Polymorphism

RESEARCH

Successful outcome in a patient of recurrent implantation failures using non-invasive PGT-A: a case report

Hrishikesh Pai, Nandita Palshetkar, Ritu Garikipati, Rishma Pai, Charumati Pekhale, Rohan Palshetkar

Bloom IVF, Mumbai, India

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

ABSTRACT

Aim: To perform and test accuracy of non-invasive chromosome screening Preimplnatation Genetic Testing for Aneuploidy (PGT A) for a couple with a history of recurrent implantation failure and male factor infertility.

Settings: In vitro fertilization (ICSI-TESA) procedure and noninvasive PGT A was performed at Bloom IVF Opera House center, Mumbai, India.

Methods: The Couple undergoing ICSI had a previous history of four failed embryo transfers. A total 10 oocytes were retrieved after standard antagonist stimulation protocol. Oocytes were fertilized using ICSI, after performing TESA on the male partner. The resulting embryos on day 5 were subjected to non-invasive PGT testing with spent culture medium. We collected the spent culture media samples on D5 and performed whole genome amplification (WGA) and sequencing by multiple annealing and looping-based amplification cycles (MALBAC) & next generation sequencing (NGS). The MALBAC NGS protocol has been previously validated in performing PGT with cleavage-stage and blastocyst-stage biopsies.

Results: Out of two tested blastocysts using spent medium, one was euploid and the other one was aneuploid. The transfer of the single euploid embryo resulted in pregnancy and live birth.

Conclusion: We report the birth of a healthy baby after non-invasive PGT-A testing for the first time in India.

KEYWORDS: NGS, noninvasive PGT A, MALBAC, blastocyst, spent culture medium.

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INTRODUCTION

Human preimplantation embryos are prone to chromosomal abnormalities and account for repeated abortions and repeated implantation failures. It's the dream of every clinician to achieve pregnancies in the first attempts of any ART cycle and its much less worrisome for patients too. Presently, most clinics perform morphological assessment of embryos and aim to transfer best looking embryos to attain pregnancy (Juanjuan et al., 2016). But morphological assessment of embryos does not alone suffice. If there is a failure to attain pregnancy after transferring good looking embryos, then clinics resort to using add ons in subsequent cycles. These are mostly empirical, expensive may or may not benefit the patient. There is enough literature suggesting the beneficial role of PGT A testing in recurrent miscarriages, advanced maternal age cases, recurrent implantation failure male factor infertility cases (Rui et al, 2019). Some clinics even perform PGT A for an elective single embryo transfer to reduce the risk of multiple pregnancies. However, despite of all these advantages, the usefulness of PGT A has always been debatable amongst clinicians across the world (Juanjuan et al, 2018).

There are various PGT A methods for comprehensive chromosome screening such as array Comparative Genomic Hybridization (aCGH) & NGS. NGS is certainly at par with array CGH because of its high accuracy, reliability, sensitivity and specificity with an enhanced ability to detect mosaic status (Fiorentino et al, 2014). Generally, PGT is practiced mostly on cells of blastocyst obtained using trophectoderm (TE) biopsy. This has been shown to yield accurate results & improve pregnancy outcome. However, trophectoderm biopsy involves the use of lasers, which is an invasive procedure and may potentially damage the embryos (Burks et al, 2021). Animal studies suggests that embryo biopsy could delay blastocoel formation and may increase the risk of neurodegeneration and dysfunction in the offspring. Less invasive procedures, like blastocoelic fluid assessment for aneuploidies have been performed. Due to the limited amount of DNA obtained and inconsistent results, it could not be applied in clinical practice. In addition, collecting blastocyst cavity fluid can be as challenging as performing an embryo biopsy.

Spent culture medium may be a more reliable source of DNA [Rui Fang et al, 2019]. Previous studies have shown that DNA testing using embryo culture medium on days 5 or 6 could detect chromosome aneuploidy with reasonable positive predictive value and high negative predictive value, suggesting that this assay could be used for selecting chromosomally normal embryos. [Jiao et al, 2019].

In an attempt to establish a Noninvasive PGT A method for our clinic, we initially optimized and then performed it in a patient with a history of recurrent implantation failure. With highly reliable, special DNA amplification methods applied in our study we could give conclusive results which resulted in a healthy live birth. Herein we present the first successful case of Noninvasive PGT A from India.

