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First live birth in Greece after blastocyst trophectoderm biopsy and preimplantation genetic diagnosis by karyomapping

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Abstract

Preimplantation genetic diagnosis for single gene defects is a well established tool in assisted reproduction. Karyomapping is a genome-wide parental haplotyping technology using a high density single nucleotide polymorphism array that allows the diagnosis of any single gene defects and the majority of aneuploidies from the same embryonic sample post embryo biopsy. A couple, carriers of different cystic fibrosis (CF) mutations and history of miscarriages due to chromosomal aneuploidies attended our clinic. Employing a strategy of accumulating embryos after blastocyst trophectoderm biopsy by vitrification cryopreservation, we managed to screen 8 embryos collected from 2 consecutive IVF cycles, employing the karyomapping genetic analysis tool. Two embryos were found normal after karyomapping analysis for both the CF and aneuploidies. The transfer of 1 normal blastocyst post-thaw resulted in a healthy and uneventful pregnancy and in the live birth of a female neonate on the 38th week of pregnancy. This is the first report of a live birth in Greece after blastocyst trophectoderm biopsy and karyomapping analysis of the biopsied cells.

Key words: preimplantation genetic screening (PGS), karyomapping, blastocyst trophectoderm biopsy *in vitro* fertilization

Introduction

Conventional preimplantation genetic diagnosis (PGD) is considered to be an alternative to prenatal diagnosis for the diagnosis in vitro and before the establishment of a pregnancy, of single gene disorders[1,2]. Preimplantation genetic screening (PGS) is a useful tool for the IVF clinic to reduce first trimester miscarriages due to aneuploidies in the preimplantation embryos.

Recently, a method known as karyomapping was developed (Handyside et al., 2010). Karyomapping uses an array platform that interrogates about 300,000 single nucleotide polymorphisms (SNP) spread across the entire human genome[3]. SNP are used to perform linkage analysis for the gene of interest in a given couple and determine whether normal or mutant copies have been inherited by embryos produced through IVF. A major advantage of the karyomapping technique is that it provides additional information on the copy number of all 24 types of chromosome within an embryo such as chromosomal loses (monosomies) or some gains (trisomies)[3].

Cystic fibrosis is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator gene[4]. The disorder produces a variable phenotype including lung disease, meconium ileus, pancreatic insufficiency and bilateral agenesis of the vas deferens causing male infertility through obstructive azoospermia[4].

We report a case of a couple where both the male and female were carriers for different mutations of the CF gene. The couple had experienced four miscarriages after natural conceptions, while conventional PGD IVF cycle resulted in a biochemical pregnancy. We employed a strategy of two consecutive IVF cycles. The embryos created underwent blastocyst trophectoderm biopsy and subsequent vitrification. The samples were batched for karyomapping analysis. The transfer of one healthy embryo post karyomapping, resulted in a healthy uneventful pregnancy and the live birth of a healthy female infant on the 38th week of gestation. This is the first case in Greece of a live birth after the combination of blastocyst biopsy and karyomapping.

In Vitro Fertilization

After initial consultation, genetic counseling was provided to the couple, biopsy procedures and embryological processes were explained and signed consents were obtained. For all the IVF attempts the GnRH antagonist stimulation protocol5 was used, according to clinic's instructions. Intra-cytoplasmic sperm injection (ICSI) was used in all IVF attempts as previously described6 in order to avoid sample contamination with paternal sperm DNA.

Blastocyst trophectoderm biopsy

All embryos created for karyomapping analysis were cultured to blastocyst stage. On day 4 of development a small opening of about 10 μm was created on the zona pellucida (ZP) of each embryo employing a non-contact laser system (Saturn, Research Instruments Ltd, UK), in order to cause an artificial herniation of trophectoderm cells. The biopsy procedure took place employing a Nikon Diaphot 300 microscope (Nikon, Japan), equipped with a Narishige micromanipulation system (HD-2106017, Nikon, Japan) and pneumatic pipette control system (SAS-SE, Research Instruments, UK).

