couple had a normal karyotype.

Table 1 summarizes the analysis of 14 embryos obtained from 3 IVF-ICSI cycles. From the 14 embryos, 1 was uninformative, 10/13 (76.9%) were unaffected with β-thalassemia, but only 3/10 (30%) were HLA matched and only 1/3 (33.3%) was euploid. From the other 7 unaffected embryos, 5 (71.4%) were euploid but not HLA matched.

Table 1. PGT analysis of 14 embryos for β-thalassemia c.126_129delCTTT mutation, HLA matching and aneuploidy screening.

Embryo No.	PGT-M (Mutation analysis) results	HLA matched results	PGT-A (aneuploidy screening) results	Suitable for Embryo Transfer?	Used for ET
1	Normal	Non-Match	Low mosaic aneuploidy: +14	NO	No
2	Abnormal	Non-Match	NA	NO	No
3	Carrier	Non-Match	Aneuploid: +1q, -19	NO	No
4	Carrier	Non-Match	Normal	YES (No HLA Match)	No
5	Carrier	Non-Match	Normal	YES (No HLA Match)	No
6	Carrier	Match	Aneuploid: -10	NO	No
7	Carrier	Non-Match	Normal	YES (No HLA Match)	No
8	Normal	Non-Match	Normal	YES (No HLA Match)	No
9	Abnormal	Non-Match	NA	NO	No
10	Carrier	Non-Match	Normal	YES (No HLA Match)	No
11	Abnormal	Non-Match	NA	NO	No
12	Carrier	Match	Normal	YES (HLA match)	YES
13	Normal	Match	Aneuploid: -14q	NO	No
14	No DNA	No DNA	No DNA	Uninformative	No

After the transfer of the β-thalassemia unaffected HLA matched euploid embryo, the woman conceived resulting in a single live intrauterine pregnancy. The CVS analysis confirmed the β-thalassemia unaffected status of the fetus. She delivered uneventfully in September 2020. A healthy baby boy with APGAR scores of 10/10 was born. The umbilical cord stem cells were collected and successfully cryopreserved for future use for USCT with bone marrow when this child would be 2-3 years old.

DISCUSSION

The couple underwent 3 IVF-ICSI cycles in order to pool 14 blastocyst stage embryos suitable for biopsy. Of 14 embryos, one was uninformative due to the absence of intact genomic DNA. Re-biopsy was not possible as the embryo did not regain its composition after thawing. Hence only 13 embryos were informative. These results showed that pooling of embryos through multiple IVF cycles was essential in order to achieve our objective.

We reported our first success with PGT-M for β-thalassemia in 2015 (Athalye et al., 2015). Subsequently, we offered this technology to many couples who have successfully delivered healthy children unaffected by β-thalassemia (Athalye et al., 2020). This is our first case of PGT-M for β-thalassemia together with HLA haplotype matching. In this couple, the 1st IVF cycle produced only 2 embryos of which 1 was affected and the other was unaffected for β-thalassemia. The 2nd IVF cycle produced 6 embryos, all of which were unaffected for β-thalassemia. In the 3rd IVF cycle, of the 6 embryos produced, 2 were affected, 3 were unaffected and 1 was uninformative. Thus, all 3 IVF cycles showed a variety of patterns of the β-thalassemia mutation status of the embryos.

Kuliev and Verlinsky in 2002 first reported the cases of PGT-M for β-thalassemia together with HLA matching (Kuliev et al., 2002). Following this, several groups all over the world have reported success. Based on these studies, in 2014, Kuliev and Rechitsky described the chance of getting unaffected euploid embryos in different situations. According to them, the chance of getting a thalassemia unaffected embryo with a full HLA match to the affected sibling is 18.5%, and getting a thalassemia unaffected HLA matched euploid embryo is 9.4% (Kuliev et al., 2014). In the present case, we found 23.1% (3/13) unaffected HLA-matched embryos and only 1 unaffected, HLA matched, euploid embryo (7.7%).

Regarding aneuploidy screening, out of 10 beta thalassemia unaffected embryos, 6 (60%) were euploid but not all the 6 were HLA matched. From the 3 β-thalassemia unaffected HLA matched embryos (No. 6, 12 and 13), embryos No. 6 and 13 were aneuploid; hence, were not suitable for implantation. Only one embryo (No. 12) was euploid, thus elucidating the importance of checking IVF-ICSI embryos for chromosomal aneuploidies. Of the 3 HLA matched embryos, 2 were β-thalassemia heterozygous (No. 6 and 12) and 1 was without any mutation (No.13). Transfer of embryo 13 without performing PGT-A could have resulted in failed implantation or miscarriage due to chromosomal aneuploidy thus indicating the importance of PGT-A for improving implantation rates and decreasing spontaneous abortions (Munne et al., 2003).