MATERIALS & METHODS

Case Report

In this case report, the couple comprised of a 31-year-old female with good antral follicle count and male partner aged 35 years with obstructive azoospermia. In the year 2014 couple have had a healthy live birth after performing TESA on the male partner. However, subsequent four Embryo transfer attempts (two frozen transfers from the same ART center & two frozen embryo transfers from another ART center) yielded one ectopic pregnancy & three failed attempts. In the year 2019, the couple was keen to have another round of ICSI. This time, the couple was counseled for both Noninvasive PGT A and invasive PGT A. The couple opted for Noninvasive PGT A and. only consented perform the noninvasive technique. The couples karyotype was normal.

The lady underwent standard antagonist stimulation protocol routinely used in our clinic. In this attempt, ICSI TESE was performed on 7 M2 oocytes. The oocytes were cultured in Quinn's media. On day 3, five 8 cell embryos were formed. They were meticulously cleaned, care being taken to remove the entire cumulus cells, to prevent maternal contamination. Each embryo was then individually cultured in numbered 20 microliters droplets, under oil at 5% CO_2, 5% O_2 & 90% N_2 at 37 deg centigrade. At the end of 5^{th} day, 2 grade 4 AA blastocysts were formed. Using special precautions, similar to standard PGT a method, the spent culture media of each droplet was individually collected and tubed, numbered & stored at minus 20 deg centigrade. Each blastocyst was numbered and individually frozen, using standard open cryotopvitrification protocol routinely used in our clinic.

Whole genome amplification (WGA) and Next Generation Sequencing (NGS)

Blastocyst spent culture medium from both the embryos was individually was subjected to WGA with the multiple annealing and looping-based amplification cycles (MALBAC) technique and library generation As a negative control, the culture medium from empty droplets (without blastocyst) was used. Protocol was followed as per the commercial kit manufacturer's instructions (NICSinst Library Preparation kit Cat# XK-011-48, Yikon Genomics). Sequencing was carried out in the Iseq Gene studio NGS machine (Ion Genestudio S5, Semicounductor Sequencer, Thermo Fisher Scientific, USA). Good quality reads were mapped against the human genome database and chromosome copy numbers were determined using the Chromago software (Thermo Fisher Scientific, USA).

RESULTS

WGA from culture media was successful in both instances. In the negative control, only non-specific random reads could be generated while specific reads that aligned to the human genome could be detected in the NGS output of the WGA products of the spent culture medium (not shown).

Of the two embryos, noninvasive PGTA revealed that one embryo was euploid (Fig 1) The other embryo was distinctly aneuploid with partial monosomy and partial gain of sequences on chromosome 5. In addition, the culture medium predicted the gain of sequences for chromosome 8 (Fig 2).

The couple was counseled regarding the limitations and false positives and false negatives of Noninvasive PGT A. After careful assessment, the couple chose to undergo a single embryo transfer only for the predicted euploid embryo.

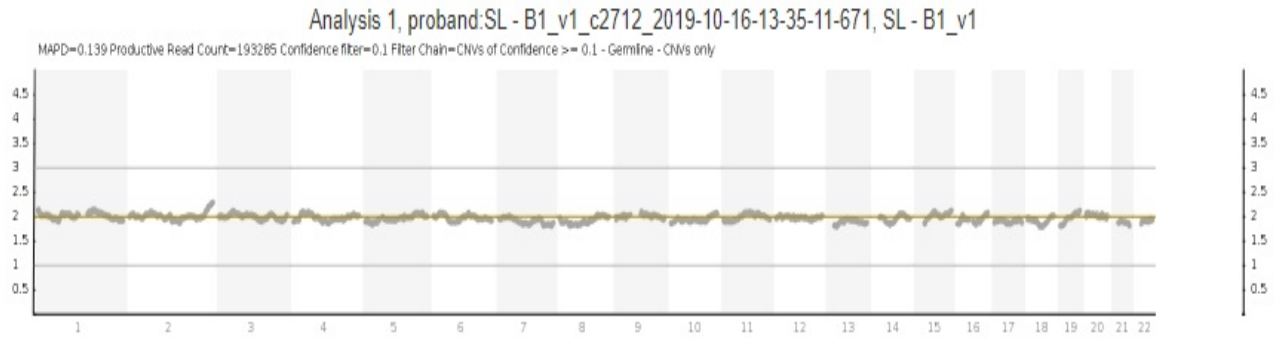

Figure 1. Euploid embryo with normal chromosome number.

