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Case Report

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First live birth in Greece after blastocyst trophectoderm biopsy and preimplantation genetic testing for hereditary angioedema

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Abstract

Preimplantation genetic testing for monogenic/single-gene defects (PGT-M) is a well established tool in assisted reproduction. A couple, where the male was carrier of the c.550G>A (p.Gly184Arg) mutation of the *SERPING 1* gene causing hereditary angioedema (HAE) attended our clinic for PGT-M. Employing a strategy of preserving embryos after blastocyst trophectoderm biopsy by vitrification cryopreservation, we managed to screen 10 embryos collected from a single IVF cycle. Three embryos were found unaffected after preimplantation genetic analysis for the paternal mutation. The transfer of one normal blastocyst post-thaw resulted in a healthy and uneventful pregnancy and in the live birth of a male neonate on the 38th week of pregnancy. This is the first report of a live birth in Greece after blastocyst trophectoderm biopsy and preimplantation genetic analysis for hereditary angioedema.

Key Words: Preimplantation genetic testing for monogenic/single-gene defects (PGT-M), hereditary angioedema (HAE), blastocyst trophectoderm biopsy, in vitro fertilization

Introduction

Conventional preimplantation genetic testing for monogenic/single-gene defects (PGT-M) 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].

Hereditary angioedema (HAE, OMIM #106100) is a rare genetic disorder characterized by recurrent episodes of swelling in different parts of the body, being potentially life-threatening when the upper airway is involved[3,4]. HAE is inherited as an

autosomal dominant trait. It is caused by a defect in the *SERPING 1* gene (alias C1NH, complement 1 inhibitor), localized on chromosome 11q12-q13.1. The mutations are heterogeneous ranging from large deletions or duplications to point mutations, small deletions or insertions[5]. Onset may occur at any age and the estimated prevalence is one in 100,000.

Gastrointestinal attacks associated with abdominal pain, nausea, vomiting and diarrhea are caused by swelling of the intestinal wall. Hand and feet swelling is extremely uncomfortable for the patients, as they become unable to continue with their daily routines. The most dangerous feature of the disease is laryngeal edema as it can block the airway and potentially cause death by asphyxiation[6]. Edema attacks occur spontaneously, while triggers like anxiety, stress, minor trauma, surgery and illness such as cold and flu have been reported[7,8]. The edema is non-pruritic and remains unrelieved with histamines and corticosteroids.

The quality of life of HAE patients is severely affected by the sudden attacks and the possibility of asphyxiation. In an attempt to prohibit HAE in next generations, HAE patients may opt for preimplantation genetic testing for monogenic/single-gene defects (PGT-M) as an alternative to prenatal diagnosis and the termination of an affected fetus. PGT-M has been made available for a large number of rare genetic disorders[9], including hereditary angioedema[4].

We report a case of a couple where the male suffers from HAE due to a *SERPING 1* gene mutation. The disease was discovered after swelling episodes of the patient. The couple visited our clinic seeking IVF and PGT-M to prohibit the passing of the disease to the offspring. We employed a strategy of a single IVF cycle. The embryos created underwent blastocyst trophectoderm biopsy and subsequent vitrification. The samples were dispatched to a genetics laboratory for PGT-M analysis. The transfer of one healthy embryo post PGT-M, resulted in a healthy uneventful pregnancy and the live birth of a healthy male infant on the 38th week of gestation. This is the first case in Greece of a live birth after blastocyst trophectoderm biopsy and PGT-M for hereditary angioedema.

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 the IVF attempt the GnRH antagonist stimulation protocol[10] was used, according to clinic's instructions. Intra-cytoplasmic sperm injection (ICSI) was used in the IVF attempt as previously described[11] in order to avoid sample contamination with paternal sperm DNA.

Blastocyst trophectoderm biopsy

All embryos created for PGT-M analysis were cultured to blastocyst stage. A novel blastocyst trophectoderm biopsy protocol employed, in order to maximize the available blastocyst number for biopsy as has been previously described[12].

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).

On day five of development a small opening of about 10 μm was created through the zona pellucida (ZP) of each tested embryo by applying three consecutive 0.7 msec pulses of a non-contact 1.48-micron diode laser system (Saturn 3, Research Instruments Ltd, UK). A mechanical micromanipulation of each blastocyst with the biopsy pipette followed in order to cause an artificial shrinkage of the blastocyst. The biopsy pipette was next inserted from the hole through the ZP and five to eight blastomeres were aspirated from the trophectoderm area.

