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## THE REPORTED SUCCESSFUL BABY DELIVERY AFTER PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDIES (PGT-A) BY MEANS OF NEXT GENERATION SEQUENCING (NGS).

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### ANNOTATION

The aim of this case report is to demonstrate a successful delivery of a baby after transfer of a blastocyst tested for aneuploidies by means of NGS. A woman aged 35 having two miscarriages decided for ICSI program with PGT-A analysis. Six eggs were fertilized out of 9 metaphase II oocytes. Five good quality blastocysts were submitted for genetic screening using 24-chromosome next generation sequencing (NGS). Two blastocysts were diagnosed as euploid and recommended for transfer. One euploid blastocyst was thawed and transferred to the patient's uterus lining. Successful pregnancy was confirmed at 7 weeks of gestation with heartbeat. Successful delivery was achieved by Caesarean section at 38-39 weeks of gestation. Karyotyping demonstrated healthy genetic constitution of a baby. This case demonstrates a good evidence and potential of a transport scheme collaboration between IVF and genetic laboratories.

**Key words:** ART; IVF; NGS; PGT-A; TE (trophectoderm).

### INTRODUCTION

Chromosomal aberrations are the most frequent etiology for early pregnancy losses in the first trimester and covers 50% of spontaneous abortions [1,2]. Preimplantation genetic testing for aneuploidies (PGT-A), previously known as preimplantation genetic screening (PGS), was developed to prevent early miscarriages at embryonic stage in conjunction with IVF [3].

Nowadays, good quality laboratory is the one offering PGT service. A variety of genetic platforms exists for screening of chromosomal aberrations. Fluorescence in situ hybridization (FISH) analysis, first clinical case published in 1993 [4], as a method is fading gradually. Such limitation, as analysis of 5 or 9 chromosomes is not satisfying anymore considering that chromosome or chromatid missegregation may occur at any chromosome set and lead to genetic malformations [5]. Array comparative genomic hybridization (array CGH) and next generation sequencing (NGS), both allow comprehensive screening of 23 pairs of chromosomes [6].

In order to detect 20% of mosaicism within an embryo by NGS, at least 5 TE cells should be biopsied [10]. Therefore, majority of laboratories moving from blastomere biopsy to trophoctoderm (TE) biopsy since several cells could be taken for genetic analysis. On average, 5 – 10 cells are recommended for analysis [10], which will provide a more strong and valid genetic result. Resolution, quality and mosaicism detection by NGS for embryo screening seems to be higher [6-9]. Embryos tested by NGS might have stronger chance for successful pregnancy and lower risk for the pregnancy loss [6,8].

Meantime, higher sensitivity for mosaicism assumes less euploid embryos, which demonstrates a risk of overdiagnosis the euploid embryos as mosaic. In all cases, both platforms are eligible and successfully applied worldwide.

However, only large scale IVF centers can afford the expense of equipment and software, the strong skills and knowledge of professionals including analysis and interpretation of bioinformatics data [11]. The transport scheme sounds feasible for small and average scale laboratories.

Here, we report a successful pregnancy and delivery of a genetically healthy baby after PGT-A by means of NGS collaborating with the genetic laboratory on transport scheme. In our report, we use PGT related terminology according to the ICMART and ASRM international glossary on infertility and fertility [3].

### CASE REPORT

#### INFERTILITY HISTORY

A couple with an infertility history of more than 5 years. At the moment of the treatment (October 2017), the female patient was 35 years old and was diagnosed with secondary infertility, pelvic pain during irregular menses, adenomyosis and polymorphism for thrombophilia. For the latter she was prescribed medication by hematologist. The husband was 51 years old; semen analysis was analyzed in accordance with WHO Lab manual [12] and showed normozoospermia. The couple has two children conceived naturally and born in 2003

and 2005. In 2008 and 2012, she experienced two pregnancy losses at the first trimester. The karyotypes for the patient and her husband are normal.

### CONTROLLED OVARIAN STIMULATION

On day 3, female patient's blood testing demonstrated FSH = 7.1 IU/ml, LH = 5.2 IU/ml, Testosterone = 0,22 nmole/L, AMH = 5.38 ng/ml.