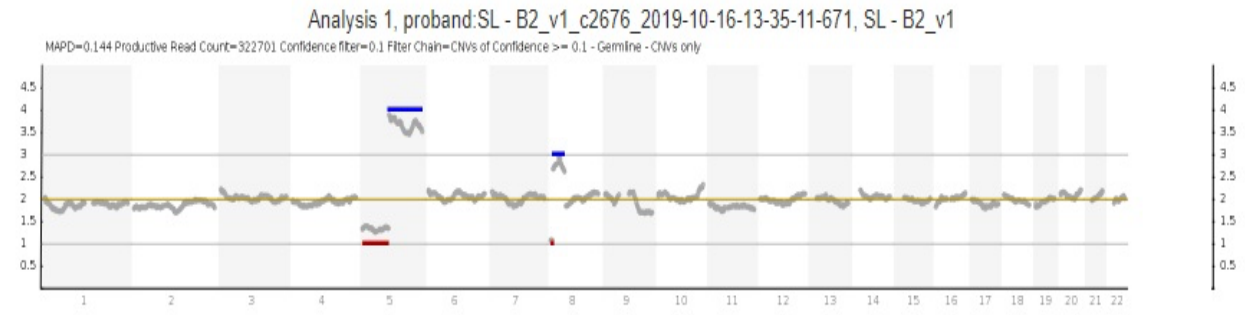

Figure 2. Aneuploidies in chromosome 5 and 8.

The female partner underwent embryo transfer as per standard protocls and she followup with our clinic. At day 15 she detected positive by biochemical pregnancy tet which was subsequently confirmed by routine ultrasound. Her pregnenty was uneventful and she delivered a single live baby at fuill term.

DISCUSSION

In clinical IVF, the gold standard method of performing PGT A, is to biopsy the trophectoderm cells of day 5 Blastocyst. This is an indirect test of assessing the embryo, as the trophectoderm develops into the placenta (Garikipati and Ashraf, 2020). This sort of invasive biopsy is still controversial among certain groups of clinicians. The TE cells biopsy requires specialized skills, and has false positives and negatives because the chromosomal component of the TE and ICM may differ widely. Noninvasive PGT A is based on sequencing DNA released in the culture medium from both TE and ICM, which develops into fetus. The DNA released by the embryo into the culture media contains a complete picture of its chromosomal content. Other advantages are that, the embryo biopsy which is an invasive procedure, can be avoided in non Invasive PGT A, without compromising the quality of clinical information. As a result of this, the potential risks associated with invasive techniques can be avoided, enabling more couples to adopt PGT techniques. Moreover, in the regular PGT-A, top quality blastocysts are required for embryo biopsy, and if some embryos fail to attain good quality, then such embryos are not considered good for biopsy and a result there are many failed PGT A cycles. This is problematic inindicated cases, especially advanced maternal age, which carries high risk of aneuploidy, can be devoid of PGT A. In case of noninvasive PGT A spent culture medium can be collected irrespective of the quality of blastocysts and relevant ploidy information can be obtained.

In this case report, the couple was wary of the issues pertaining to the biopsy procedure and only opted for the Noninvasive PGT A although we had informed them about the potential false positives and false negatives. After optimizing the process of the laboratory and preventing contamination of extrinsic contamination of DNA. Special care was taken to clean the embryos and make them completely devoid of cumulous cells to prevent signals from the maternal cells. After the necessary precautions and a steep learning curve we successfully optimized and employed this technology to our patient and herein we report a successful live birth after noninvasive PGT A in a couple with repeated failures. TO the best of our knowledge, this si the first case of a live birth using Noninvasive PGT A resulting in a live birth being reported from India.

Before offering Noninvasive PGT A, extensive genetic counseling, is a must, to educate the couple about several advantages as well as the limitations of the technique. In this case, the couple opted for noninvasive over invasive PGT A. Every noninvasive

PGT A must accompany either a Noninvasive prenatal testing after pregnancy or an invasive prenatal procedure to confirm the PGT A results.

Our non-Invasive PGT A employs highly specialized whole genome amplification protocols as per manufacturer's instructions with nearly 99.5% concordance which is already validated extensively. We feel that Noninvasive PGT A will play a significant role, in the future of euploid embryo selection [Liu et al 2017; Huang et al 2019].

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Conflict of interest statement

The authors have declared to have no competing interests or conflict of interest.

Authors' contributions

Conception and design: Ritu G and Hrishikesh Pai; data acquisition, analysis, interpretation and drafting of the article: Ritu G; study supervision: Hrishikesh Pai and Ritu G. Nandita Palshetkar, Rishma pai, Charumati Pekhele and Rohan Palshetkar have counselled and recruited the patient as an IVF team. The final version of the article is approved by all authors.

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Declaration of originality

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