PGT-M with HLA haplotype matching followed by PGT-A is a complex procedure that must be well orchestrated between specialists of different disciplines such as hematologists, assisted reproductive technology (ART) specialists, embryologists, molecular biologists, cytogeneticists, physicians, counselors and psychiatrists. Genetic counselling plays a vital role in preventing the birth of children affected with genetic disorders and also offers couples the option of curing their affected child with the assistance of a saviour child (Carvalho et al., 2020, Madon et al., 2022). Thalassemia and sickle cell anaemia are two common diseases that can be cured by HSCT from HLA haplotype-matched identical donors (Kakourou et al., 2017).

PGT-M with HLA matching is particularly useful for allogeneic HSCT from bone marrow, peripheral

blood, or utilizing umbilical cord blood stem cells. All these techniques help to repopulate and replace the hematopoietic system of the recipient. The number of transplants that have occurred over the years has increased (Saikia et al., 2020) and PGT-HLA plays a significant role in treating hematological conditions (Kakourou et al., 2017, Kakourou et al., 2019). As advances occur in hematological sciences, there is a distinct possibility of refining the technique of USCT to repopulate the hematopoietic system. Hence, the use of bone marrow stem cell transplantation may be replaced by this more affordable and painless procedure.

The overall success of the procedure is closely associated with the number of oocytes collected and fertilized, the number and quality of the embryos, the number of embryos biopsied, the genetic chance of detecting a matched unaffected embryo and the chance of achieving a pregnancy in the IVF cycle. All these steps need careful co-ordination. Embryo biopsy is a delicate procedure, requiring trained and experienced embryologists as the genetic material available for analysis is very limited. External and internal DNA contamination should be avoided while tubing the biopsied cells (Athalye et al., 2015).

In spite of being fertile, the couple has to undergo the entire IVF-ICSI cycle for PGT with HLA haplotype matching in order to obtain adequate number of blastocyst stage embryos. PGT is helpful in preventing repeated medical termination of affected pregnancies to decrease the toll on the physical and mental health of the couple. This is a cost-effective option considering the incremental costs of future blood transfusions, chelations and hospitalization. As thalassemia is one of the most common haematological disorders detected in very diverse Indian populations, the evolving option of PGT-M with HLA haplotype matching would alleviate the burden of this disease. This option is also helpful where pregnancy termination is not permitted due to ethical or religious reasons. The main ethical argument against this technology is the possible exploitation of the child, with potential adverse psychological effects on a child born with the ability to save another, and the possible future emotional reactions of the savior sibling. In sum, the conception of saviour siblings is considered acceptable since its benefit, namely, the potential to cure another child through HSC transplantation, is likely to outweigh its other possible emotions and psychological ramifications. Today stakeholders accept this concept since its benefits outweigh the risks of embryo biopsy and stem-cell donation (Kakourou et al., 2017). With the advent of USCT, the process is likely to be less invasive, potentially lifesaving, and quality-of-life enhancing, improving outcomes for the family and society at large.

Though the technology of PGT-M for thalassemia with HLA matching was developed in 2002, in India this specialized technology was available only after 2014. Since then, couples were opting for the use of this technology to cure their previously affected child. Due to two major concerns, this technology is still not used by couples so frequently in India. The main reason is the lack of public awareness. If the haematologist treating thalassemia major children is not aware of the available technology of getting a Savior Sibling using the PGT-M technique, parents will not receive the necessary counselling for a future pregnancy from the haematologist. The other concern is though fertile, the couple has to undergo IVF cycle, may even be multiple times, to get successful implantation with one thalassemia unaffected HLA matched euploid embryo. The cost involved is yet another issue, though in the long run, it will compensate for life long treatment.

The role of PGT is shifting from diagnostics to therapeutics, being used not only to avoid the conception of affected children but also to give birth to healthy children who would play a large potential role in alleviation of the burden of the disease. The medical community and society should be aware of the availability of these advanced techniques so that children and their families affected with these lethal haematological disorders can be helped. Although PGT-M with HLA haplotyping involves intricate processes, every success means that one more child is saved from a dreaded and potentially lethal disease.

Acknowledgement

We are thankful to the family who allowed us to describe this case. We are also thankful to the entire team of IVF-Genetics of our department who helped us to take this case successfully to birth of the healthy child.

Author's contribution

AA and PM did the PGT work up counselling, coordination in the entire PGT procedure and prepared the draft of case report. DN and RS had carried out actual embryology laboratory procedures required for PGT. DW obtained and maintained all the informed consents from the patient and generated the records for each IVF cycle and also helped in article preparation. FP was the main fertility consultant who carried out actual IVF procedures and supervised on the entire work and finalized the case report draft. All authors have read and approved the final version of the manuscript for submission.

Conflict of interest

The authors declare to have no conflict of interest or competing interest.

Source of Funding

The authors declare that this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of originality

The authors have declared that the data/text presented in this manuscript is original and no text, figure or data has been copied from any other source without appropriate citation.