From day 3 of menstruation, the female patient was prescribed with 150/75 IU of recombinant FSH and LH (Pergoveris; Merck Serono), 75 IU of recombinant FSH (Gonal F; Merck Serono). From the 6th day of stimulation 0,25 mg of antagonist (Orgalutran; Merck Serono) was added to the treatment. After 9 days of ovarian stimulation, recombinant human chorionic gonadotropin (rhCG; 250 mg of Ovitrelle; Merck Serono) as a trigger was administered. Eleven cumulus-oocyte complexes (COC) were aspirated under ultrasound, 37 hours after rhCG injection.

### INTRACYTOPLASMIC SPERM INJECTION, EMBRYO CULTURE, BIOPSY AND NGS

Nine mature eggs were subjected to injection in 41 hours post rhCG injection. The fertilization was assessed in 18 hours after ICSI, 6 oocytes were fertilized. Fertilization pattern was analyzed according to modified and adapted in our lab Z-score, initially proposed by Scott et al. [13], along with the presence or absence of halo for each zygote.

Zygotes were cultured in Sage HSA mono-step medium until Day 7. On Day 3, clinical embryologist performed Zona pellucida opening for 6 cleaving embryos. The embryos were cultured up to day 7 to allow blastulation and biopsy. Four good quality blastocysts (5AB, 5AA, 5AB and 5BB) according to the Gardner and Schoolcraft [14-15] grading system were subjected to biopsy on day 6 conducted by experienced embryologist. The 5th blastocyst (5BA) was biopsied on day 7. On average, 4-8 trophoctoderm cells were biopsied and inserted into PCR tubes with PBS/PVP buffer. The PCR tubes along with biopsy protocol were sent to Genetic lab "Medical Genomics" for NGS analysis. Two out of 5 embryos were euploid; 1 embryo had monosomy for the 22nd chromosome, 1 embryo – monosomy for the 14th chromosome and detected mosaicism for the 6th chromosome, 1 embryo had trisomy for the 21st chromosome. The embryo transfer was performed in frozen embryo replacement (FER) cycle. The vitrification and thawing procedures were conducted with the Cryotop method (Kitazato Biopharma Co., Japan) initially described by Kuwayama [16].

### FER/FET CYCLE

Due to severe adenomyosis, the female patient was prescribed agonist treatment, 3.75 mg (Diphereline, Ipsen) 3 times a day for 3 months before the embryo transfer. After the treatment, endometrium preparation was initiated with estradiol, 1 tablet three times a day (Progynova, Bayer Co). When endometrium thickness reached 8-9 mm, 800 mg Utrogestan (Besins Healthcare, SA) was added to the treatment. Two days before FET, intramuscular progesterone was added. On the 6th day of Utrogestan supplementation, the recommended blastocyst was thawed (Kitazato Biopharma Co., Japan) in the morning and hatching blastocyst was

transferred in culture medium Sage HSA at 1 pm. At the day of transfer, endometrium thickness was 11.5 mm.

### OUTCOMES

Clinical pregnancy was confirmed at 7 weeks of gestation with a heartbeat. A healthy baby was born. The karyotyping of a baby demonstrated no presence of chromosomal aberrations.

### DISCUSSION

The prevalent method for PGT was FISH analysis for decades worldwide. FISH analysis seems to be the cheapest amongst the methods, although it encounters well-known limitations like a limited number of chromosomes to be analyzed, availability of the cytogenetic laboratory, labor-intensive work [17].

Array CGH for preimplantation genetic analysis is a good tool. It was successfully implemented for all the stages of biopsy including randomized controlled trial and retrospective case-control studies [23]. It was demonstrated that NGS results 100% correlate with array CGH results for aneuploidy [18-19]. However, NGS protocols provide enhanced detection of segmental aneuploidies and detect mosaicism from 20% [8,19]. Meantime, array CGH detects 40-60% mosaicism [8]. Therefore, higher resolution of NGS allows less mistakes and a positive impact on the clinical outcome decreasing the miscarriage rate.

The clinical implementation of NGS into the laboratory lies in patients' strong wish to have a healthy child and staff's determination to offer the best possible in IVF market. Moreover, it is pivotal in competitive environment to offer a range of services: aneuploidy screening, genetic testing for monogenic diseases (PGT-M), for structural rearrangements (PGT-SR) and Rhesus factor of an embryo alone and along with comprehensive chromosome screening. Implementation of a genetic unit into the fertility clinic is not affordable for majority of IVF units. Therefore, the transport scheme seems a viable option for small and average size IVF units, considering that successful biopsy rate is 98.2% in our clinic, which is acceptable according to the ESHRE Vienna consensus (ESHRE SIG of Embryology, 2017) [20].

