



Diagnosis, Management and Clinical Prioritization of Mosaic embryos: A Case Study

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Abstract

We report a case of normalization of mosaic embryo transfer resulting in the successful delivery of the healthy live baby. The patient was having advanced maternal age and poor ovarian reserve and previous implantation failure. The conventional peripheral blood karyotypes of the couple were normal 46, XX, and 46, XY. The patient underwent infertility treatment at our centre, ovulation was triggered and three oocytes were retrieved which developed into blastocysts. The trophoctoderm cells were biopsied on day 5 after fertilization and were subjected to Preimplantation Genetic Screening (PGS) using next generation sequencing (NGS) for the detection of the presence/absence of any chromosomal aneuploidy in the embryos.

The whole genome analysis for three embryos showed that the first was complex aneuploid, the second was chaotic aneuploid and the third embryo was mosaic (<40%). After careful consideration and in absence of any other option, the intervention was made and the embryo with mosaicism of chromosome 3 was transferred after taking the couple's consent. Prenatal and postnatal follow-up was done, no pathological findings were reported and the foetus was observed with absolutely normal growth during pregnancy and a phenotypically and genotypically normal healthy baby was delivered after 36 weeks and 2 days. The blood karyotype of the baby and aneuploidy screening using NGS showed the normal chromosomes of the baby validating the normalization or self-correction of the mosaic embryo.

Our study suggests that PGS assisted In Vitro Fertilization (IVF) treatment has paved the way for undertaking appropriate clinical risk calculation for the transfer of potential mosaic embryos resulting in a healthy euploid embryo.

Keywords: Embryo, In Vitro Fertilization (IVF), Postnatal, Prenatal, Preimplantation genetic screening, Mosaicism, euploid, Aneuploid,

Introduction

Chromosomal mosaicism is frequently observed in IVF derived human embryos¹. Mosaic embryos originate as a consequence of diverse molecular mechanisms during post zygotic development from mitotic events^{2,3}. The mosaic embryos have a

mixture of diploid and aneuploid cell lines, are not recommended to transfer due to the unknown effect of mosaicism on implantation success of these embryos³. Mosaicism in embryos is assumed to reduce the chances of successful implantation, increase the risk of miscarriages and may have

deleterious consequences on developing embryo's overall functions^{3,4}. In Preimplantation genetic screening or test PGS, an embryo is considered to be mosaic if it contains $\geq 20\%$ mosaic cells⁵. Numerous factors contribute to mosaicism, including advanced maternal age leading to aneuploid and mosaic embryos during IVF⁶.

Since, the clinical impact of mosaicism on either IVF outcome (e.g., pregnancy and miscarriage rates) and ensuing offspring (e.g., chromosomal abnormalities), is not known precisely, therefore, euploid embryos are selected for transfer to the patient on a priority basis, whilst the putative mosaic embryos are given low priority and often discarded⁷. Moreover, the direct effects of mosaicism on early pregnancy remain unknown. Evidence from actual clinical data indicate that fewer than 3% of embryos with a putative mosaic diagnosis are selected for clinical use⁸

Case Report

We represent a case of a successful pregnancy resulting in a healthy live birth after the transfer of a mosaic embryo with partial monosomy of chromosome 3. A couple with a previous history of infertility, advanced maternal age 46 years, poor ovarian reserve, previous implantation failure, underwent IVF treatment at our centre. Both female and male partners had normal peripheral blood lymphocyte karyotyping reports 46, XX and 46, XY respectively. The procedure was carried out after genetic counselling explaining to the couple all the options available and taking written informed consent. Ovulation was triggered using recommended clinical

protocol by the clinician, after the controlled ovarian stimulation procedure, 3 oocytes were retrieved. After fertilization day 5 embryos were biopsied to obtain 5-8 cells of trophectoderm (with grade AA) using an advanced diode laser (Octax Laser Shot) without loss of inner cell mass and vitrified using Kitazato media (Bio Pharma Co. Japan), as per guidelines and manufacturer's protocol. The biopsied embryos were subjected to NGS using the standard operating protocol. DNA was extracted from the trophectoderm, followed by whole genome amplification (WGA) using Ion Torrent Ion SingleSeqTM96 kit (Thermo Fisher Scientific, USA). Strict adherence to quality control was maintained during all the technical procedures. Positive and negative controls were added to the test with the samples to be tested. The DNA libraries were purified and pooled followed by gel electrophoresis or DNA concentration using QubitTM dsDNA HSKit, which was done as a measure of quality control. Ion chef loading was done using the required chip after diluting the purified and pooled library. The NGS procedure was completed using the Ion Chef System (Thermo Fisher Scientific, USA) and Ion S5 sequencer (Thermo Fisher Scientific, USA). The sequencing data were analysed with Ion Reporter Software 5.10.5.0 (Thermo Fisher Scientific, USA) which aligned the readings with the human genome (hg19) using Repro Seq Mosaic PGS w1.1 whole-genome aneuploidy (5.10) workflow. The samples which showed variation from diploid or expected ploidy were treated as aneuploid.