Jurisdiction and maps

Polymorphism and Peer Publishers remain neutral to the jurisdictional claims, maps, boundaries and institutional affiliations shown or claimed in any of the articles published.

REFERENCES

Angelucci, E, Matthes-Martin S, Baronciani D, Bernaudin F, Bonanomi S, Cappellini MD, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica. 2014;99:811–20.

Athalye AS, Madon PF, Parikh,FR. Preimplantation genetic diagnosis for single gene disorders. In: Agarwal MB, ed. Hematology Today – A case-based approach. 2015;125-8.

Athalye AS, Naik DJ, Sanap RR, Nair SB, Naik NJ, Sanap MV et al. Live births and ongoing pregnancies after Preimplantation Genetic Testing (PGT) for beta thalassemia and other hematological disorders. In: Program and Abstract book of 43rd Annual conference of Mumbai Hematology Group (MHG), March 2020, P12, 289-90.

Carvalho F, Coonen E, Goossens V, Kokkali G, Rubio C, Meijer-Hoogeveen M et al. ESHRE PGT Consortium good practice recommendations for the organisation of PGT. Hum Reprod Open. 2020:1-12 doi:10.1093/hropen/hoaa021.

Chandy M. Developing a National Thalassemia Control Programme for India. In: Ghosh K, Colah R editors. Control and management of Thalassemia and other Hemoglobinopathies in the Indian Subcontinent Synoptic Views. National Institute of Immunohaematology, Mumbai; 2008:46-9.

Ghosh K, Ghosh K, Agrawal R, Nadkarni AH. Recent advances in screening and diagnosis of hemoglobinopathy. Exp Rev of Hemat. 2020;13(1):13-21.

Griffin D, Wilton L, Handyside A, Winston R, Delhanty J. Pregnancies following the diagnosis of sex in preimplantation embryos by fluorescent in situ hybridisation. Br Med J 1993;306:1382-3.

Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990;344:768-70.

Kakourou G, Mamas T, Vrettou C, Traeger-Synodinos J. Preimplantation Genetic Testing for HLA-matching: An Overview of Clinical Application and Utility. OBM Genet. 2019;3(3):1-13.

Kakourou G, Vrettou C, Moutafi M, Traeger-Synodinos J. Pre-implantation HLA matching: The production of a Saviour Child. Best Practice & Research Clinical Obstetrics and Gynaecology. 2017;44:76-89.

Kuliev A, Rechitsky S. Preimplantation HLA typing: Practical tool for stem cell transplantation treatment of congenital disorders. World J Med Genet 2014;4(4):105-109.

Kuliev A, Rechitsky S, Verlinsky O, Ivakhnenko V, Evsikov S, Wolf G et al. Preimplantation diagnosis of thalassemias. J Assist Reprod Genet. 1998;15(5):219-25.

Kuliev A, Verlinsky Y. Current features of preimplantation genetic diagnosis. Reprod Biomed Online. 2002;5(3):294-9.

Madon P, Athalye A, Parikh F. Genetic counseling approach in preimplantation genetic diagnosis. In: Genetic counseling clinical and laboratory approach. Chapter 31. Ed: Usha Dave. Jaypee Brothers Medical Publishers, New Delhi. 2022;387-92.

Munné S, Sandalinas M, Escudero T, Vellila E, Walmsley R, Sadowy S et al. Improved implantation after preimplantation genetic diagnosis of aneuploidy. Reprod Biomed Online. 2003;7:91-7.

Munné S, Weier HU, Stein J, Grifo J, Cohen J. A fast and efficient method for simultaneous X and Y in situ Hybridization of human blastomeres. J Assist Reprod Genet. 1993;10:82-90.

National Health Mission Guidelines on Hemoglobinopathies in India. Prevention and control of hemoglobinopathies in India-Thalassemia, Sickle cell disease and other variant hemoglobins, Ministry of Health and Family Welfare, Govt of India; 2016.

Roy P. Beta Thalassemia: an Indian Perspective. Adv Biotechnol Microbiol. 2019;14(4):555893.

Saikia TK. Blood and bone marrow transplantation in India: Past, present and future. Indian J Med Paediatr Oncol. 2020;41:308-11.

The Pre-conception and Pre-natal Diagnostic Techniques (Prohibition of Sex Selection) Act, 1994 [PC-PNDT]. Bare Act with Short Comments 2017 by Professional Book Publishers; 2017.

Tiwari AK, BhatiKushwaha H, Kukreja P, Mishra VC, Tyagi N, Sharma A, et al. Probability of finding marrow unrelated donor (MUD) for an Indian patient in a multinational human leukocyte antigen (HLA) registry. Indian J Hematol Blood Transfus 2015;31:18695.

Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for fanconi anemia combined with HLA matching. JAMA 2001;285:313