The trophectoderm biopsy took place as has been previously described[7] with a slight modification: in order to avoid excessive laser use that could potentially affect the chromatin quality of the biopsied cells and also harm the rest of the embryo, only two laser pulses of 1.10 msec irradiation time each were applied and the trophectoderm cells were then dissociated from the blastocyst by fine mechanical movements and by pressing the biopsy pipette against the holding pipette with simultaneous application of negative pressure in the biopsy pipette. The technique resulted into five to eight trophectoderm cells being biopsied from the herniated part of each blastocyst.

Embryo vitrification and thaw

All blastocysts biopsied for karyomapping analysis were vitrified right after the biopsy procedure employing the Kitazato vitrification protocol[8] (Kitazato BioPharma Co., Ltd., Japan). After karyomapping diagnosis, a healthy blastocyst was thawed

Table 1: Results of karyomapping PGD analysis: +16= trisomy 16, +6= trisomy 6, -10q= deletion of 10q arm (partial monosomy), -8= monosomy 8

Embryo Number	Paternal Chromosome Carrying the Mutation	Maternal Chromosome Carrying the Mutation	Chromosomal Abnormalities	Final Interpretation
1	Detected	Not detected	+16	Abnormal
2	Not detected	Detected	None detected	Carrier
3	Detected	Detected	None detected	Abnormal
4	No result	No result	No result	No diagnosis
5	Detected	Detected	None detected	Abnormal
6	No result	No result	No result	No diagnosis
7	Not detected	Detected	+6	Abnormal
8	Not detected	Detected	-10q	Abnormal
9	Detected	Detected	-8	Abnormal
10	Not detected	Not detected	None detected	Unaffected

according to the same protocol (Kitazato BioPharma Co., Ltd., Japan) and was left in culture for at least two hours before embryo transfer.

Sample preparation and Karyomapping

Biopsied cells were thoroughly washed in four microdroplets of sterile non-stick washing buffer (NWB) (Phosphate-Buffered Saline, without magnesium or calcium [Invitrogen Life Technologies, USA] + 0.1% polyvinyl alcohol [Sigma-Aldrich, USA]) and transferred to sterile 0.2 ml polymerase chain reaction (PCR) tubes in 1 μl of clean NWB. All biopsied samples were transferred for karyomapping analysis and diagnosis to Reprogenetics UK, Ltd. (Oxford, UK).

Each trophectoderm biopsy was amplified using multiple displacement amplification (SureMDA, Illumina). For karyomapping, 8 μl of MDA product was used. Amplified products were tested according to the manufacturer's protocol (Illumina, USA) and results were evaluated using dedicated software (BlueFuse Multi V4.0, Illumina)[3]. Additionally, direct mutation detection was carried out by taking a further 1 μl of MDA product, amplifying the mutation sites and performing minisequencing to reveal the presence/absence of each mutation, according to Reprogenetics protocols. Although diagnosis can often be accomplished using karyomapping alone, the addition of direct mutation detection further enhances the accuracy of testing. Furthermore, supplementary mutation analysis is essential in cases where the only DNA samples available are those from the couple. Samples from additional family members are essential for understanding which of the parental SNP alleles are associated with the inherited disorder (Table 1).

Case Report

We report a case of a couple with CF mutations in both partners: the male partner was carrier of the P.GLU826LYS mutation and the female partner of the ΔF508 mutation of the CF gene. The couple presented in our IVF clinic in 2013. The female and male ages were 33 and 35 respectively at the time of referral. The couple had experienced four miscarriages after natural conceptions, two of which were confirmed as aneuploidic (one fetus had trisomy 22 and one had trisomy 17).

In their first IVF cycle in our clinic eight ova were collected, six were fertilized after ICSI and all six were submitted to blastomere biopsy. Conventional PGD analysis for CF revealed three unaffected embryos, two of which were suitable for embryo transfer at blastocyst stage. The transfer of these embryos resulted in a biochemical pregnancy.