Table: 1 Detailed Analysis of all the Embryos screened for chromosomal aneuploidies

Sample ID	Interpretation (Gain + and Loss -)	Result	Remarks
Embryo E1	-11, -19, and +21	Complex Aneuploid	Abnormal/ Not recommended for transfer
Embryo E2	Multiple chromosomal aneuploidies	Chaotic Aneuploid	Abnormal/ Not recommended for transfer
Embryo E3	Mosaicism of chromosome 3 (<40%)	Mosaic	Genetic counselling offered to the couple, for ultimate decision making discussed with Clinician

NGS results showed that out of the three embryos E1 had a high level of mosaicism of chromosome

11 (mosaic loss), chromosome 19 (mosaic loss) and gain on chromosome 21 and was complex

aneuploid. Another embryo E2 was a chaotic aneuploid with multiple chromosomal abnormalities. The third embryo profile E3 had

mosaicism of chromosome 3 (Monosomy) less than 40% mosaicism (Figure. 1).

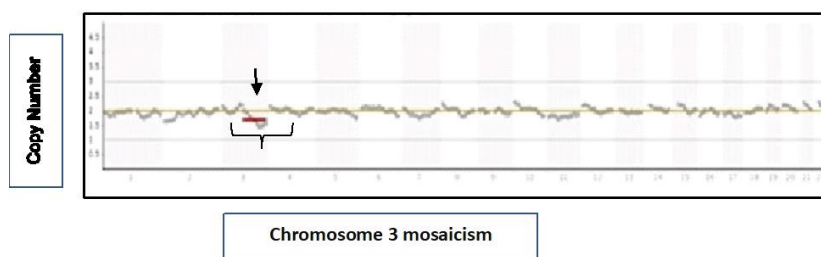


Figure 1: Whole Genome view representing Mosaicism of chromosome 3 after NGS

The concerned clinician was informed about the results of all the embryos with available information. The only option left was the transfer of embryo with less than 40% mosaicism and with associated risks. The risk over benefit ratio was taken into account and a decision was made to transfer the mosaic embryo. The couple strongly insisted to transfer the mosaic embryo because they had multiple problems in conceiving a healthy baby and another cycle of IVF was not worth considering, and third-party reproduction was not acceptable. Considering all these factors the couple was genetically counselled with detailed discussion regarding the risk of miscarriage post-transfer and other options.

Close observation of the case with extensive counselling in the pre-and post-natal period was done. The written informed consent was obtained from the couple and the procedure was completed

at our IVF centre. The endometrium was prepared and the vitrified and selected embryo was transferred after a modified natural cycle using the recommended clinical protocol at our IVF centre.

The serum level of β -human chorionic gonadotropin (hCG) on the 15th day after the transfer was ≥ 155 mIU/mL confirmed the clinical pregnancy. The transvaginal ultrasound was performed on the 7th and 12th gestational week and gestational sac and foetal heartbeat confirmed the pregnancy. The follow-up went well without noticing any undesired event. No sign of morphological abnormality was found in the developing foetus.

A normal healthy baby was delivered at 36 weeks and 2 days. Further, the NGS and blood karyotype of the baby (Figure: 2 and 3) showed perfectly normal chromosomes without any aneuploidy or mosaicism.



Figure 2: The Giemsa banding from peripheral blood lymphocytes of the infant showing normal male 46, XY karyotype.

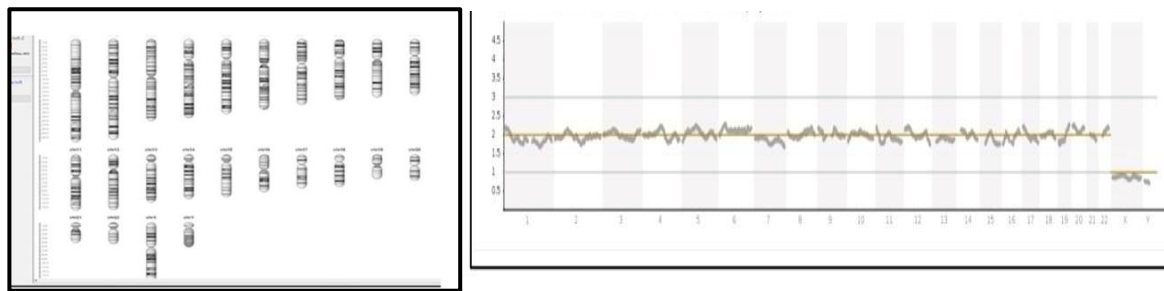


Figure 3: Whole genome view and karyomaps showing all normal chromosomes

Postnatal examination of the baby was normal, and the hospital stay went normal without any undesired event. Both the mother and baby were followed up and there was normal development of the infant born.

