Genetic analysis of recurrent parthenogenesis: A case report and literature review

YAN JIANG, GE SONG, JING-CHUAN YUAN, XU-HUI ZHANG and XIAO-HUA WU

The Center for Reproductive Medicine and Infertility, The Fourth Hospital of Shijiazhuang, Hebei Medical University, Shijiazhuang, Hebei 050011, P.R. China

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Abstract. The present study reported a case of bilateral salpingectomy for an ectopic pregnancy with recurrent parthenogenesis over two in vitro fertilization (IVF) cycles. The first IVF cycle resulted in short-time fertilization. Two cleaved embryos were present after removing the cumulus cells. In the second cycle, intracytoplasmic sperm injection (ICSI) was performed directly and two 6-cell embryos were discovered again prior to the injection. Embryo biopsy, genome amplification, copy number variation (CNV) and single nucleotide polymorphism (SNP) analysis were performed on the two 6-cell embryos of the second cycle. The results of the CNV analysis indicated a genotype of 39,XX,+1,+1,+1,+1,+6q,+6q,+6q,-7p(x1),-10(x1),-13(x0),-15(x0),-17(x1),-18(x1),-19(x1),-20(x1) and the SNP analysis reported that only those chromosomes with one copy had a signal pattern similar to that obtained for an uniparental disomy. Although repeated spontaneous parthenogenesis was observed, the other metaphase II oocytes were fertilized normally after ICSI and the patient became pregnant.

Introduction

It is common to obtain metaphase II (MII), MI or germinal vesicle (GV) oocytes in in vitro fertilization (IVF). Cases of spontaneous oocyte activation (parthenogenesis) in vivo have previously been reported (1-4). However, the mechanisms of the oocyte activation in natural or stimulated cycles have remained to be clarified. In the present study, a case of recurrent parthenogenesis was reported and it was attempted to find common characteristics through reviewing the literature.

Case report

Patient history. A 33-year-old female patient and her 32-year-old husband presented at The Center for Reproductive Medicine and Infertility, The Fourth Hospital of Shijiazhuang, Hebei Medical University in March 2021 due to infertility for a year. The patient had a regular menstrual cycle and remarried a year ago. The patient had delivered two full-term babies with her ex-husband in 2012 and 2014. In 2016, a laparoscopic bilateral salpingectomy had been performed for an ectopic pregnancy. Chromosomal analysis indicated a normal female chromosome complement (46,XX). The patient's husband's semen parameters were in the normal range and the chromosome karyotype was normal (46,XY).

Cycle 1 (IVF+rescue ICSI). In June 2021, a long program of fertility treatment due to infertility following salpingectomy was adopted using triptorelin, in association with follicle-stimulating hormone (FSH) and human menopausal gonadotropin (5). On the day of the trigger, the patient produced four follicles of 18 mm, two of 17 mm and one of 16 mm under ultrasound. Ovum pick up was scheduled after 36 h. This time, seven oocytes were obtained and short-time fertilization was performed. A total of four MII oocytes with only one polar body, one MI oocyte and two 6-cell embryos were retrieved. Consequently, rescue ICSI was performed and three out of four injected oocytes were fertilized normally, while one remained unfertilized. Embryo transfer was performed 72 h after oocyte retrieval, resulting in no pregnancy (Fig. 1).

Cycle 2 (ICSI). The patient underwent a second cycle after an interval of one month. An antagonist protocol commenced after the baseline scan and then hormonal treatment on day two (6). The patient was stimulated with urinary (u)FSH 225 IU. The antagonist was given from day seven. The trigger was administered on day 17 of stimulation with recombinant human chorionic gonadotropin, 0.25 µg subcutaneously. The patient had eight follicles of 18 mm and four follicles of 15-17 mm in
end on the day of the trigger under ultrasound. Egg retrieval was performed 36 h after the trigger. A total of twelve oocytes were recovered by ultrasound-guided transvaginal aspiration. After removing the cumulus cells, seven MII, two MI and one GV oocyte, as well as two 6-cell embryos were retrieved. A total of seven MII oocytes were injected and five 2PN were observed (D1); two good-quality 8CI (cell) embryos were formed and frozen (D3). Subsequently, two frozen embryos were thawed and transferred to the patient, resulting in a normal single pregnancy (Fig. 2).

Genetic analysis. Due to IVF, the two 6-cell embryos in the first cycle were not further analyzed. The two 6-cell embryos in the ICSI cycle were observed for three days and exhibited no changes from their previous state. The embryos had a normal morphology and certain blastomeres had distinct nuclei. A blastomere biopsy was then performed on them using a laser (Hamilton Thorne) (Figs. 3 and 4). The blastomere was aspirated from the embryo and released into the medium for amplification (YK001B; Yikon). The amplification results were detected by copy number variation (CNV) analysis and next-generation sequencing (NGS) (Illumina, Inc.) (7). The NGS data that were used for SNP chip (Illumina, Inc.) analysis indicated that one 6-cell embryo was not able to be obtained using the amplification concentrate (Fig. 5). The other 6-cell embryo SNPs reported those chromosomes with one copy had a signal pattern similar to that obtained for uniparental disomy (UPD) based on the B-allele frequency value combined with the LogR Ratio, which may be ascertained by the absence of heterozygous sites on certain chromosomes (Fig. 6). The CNV results indicated a genotype of 39,XX,+1,+1,+1,+6q,+6q,+6q,-7p(x1),-10(x1),-13(x0),-15(x0),-17(x1),-18(x1),-19(x1),-20(x1) (Fig. 7). The participant provided written informed consent and the ethics committee of The Fourth Hospital of Shijiazhuang (Shijiazhuang, China) approved the present study.