The PGT also demonstrates indirect benefits. One of them is to reduce amount of embryos to store. The non-recommended ones are being discarded upon informed consent from the patient. The embryos with detected mosaicism is a subject of debate inquiring extensive genetic counseling, but final decision should be up to the patients.

Furthermore, PGT is a good mean to reduce amount of transferred embryos since only one blastocyst is vitrified per straw. Multiple pregnancies are still a milestone that demands a solution and are associated with elevated risks of obstetric complications [21]. Up to date, in Kazakhstan, three embryos are allowed for intrauterine transfer [22]. However, the maximum to be transferred is, predominantly, one or two as a policy of the majority (personal communication). Therefore, in order to reduce the number of embryos for transfer, especially, in advanced maternal age group, PGT implementation might assist in that by choosing a single genetically "healthy" embryo [21].

The above clinical case demonstrates the efficiency of clinical implementation of NGS on the transport scheme.

Nowadays, collaborating with large genetics laboratories with well-established logistics the IVF center of any size can afford PGT service to the patients. However, it is crucial to choose the lab based on not only the quality of services, but also its variety.

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## РЕЗЮМЕ

### ОПИСАНИЕ КЛИНИЧЕСКОГО СЛУЧАЯ ПО ИСПОЛЬЗОВАНИЮ СЕКВЕНИРОВАНИЯ НОВОГО ПОКОЛЕНИЯ (NGS) В ЦЕЛЯХ ПРОВЕДЕНИЯ ПРЕИМПЛАНТАЦИОННОГО ГЕНЕТИЧЕСКОГО ТЕСТИРОВАНИЯ НА АНЕУПЛОИДИИ (ПГТ-А)

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Описание клинического случая демонстрирует успешное завершение программы ЭКО рождением ребенка используя преимплантационное генетическое тестирование (ПГТ) методом NGS (секвенирование нового поколения). Пациентка в возрасте 35 лет с наличием двух замерших беременностей обратилась в клинику. Оплодотворение проведено методом ИКСИ, 6 из 9 зрелых яйцеклеток оплодотворились. Пять blastocист хорошего качества и выше отправлены на генетический анализ методом NGS. Две blastocисты диагностированы euploidными и рекомендованными к переносу. Одна blastocиста разморожена и перенесена. Беременность завершилась родами на 38-39 неделе кесаревым сечением. Каритипирование выявило здоровый генетический набор хромосом у ребенка (девочки). Данный клинический случай демонстрирует, что транспортная схема является отличной альтернативой сотрудничества между генетическими и ЭКО лабораториями.

**Ключевые слова:** BPT; IVF; NGS; ПГТ-А; TE (трофэктодерм)

## ТҮЙІНДЕМЕ

### АНЕУПЛОИДИЯ (PGT-A) ҮШІН ИМПЛАНТАНТҚА ГЕНЕТИКАЛЫҚ ТЕСТІЛЕУ ҮШІН КЕЛЕСІ БУЫННЫҢ РЕТТІЛІГІН (NGS) ҚОЛДАНУДЫҢ КЛИНИКАЛЫҚ ЖАҒДАЙЫНЫҢ СИПАТТАМАСЫ

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Клиникалық жағдайларды сипаттау барысында ЭКҰ бағдарламасы бойынша имплантация алдында эмбрионға жүргізілетін генетикалық тасдаудың (ПГТ) NGS (жаңа буындағы секвенирлеу) әдісін қолдануда бала туылуының сәтті аяқталатынын көрсетті. Клиника көмегіне 35 жастағы екі рет жүктілігі сәтсіз аяқталған науқас жүгінді. Ұрықтандыру ИКСИ әдісімен жасалынды, 9 толық жетілген аналық жасушадан 6 жасуша ұрықтанды. Сапасы жақсы 5 blastocиста NGS әдісі бойынша генетикалық талдауға жіберілді. Оның ішінде екі blastocиста euploidты және жатырға көшіруге жарамды болды. Соның бірі ерітіліп жатырға көшірілді. Жүктілік 38-39 аптада қарынды жарып алу әдісімен сәтті аяқталды. Баланың(қыз бала) каритиптік талдауы бойынша генетикалық хромосом жиынтығы салауатты екенін көрсетті. Осы клиникалық жағдай транспорттық жүйенің генетикалық және ЭКҰ зертханалардың арасындағы жақсы балама екендігін көрсетеді.

**Түйін сөздер:** ART; IVF; NGS; PGT-A; TE (трофэктодерма).