Discussion

Mosaicism is a commonly encountered phenomenon during IVF procedures. The main reasons behind chromosomal mosaicism are anaphase lagging, nondisjunction, premature cell division before DNA duplication⁹. We report a case of healthy live birth of mosaic embryo identified using NGS. The patient was having advanced maternal age, poor ovarian reserve and previous implantation failure. PGS is an established technique for improving IVF outcomes in certain patients with advanced maternal age and repeated and recurrent implantation failures due to chromosomal aneuploidies¹⁰. As per assumptions, in case of unavailability of the euploid embryo for transfer and if the patient can't go for another IVF cycle due to advanced age and other clinical factors, prioritization of mosaic embryos for transfer based on the guidelines of the 2016 PGDIS newsletter and after taking couple's consent is recommended¹¹. Grati et al had established a scoring system for mosaic embryo transfer for helping the clinicians in decision making¹². So far, the embryo transfer with chromosome 3 mosaicism has not been reported in the literature, and this is the first case report with prenatal as well as post-natal follow up. The human chromosome 3 is a metacentric chromosome and spans 200 million base pairs and consists of 1024

genes¹³. Many abnormal conditions may arise due to loss or gain of chromosome 3 such as 3p deletion, microdeletion or 3p micro duplication syndrome, trisomy resulting in alteration in structure and function of genes located at that particular region affecting the individual.

As per our algorithm, the mosaic embryos in PGS results are reported as: if an embryo consists of less than 20% abnormal cells then it is considered normal. More than 20% and less than 40% - low mosaic, more than 40 and less than 80% as high-level mosaicism and more than 80% as aneuploid. The embryo selected for transfer, in this case, had mosaicism of chromosome 3 (less than 40%). We hypothesize that post-transfer, the mosaicism was rectified due to a self-correction mechanism resulting in a healthy live birth. The ultrasonography findings showed normal growth of the foetus. The mosaic embryos potentially undergo a self-correct mechanism before the 18th week of gestation and showed a normal karyotype using amniotic fluid after the transfer has been reported¹⁴. The peripheral blood karyotype and NGS of the baby were normal in our case.

A few studies have already established the successful transfer of mosaic embryos^{7,14,15} (with different levels). The suggested mechanisms of self-correction of mosaic embryos involved the better growth of euploid cells or favourable allocation of normal cells to inner cell mass, postzygotic chromosome gain or chromosome loss, mitotic nondisjunction, and trisomic rescue⁹. Liu et al., reported that even when selected the embryos with high mosaicism successfully produced healthy euploids¹⁴. This study reported two cases where embryos with a high level of

mosaicism (68% and 50%) were transferred resulting in healthy live births¹⁴. As per guidelines that “embryos revealing mosaic euploid/monosomy are preferable to euploid/trisomy, given that monosomic embryos (excepting 45, X) are not viable¹⁴. Therefore, in absence of any euploid embryo for transfer in IVF patients undergoing PGT-A, prioritize mosaic embryos for transfer based on the guidelines of the 2016 PGDIS newsletter¹³. Viotti et al., 2021², documented in a multicentre study of 1000 transferred mosaic embryos outcome,⁴ one of the largest datasets with mosaic embryo transfer outcomes. The authors found that the level and type of mosaicism significantly affects the embryo transfer outcome⁴. The mosaic embryos develop into physiologically healthy babies and they proposed a classification system for these embryos⁴. The ranking system was accessible as a freely available web-based tool.¹⁶ However, there is always a clinical dilemma for transferring mosaic embryos to achieve a clinically healthy pregnancy. To overcome this dilemma, a safer choice is to consider the recommended guidelines and use of genetic counselling with a detailed discussion of results of embryo transfer and close observation of the patient during pre-and post-pregnancy using advanced prenatal diagnostic techniques.

To conclude our study clearly showed that there is a high potential of mosaic embryos with a certain level of mosaicism to develop into healthy euploid new-born. The couple should be genetically counselled explaining the potential consequences (pros and cons) of the intervention of transferring a mosaic embryo and should be transferred only with their consent. The mosaic embryos transfer has implications for the couples with no euploid embryos available and with other clinical constraints. Future studies with prospective follow up will help in elucidating the potential long-term implications of mosaic embryo transfer.

Conflicts of interest

The authors declare no conflicts of interest.

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