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CASE REPORT

Successful Pregnancy Following ICSI Using Array Comparative Genomic Hybridization for Preimplantation Genetic Screening

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ABSTRACT

A 41-year-old woman with 2 previous failed IVF cycles was reported. After controlled ovarian hyperstimulation and then intracytoplasmic sperm injection (ICSI) of 4 metaphase II oocytes with ejaculated sperm was performed, 4 fertilized oocytes occurred. Two blastocysts were biopsied on day 5, one of them was chromosomally normal after array Comparative Genomic Hybridization (aCGH)-based preimplantation genetic screening (PGS). The successful pregnancy was achieved after blastocyst transfer. The aCGH-based PGS may be the preferred method to select the balanced and euploid embryo before transfer in IVF.

Keywords: PGS, aCGH, ICSI, repeat IVF failure

Background

In vitro fertilization (IVF) treatments typically results in many embryos. However, the quality of the sibling embryos may vary greatly. Euploid embryos have higher chance to get successful pregnancy. Morphological assessment is not a reliable method to check for chromosome abnormalities. This step is the most important to ensure the embryo viability. Screening of all 24 chromosomes using array comparative genomic hybridization (aCGH) may be a better strategy than limited chromosome assessment with fluorescent in situ hybridization (FISH) technique.

Case

A 41 year-old woman, with history of idiopathic infertility came for assisted reproduction, She had history of failed intrauterine insemination (IUI) (4 cycles) and failed in vitro fertilization (IVF) (2 cycles). The semen analysis of her husband was normal. Long Gonadotropin-Releasing Hormone (GnRH) agonist protocol with recombinant follicle-stimulating hormone (rFSH) was used for controlled ovarian hyperstimulation

in her previous cycle.

In the first cycle, 5 embryos were obtained, Four of themhad normal chromosomeaccording to 5-probes fluorescent in situ hybridization (FISH). Three embryos were transferred in fresh cycle, but she did not pregnant. In the second cycle, 16 embryos were obtained. Eight of them were cryopreserved at the 2 pronuclei (PN) stage. Eight embryos were then cultured to cleavage stage. Blastomere biopsy was performed for aneuploidy screening. All 8 embryos were chromosomally normal according to 5-probe FISH. The best 2 blastocysts were transferred in stimulated cycle, but she failed to get pregnant.

In frozen-thaw embryo transfer cycle, 8 embryos were thawed and cultured to cleavage stage. Seven embryos were then biopsied and 6 of them had normal chromosome after checking with 5-probe FISH. The best 2 blastocysts were transferred, but again, no successful pregnancy.

The next IVF cycle, GnRH antagonist protocol was used to ovarian hyperstimulation. Daily injection of 150 units of rFSH (Gonal-F; Merck Sereno, Switzerland) and 150 units of highly-purified human menopausal gonadotropin (Menopur; Ferring, Switzerland) were given, starting on the second day of the menstrual cycle. Six day after stimulation, 4 follicles, with diameter $\geq$ 16 mm were observed. The level of serum E2 level was 3,709 pg/mL Cetrorelix (Cetrotide; Merck Serono, Germany), 0.25 mg, was then given daily. Oocyte maturation was triggered by injecting 10,000 I.U. of human chorionic gonadotropin (Pregnyl; Merck, Canada), 7 days after the gonadotropin administration (total 2,100 units). Thirty-seven hours later, transvaginal oocyte retrieval was performed under sonographic guidance. Thirteen oocytes were retrieved. Six oocytes were mature (metaphase II) and considered to be suitable for insemination by the ICSI procedure. Five oocytes were fertilized and incubated in the medium with 10% human serum albumin. All embryos were cultured in sequential media (Cook Blastocyst medium, Australia) 3 days after performing ICSI. Two blastocysts (grading 2AB and 2BB by Gardner's blastocyst evaluation criteria[1]) were biopsied for aneuploid testing with array comparative genomic hybridization (aCGH).

Samples were lysed, fragmented, and amplified using the SurePlex kit. Amplified samples were processed according to the 24 SURE Version 2 BlueGnome CytoChip protocol (BlueGnome, Cambridge, UK). Scanned images were analyzed and qualified. Whole chromosomal copy number ratios were reported using the CytoChip algorithm fixed settings in BlueFuse Software (BlueGnome). The results showed that there was no aneuploidy found/XX, and monosomy 19/XY. The normal blastocyst was then cryopreserved with the cryotop vitrification protocol[2]. For vitrified-warmed embryo transfers, patient was given estrogen (Progynova; Bayer Schering Pharma, Germany) and progesterone vaginal suppositories (8% Crinone; Serono, Switzerland) replacement for endometrial preparation. Single blastocyst was transferred.Luteal support was preparedby daily 8% Crinone, oral progesterone (Duphaston; Abbott, The Netherlands), and oral estradiol (progynova; Bayer Schering Pharma, Germany).