Literature review (search terms: Parthenogenesis in human case IVF in PubMed). Parthenogenesis and spontaneous oocyte activation in unfertilized oocytes has been reported previously in humans and cases are presented in Table I (1-4). For a 38-year-old female patient with a history of bilateral tubal obstruction, one pronucleus was retrieved prior to ICSI and it developed into an embryo 30 h post-retrieval. The genome electrophoresis assay of the individual blastomere indicated that only one of eight blastomeress contained the genome. The CNV results indicated a genotype of 48, XX,+17,+17. The NGS data were used for MultiSNPs analysis and the results indicated that the SNPs of the biopsied embryo...
were exclusively consistent with those of the maternal side (1) (Table I).

Combelles et al (2) reported a case with recurrent miscarriages and prolonged infertility in which spontaneous activation of oocytes with parthenogenetic evolution was the likely cause. Following the unexpected presence of cleaved embryos at the fertilization check at the first IVF attempt, oocytes and embryos were subsequently analyzed in an ICSI/preimplantation genetic diagnosis (PGD) case. Fluorescence in situ hybridization (FISH) revealed aneuploidy in all seven blastomeres analyzed, with all but two lacking Y chromosomes. Microarray SNP analysis indicated an exclusively maternal origin of all blastomeres analyzed, which was further confirmed by PCR (Table I).

A 29-year-old female had undergone left oophorectomy due to an ovarian teratoma. The patient had 13 MII oocytes
and one 4-cell embryo retrieved prior to injection in an ICSI cycle. The 4-cell (parthenote) embryo developed into a 7-cell embryo by the next day. FISH returned two signals for the X chromosome in each blastomere that was analyzed. Other oocytes were fertilized normally and three were transferred, resulting in a normal singleton pregnancy and the birth of a healthy baby (Table I) (3).

In another study, the patient was a G1P0 38-year-old female of African ethnicity who had experienced a previous ectopic pregnancy, which had been treated by salpingectomy in search of an IVF with donor sperm. One oocyte was retrieved, donated sperms were used for IVF and an extruded oocyte with one nucleus separate from the granulosa cell wall was described. At 40 h, the aspect was of parthenogenic oocytes in a three-cell cluster, one cell with one nucleus, the others with high granulation and no visible pro-nucleus (4) (Table I).

Through the literature review, it was discovered that parthenogenesis and spontaneous oocyte activation may occur in humans of different ethnicities/regions, such as Chinese, American, Brazilian and African ethnicity patients. All of these patients had a history of either recurrent miscarriages or bilateral tubal obstruction with or without ovarian/fallopian tube surgery. In certain cases, embryos appear as 1PN and cleave later and in others, they appear as four-to six-cell embryos directly.

**Discussion**

In the studies retrieved in the literature review, oocyte activation, parthenogenesis and UPD were mentioned. This raises the question of whether there are any differences and correlations between oocyte activation, parthenogenesis and UPD.

Oocyte activation is a series of events that converts an MII-arrested oocyte into a fertilized egg ready to begin embryogenesis. In most mammals, oocyte activation is a spatial-temporal regulated process triggered by sperm entry (8).

By activating mouse eggs with experimentally controlled and precisely defined calcium transients, Ducibella et al (9) demonstrated that each of the early events of mammalian oocyte activation is initiated by a different number of calcium transients, while their completion requires a greater number of calcium transients than for their initiation (8). A variety of artificial activating methods is used in human assisted reproduction treatment, including physical, mechanical or chemical stimuli, which provoke one or more calcium rises in the oocyte cytoplasm (8). However, spontaneous oocyte activation may occur under experimental conditions, providing genetic material for PGD diagnostics, as reported by Paffoni et al (10).

Parthenogenesis, a unique form of reproduction, is normally inhibited in mammals and a human embryo with parthenogenetic origin is not considered capable of producing offspring (1). It was reported that parthenogenetic activation took place around the time of fertilization of a sperm missing a sex chromosome, resulting in the generation of the upid(AC) mat 46,XX cell lineage by endoreplication of one blastomere containing a female pronucleus and the 45,X cell lineage by union of male and female pronuclei (9,11).

Artificial activation may also be used to obtain parthenogenetic mammalian embryos that may serve as a model to...
study biochemical and morphological events. Fresh and aged human oocytes may be activated parthenogenetically using a calcium ionophore. Ethanol was proven to be a poor activating agent. Human parthenotes may complete division to the eight cell stage (11,12). Parthenogenesis may also be induced by exposure of unfertilized oocytes to strontium-containing medium (13). Certain early human pregnancy losses may involve oocytes that have been parthenogenetically activated spontaneously (2,14).