In order to avoid excessive laser use that could potentially affect the chromatin quality of the bi-

opsied cells and also harm the rest of the embryo, only two laser pulses of 0.7 msec irradiation time each were applied at the cell junctions on the biopsied trophectoderm mass. The trophectoderm cells were then dissociated from the blastocyst by fine mechanical movements and by pressing the biopsy pipette against the holding pipette (flicking method) with simultaneous application of negative pressure in the biopsy pipette. The technique resulted into five to eight trophectoderm cells being biopsied from each blastocyst. Special attention was paid to leave the inner cell mass of each biopsied blastocyst unharmed by the procedure.

Embryo vitrification and thaw

All blastocysts biopsied for PGT-M analysis were vitrified right after the biopsy procedure employing the Kitazato vitrification protocol[13] (Kitazato BioPharma Co., Ltd., Japan). After PGT-M a healthy blastocyst was thawed 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 PGT-M analysis

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] and transferred to sterile 0.2 ml polymerase chain reaction (PCR) tubes in 1 μl of clean NWB. All biopsied samples were transferred for PGT-M analysis and diagnosis to Alpha Lab S.A., (Athens, Greece).

Confirmation of the genetic status of the couple was performed as previously described[14]. PCR amplification of exon 3 of the *SERPING 1* gene was performed using the OUT and IN primer pairs listed in Table 1, using a hemi-nested approach. Short tandem repeat (STR) markers (Table 1), closely linked to the disease-causing gene *SERPING 1*, that were informative for the couple, were also included to avoid a possible misdiagnosis resulting from allele dropout (ADO). All primer pairs were designed using "primer 3 (v. 0.4.0)" program. The reliability of the protocol was evaluated before clinical application on single lymphocytes collected from both parents, as previously described[14].

DNA analysis of blastomeres was carried out on the same day as biopsy. Before proceeding to multiplex PCR, cells were lysed by incubation at 37°C for 1h in 10μl of proteinase K/SDS buffer and at 95°C for 10 min for proteinase K inactivation.

For the diagnosis of the disorder a nested multiplex PCR assay was used. The first round of PCR, that contained the external primers for the amplification of the gene region of exon 3 containing the mutation and STR markers linked to these regions for ADO detection, was performed in a total volume of 50μl containing 3.5 mmol/l MgCl2, 200mmol/l of each dNTP (Roche Diagnostic, Italy), 2.5 IU AmpliTaq Polymerase (Hot Start Taq Qiagen) and 10 pmol of each outer primer pair. The program used consisted of 35 cycles of 30 sec at 95°C, 30 sec at 60°C, 1min at 72°C. Each round of PCR was preceded by an initial 4 min denaturation step at 96°C and followed by a final extension step of 10 min at 72°C. The first round of multiplex PCR was followed by three separate second round PCRs, one for the exon and two multiplex PCRs for the STRs (PLEX1 and PLEX2 as listed in Table 2) using the inner primer pairs. Same conditions were used, only this time each PCR consisted of 22 cycles. Fluorescent fragments

Table 1. The OUT and IN primer pairs employed for the PCR amplification of exon 3 of the SERPING 1 gene.

SERPING1 EX3END FOUT	5' TTCTGCCCAGGACCTGTTAC 3'
SERPING1 EX3END R	5' TGGGAGTGTCCAACAAATGA 3'
SERPING1 EX3END FIN	5' GGGGGATGCTTTGGTAGATT 3'
SNAP Gly184Arg (R)	5' TTT TTT TTT GAG AAT TCA AGC AGG GTC TTA C 3'

Table 2. Relative position of markers: D11S1395; D11S2365; D11S1313; SERPING 1; D11S1777; D11S4202; D11S1983

D11S1395 FOUT	5' ACAAAAACAGAGGGCAAAAA 3'		
D11S1395 R	5' TGAATGAATAGAGGCAATGTGAC 3'		PLEX1
D11S1395 FIN	5' GGTGATGTAAGGTACAGCATAATGTC 3'	ROX	
D11S1777 FOUT	5' AGGCAGGAGAATCGTTTGAA 3'		
D11S1777 R	5' TCCACGAATGCCAACAATTA 3'		PLEX1
D11S1777 FIN	5' GCCAAGATTGCACCATCAC 3'	HEX	
D11S2365 FOUT	5' GTGAATAGCGCTGCAATGAG 3'		
D11S2365 R	5' GCCCTGAACTGGAATAAGCA 3'		PLEX1
D11S2365 FIN	5' TCACATGCATATGTCTTTATGGTAGA 3'	FAM	
D11S1313 F	5' TTGGAAAAGAAGAAAGAGTGTGTG 3'		
D11S1313 ROUT	5' GCATTTCCAACGTCTAAGCA 3'		PLEX2
D11S1313 RIN	5' TCCAACGTCTAAGCATGAAGC 3'	FAM	
D11S4202FOUT	5' ATGGGTACAGGGGTCTCCTT 3'		
D11S4202R	5' CTTTTGTGCACGTGTGTGTG 3'		PLEX2
D11S4202FIN	5' TCCTTTGTTGGTGGTGATGA 3'	HEX	
D11S1983 FOUT	5' CTCCAGCTGGGTGACAGAAT 3'		
D11S1983 R	5' GGGGAATCAAATTCAACCAA 3'		PLEX2
D11S1983 FIN	5' CAGCTGGGTGACAGAATGAG 3'	FAM	