The levels of serum β-hCG and serum progesterone (P4) was 58.81 IU/ml and 10.47 ng/ml, respectively on day 9th. She had vaginal spotting on day 12th. Serum β-hCG was 251.8 IU/mlwhile serum P4 was 10.52 ng/ml. Hydroxyprogesterone caproate (Depot proluton; Bayer Schering Pharma, Germany) was then injected. The serum β-hCG rose to 2,462 IU/ml, and serum P4 was 12.9 ng/ml on the 3rd weeks after embryo transferred. Transvaginal ultrasonography revealed the intrauterine gestational sac. The embryo length was 22.7 mm. with positive cardiac activity. Double screening for Down syndrome was performed on weeks 12th. The level of PAPP-A was 6,767 mU/L whilefree β-hCG was 68.13 ng/ml. Nuchal translucency was 1.2 mm on ultrasonography. Her screening result was negative, with 1:450 risk of Down syndrome at term.

Discussion

This couple experienced 2 unsuccessful IVF cycles with FISH-based preimplantation genetic

screening (PGS). In the 1st and 2nd cycle, 19 embryos were biopsied on cleavage stage. Eighteen of them were diagnosed as chromosomally normal with 5-probe FISH. The best 7 blastocysts (morphologic) were transferred for 3 cycles but failed to get pregnant. Two more blastocysts were biopsied and sent for aCGH test. Only one of them had normal chromosome and resulted in successful pregnancy.

Chromosomal aneuploidy is now well established as a major cause of repeated implantation failure and miscarriage in IVF[3]. Aneuploid embryos are associated with unsuccessful implantation. Euploid embryos had higher chance for successful outcomes[4]. FISH-based PGS is not possible to analyse more than 12 chromosomes in each biopsied cells. Aneuploidy of other chromosomes is also relatively common[5]. Data from randomized controlled trials does not show any improvement on the implantation rates after IVF-ICSI in women with recurrent implantation failure[6,7]. Furthermore, women with advanced maternal age and women with repeated implantation failure, FISH-based PGS may significantly decreased live birth rates after IVF[7] This may be due to the invasiveness of the biopsy or mosaicism of the embryos. Thus, FISH-based PGS is no longer recommended in routine clinical practice[8,9].

Comprehensive chromosome screening on human embryos can be done by aCGH technique. Abnormalities of all chromosomes, both numerically and structurally can be detected by aCGH while 60-70% of these abnormalities cannot be detected by FISH[10]. Since trophectoderm biopsy is performed for aCGH test, mosaicism of the blastocysts can be detected. Normal embryos from comprehensive chromosome screening should have a high possibility for implantation and pregnancy. This report showed that only 1 blastocyst with normal chromosome from aCGH-based PGS can result in successful pregnancy.

However, aCGH technique has some limitations. It cannot detect whole ploidy errors, such as haploid, triploid, tetraploid cells from the diploid embryos[11]. Fortunately, these abnormalities can be detected in zygotic stage as the abnormal number of 2 PN. Fiorentino et al, showed that only 22.5% of the trophectoderm biopsy (Day5/6 after fertilization) was balance and euploid. This resulted in fewer embryos left for transfer. However, in the pregnancy rate went up to 66.5%.[12].

Biopsy may not be able to performin up to 58%, and almost half of the biopsied cycles have no euploid embryos left for transfer[13]. Excellent cryopreservation method is necessary for subsequent frozen-thaw embryo transfer after blastocyst biopsy.

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ความสำเร็จในการตั้งครรภ์จากการฉีดอสุจิเข้าไปในซัยโตพลาสมของไข่ และการคัดกรองทางพันธุกรรมระยะก่อนการฝังตัวของตัวอ่อนด้วยวิธี *array Comparative Genomic Hybridization*

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รายงานผู้ป่วยหญิงอายุ 41 ปี ที่เคยล้มเหลวในการทำเด็กหลอดแก้วมาแล้ว 2 ครั้ง หลังจากได้รับการกระตุ้นตกไข่ และได้รับการฉีดอสุจิเข้าไปในซัยโตพลาสมของไข่ในระยะเมตาเฟส II ทั้งหมด 4 ใบ จากนั้นได้ทำการตัดเซลล์ของตัวอ่อนระยะบลาสโตซิสต์ 2 ตัว เพื่อตรวจโครโมโซมในวันที่ 5 พบว่าตัวหนึ่งปกติ จากการตรวจคัดกรองทางพันธุกรรมในระยะก่อนการฝังตัวของตัวอ่อนด้วยวิธี aCGH และการตั้งครรภ์ประสบความสำเร็จหลังจากการย้ายตัวอ่อนนี้กลับเข้าสู่โพรงมดลูก การตรวจคัดกรองทางพันธุกรรมระยะก่อนการฝังตัวด้วยวิธี aCGH อาจเป็นวิธีที่ช่วยเลือกตัวอ่อนที่มีโครโมโซมปกติก่อนการย้ายกลับเข้าสู่โพรงมดลูกในการทำเด็กหลอดแก้วปฏิสนธิภายนอกร่างกาย