UPD refers to the situation in which both homologues of a chromosomal region/segment have originated from only one parent. UPD and mosaic aneuploidy arise from mitotic or meiotic events (15). As a consequence of UPD, or deficiency of part of a chromosome, there are two types of developmental risk: Aberrant dosage of genes regulated by genomic imprinting and homozygosity of a recessive mutation (16). This may involve the entire chromosome or only a small segment. Uniparental disomy in the human blastocyst is exceedingly rare (17).

UPD almost always arises in connection with a numerical or structural chromosomal aberration. UPD cases in which the causative cytogenetic event is still present, even if only in the mosaic state, provide deep insight into the abilities of gametes or embryonic cells to repair chromosomal imbalances and/or rearrangements (18).

In conclusion, there are certain correlations between oocyte activation, parthenogenesis and UPD, but the following are the differences, which are well established: The incidence of UPD of any chromosome is estimated to account for ~1:3,500 live births. For certain chromosomes, UPD does not exert any adverse effect on any individual. However, for other chromosomes, it may result in abnormalities due to aberrant genomic imprinting (16). In humans, parthenogenesis always leads to teratoma and never to a viable individual (1).

In the present study, the first cycle used was IVF short-time fertilization. After the removal of the cumulus cells, two early cleavage embryos were discovered. ICSI was performed in the second cycle and prior to injection, two 6-cell embryos were discovered and tested. Although recurrent spontaneous parthenogenesis was observed, this did not influence the other MII oocyte fertilization and the achievement of pregnancy. It was hypothesized that the bilateral salpingectomy resulted in recurrent spontaneous oocyte activation. This is a similar case to that reported by Combelles et al (2), where the patient had two cycles of spontaneous oocyte activation and underwent left oophorectomy due to an ovarian teratoma.

In humans, parthenogenesis never results in a viable individual. In the present study, the parthenogenetic embryos were observed for three days and exhibited no changes. Although there were two parthenogenetic embryos, this did not influence the other oocyte fertilization and the resulting pregnancy. In the present case, the healthy baby that was born originated from the other normal oocyte.

The present results indicated that the first 6-cell embryo was not able to be observed in the amplification concentrate. The reason was probably that not every blastomere contains genomes. As with the previously reported case, only one of eight blastomeres contained genomes, indicating that the oocyte underwent cleavage without genome replication, or another probability was that what was assumed to be the 7 blastomeres were actually embryo fragments rather than blastomeres (1).

In the present study, the SNP and CNV results for the second 6-cell embryo revealed a genotype of 39,XX,+1,+1,+1,+1,+1,+6q,+6q,+6q,+7p(x1),-10(x1),-13(x0),-15(x0),-17(x1),-17(x1),-18(x1),-19(x1),-20(x1). Only those chromosomes with the presence of one copy exhibited a signal pattern similar to that of a UPD and other chromosomes rather appeared to have a trisomy or tetrasomy copy. The reason may be due to amplification concentration, or other unknown aspects. The mechanisms of the formation of the UPD included trisomy rescue, with and without concomitant trisomy, monosomy rescue and mitotic formation of a mosaic segmental UPD (15). UPD was also identified with one cell line having complete maternal UPD consistent with a parthenogenetic origin (14). Chromosomal segregation was analyzed, revealing a highly probable UPD as the reason for parent/child allele mismatches (19). This indicates that massively imbalanced embryos were identified, as new single-cell genomic methodologies have further pinpointed the existence of mixoploidy in cleavage-stage embryos (20).

Although repeated spontaneous parthenogenesis appeared in the patient of the present study, other oocytes were able to be fertilized normally and the patient became pregnant. The clinical value of the case report was that it demonstrated that pregnancy outcomes were not influenced by the fact that parthenogenesis was observed in part of the oocytes. As with the case reported by Oliveira et al (3), although there was a parthenogenetic origin of the ovarian teratoma, there were eight normally fertilized embryos, three of which were transferred, resulting in a normal single pregnancy and the birth of a healthy baby.

In conclusion, in the present study, a massively imbalanced parthenogenesis embryo was detected, which may occur in patients from various regions, who always have a history of either recurrent miscarriages or bilateral tubal obstruction with or without ovarian/fallopian tube surgery.

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Availability of data and materials
The sequencing data are available from a curated repository (https://submit.ncbi.nlm.nih.gov/sra/metadata_file/SUB11494870/processed-ok; Homo sapiens genome sequencing, SRR19261840, PRJNA838268). All other data are available from the corresponding author upon reasonable request.

Authors’ contributions
YJ conceived the study and wrote the manuscript. GS, JY, XZ and XW performed experiments and analyzed data. YC,
ZH and WH supervised the study. GS and XW confirm the authenticity of all raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Fourth Hospital of Shijiazhuang ethics committee approved this study (approval no. 20210081; July 06, 2021). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Patient consent for publication

The patient gave written informed consented to the publication of data and images.

Competing interests

The authors declare that they have no competing interests.

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