were analysed by 20 min of capillary electrophoresis on an automatic DNA sequencer ABI 3500™ (Applied Biosystems). Mutation analysis was performed using the minisequencing method, as previously reported[14] and the specific primer for the mutation SNAP Gly184Arg (reverse strand).

Case Report

We report a case of a couple where the husband was affected by HAE. The male partner was carrier of the c.550G>A (p.Gly184Arg) mutation of the *SERPING 1* gene. Sudden swelling episodes resulted in him being diagnosed with HAE. The couple presented in our IVF clinic in 2018. The female and male ages were 33 and 40 respectively at the time of referral.

In their scheduled IVF cycle in our clinic on 01/12/2018, 19 ova were collected, 17 were mature post-cumuli/corona cell-denudation and 15 were fertilized after ICSI. Ten blastocysts were submitted to blastocyst trophectoderm biopsy on day five of development.

The biopsied cells were transported to Alpha Lab S.A., Athens, Greece for PGT-M analysis. Three embryos were diagnosed as unaffected for HAE (Table 3). All three were at blastocyst stage on the fifth day of embryonic development with qualities 5AA, 4BA and 4BB according to Gardner's blastocyst scoring criteria[15].

On 3/12/2019 the couple presented in our clinic for a frozen embryo replacement cycle. The 5AA blastocyst, unaffected by HAE (Table 3), was thawed intact for the embryo transfer procedure. This frozen

Table 3. Results of PGT-M analysis for the paternal c.550G>A (p.Gly184Arg) *SERPING 1* gene mutation causing hereditary angioedema; *Unaffected embryo transferred to the patient

EMBRYO NUMBER	BLASTOCYST QUALITY	PATERNAL MUTATION C.550G>A (P.GLY184ARG)	FINAL EMBRYO INTERPRETATION
1	4BB	Detected	Affected
2	4BB	Not Detected	Unaffected
3	3BB	Detected	Affected
4	4BB	Detected	Affected
5	4BB	Detected	Affected
6	4AB	Detected	Affected
7	4BA	Detected	Affected
8	4BA	Not Detected	Unaffected
9	6AA	Detected	Affected
10*	5AA*	Not Detected*	Unaffected*

embryo replacement resulted in a positive outcome with a 61 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 the 6th of August 2020 the patient underwent vaginal delivery and gave birth to a healthy male neonate at 38 weeks of gestation, weighing 2510 g and with an Apgar score of 10/10 without intercurrences.

Discussion

We report the first case in Greece of a hereditary angioedema PGT-M 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 was carrier of the c.550G>A (p.Gly184Arg) mutation of the *SERPING 1* gene, suffering from frequent swelling episodes.

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, provides the advantage of a more robust outcome for the genetic analysis, since more than one cells can be analyzed. Blastocyst biopsy has now become the gold standard in order to obtain embryonic material for PGT-M purposes[16].

A novelty reported here is the direct hatching of the zona pellucida (ZP) and biopsy of the trophectoderm cells on day five of embryonic development, instead of a prior day three or day four ZP assisted hatching. This methodology has been reported to increase blastulation rate since the extra embryo manipulation taking place during day three or day four assisted hatching, may negatively affect embryo blastulation[12]. Our increased blastulation rate of 67% (10/15 embryos) amplifies the finding of a previous study[12] shown that performing direct ZP hatching right before trophectoderm cell aspiration may increase the available blastocyst number for biopsy.

This is the first report in Greece of the live birth of a healthy infant after trophectoderm biopsy of blastocysts, vitrification and thaw of a biopsied embryo, in order to identify unaffected embryos by HAE suitable for embryo transfer. Two surplus healthy and good quality blastocysts (4BA and 4BB) remain in cryopreservation in our clinic for the

study couple and could potentially lead to the birth of more healthy infants if transferred to the patient in the future.

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