A year after a second IVF cycle was attempted,

where 13 ova were collected and eight were fertilized and submitted to blastomere biopsy. The biopsied cells were transported to Reprogenetics UK for karyomapping analysis this time. Three embryos were diagnosed as unaffected for CF and chromosomally healthy. All three were at morula stage on the fifth day of embryonic development. The transfer of two such morulae did not result in a pregnancy.

Two years later the couple visited our clinic and a new strategy was planned: two consecutive IVF cycles were performed in order to accumulate more embryos for karyomapping analysis, increasing the likelihood of finding genetically normal embryos of good morphological grade. In the first IVF cycle 14 ova were collected, 11 were fertilized, six embryos were biopsied at blastocyst stage this time and vitrified, while the trophectoderm samples were transferred to Reprogenetics UK. A second cycle was attempted three months later: nine ova were collected, five were fertilized and four blastocysts were successfully biopsied and vitrified.

A total of 10 biopsied trophectoderm samples were analyzed by karyomapping for both the CF mutations and chromosomal aneuploidies. A definite diagnosis was possible in eight embryos: one embryo was unaffected by the CF and chromosomally normal, one embryo was carrier for the CF and chromosomally normal and six embryos yielded abnormal results for the CF or/and the chromosomal status (data not shown).

Five months after the last cycle, a thaw and embryo transfer of the completely unaffected embryo took place. This resulted in a positive outcome with an 84 mIU/ml initial β-human chorionic gonadotropin (β-HCG) result 10 days post embryo transfer. Transvaginal ultrasound at eight weeks of gestation confirmed a clinical pregnancy identifying one endometrial sac with one positive fetal heart activity. On July 26, 2017 the patient underwent caesarean section and gave birth to a healthy female neonate at 38 weeks of gestation, weighing 3270 g and with an Apgar score of 10/10 without intercurrences.

Discussion

We report the first case in Greece of a karyomapping PGD that resulted in the birth of a healthy infant after blastocyst biopsy, vitrification and thaw of a healthy embryo used in embryo transfer. The father had the P.GLU826LYS CF mutation and the mother the ΔF508 one. The couple had achieved four natural conceptions all of which miscarried, while in two of those a chromosomal aneuploidy was defined as the cause of miscarriage.

Karyomapping has the great advantage against conventional PGD that allows the simultaneous detection of single gene disorders such as CF and the majority of chromosomal aneuploidies, from the same embryonic material3. This is why it was the method of analysis we chose after the first failed conventional PGD cycle of the couple in our clinic. In general, the use of karyomapping reduces the amount of patient-specific customization necessary when developing a PGD test for a couple. This typically reduces the time required for work-up of a new test from months to just a few weeks.

This live birth was achieved by applying trophectoderm biopsy on the embryos to be analyzed, on the fifth day of in vitro development. The biopsy of five to eight trophectoderm cells compared to single blastomere biopsy employed in the first PGD attempts, provided the advantage of a more robust outcome for the genetic analysis, since more than one cells were analyzed. Blastocyst biopsy has now become the gold standard in order to obtain embryonic material for preimplantation diagnosis purposes[9].

A novelty reported here is the vitrification of the biopsied blastocysts in order to significantly increase the available embryo number for karyomapping diagnosis. There are only a couple of studies reporting this strategy[10,11] and it seems that it could be a powerful tool in the event of complicated PGD cases such

as the one reported here, where a large number of embryos is necessary to ensure the identification of healthy embryos suitable for embryo transfer.

This is the first report in Greece of the live birth of a healthy infant after trophectoderm biopsy of blastocysts, vitrification and thaw of the biopsied embryos in order to increase embryo availability for analysis and karyomapping analysis of the biopsied material. This strategy proved to be quite efficient and could be the method of choice for couples seeking PGD where a single gene disorder and chromosomal abnormalities may coexist.

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