Live euploid birth and complete hydatid mole, followed by partial hydatid mole after ICSI

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Introduction

Hydatidiform mole, also known as molar pregnancy, occurs in approximately one in every 600–800 pregnancies [1–3] and is the result of a genetic abnormality that causes growth of atypical in uterohyperplastic trophoblasts and hydropic villi. After one, or two consecutive, complete hydatidiform moles (CMs), the risks of a subsequent CM are 1 in 100 or 1 in 4, respectively; a partial hydatidiform mole (PM) only slightly increases the risk of a subsequent molar pregnancy [4]. Molar pregnancies carry the risk of the development of gestational trophoblastic neoplasia (GTN), with approximately 10% of patients with CM and 1% of patients with PM requiring chemotherapy for GTN [2]. van der Ven et al. reported an 8–10% incidence of PM following assisted reproductive procedures [5], including intracytoplasmic sperm injection (ICSI). Here, we describe a rare case of a patient who experienced a live euploid birth and spontaneous CM, followed by PM after ICSI.

Case report

A 36-year-old, gravida 4, para 1 patient at our clinic was receiving in vitro fertilization (IVF) with ICSI. According to her medical records, she gave birth to a normal cytogenetic son in 2013, had CM in 2015, and experienced a spontaneous

abortion (cause unknown) in 2016. The IVF/ICSI-embryo transfer cycle was undertaken in 2017. For IVF/ICSI, controlled ovarian hyperstimulation was achieved using a combination of human menopausal gonadotrophin (hMG) and follicle stimulating hormone (FSH) with luteal phase gonadotrophin releasing hormone analogue (GnRHa). Ovarian response was monitored via vaginal sonography and estimation of serial serum estradiol concentrations. Optimal ovarian stimulation was obtained with a total of eight follicles of > 16 mm in diameter on cycle day 12, with the two leading follicles of 18 mm in diameter and serum estradiol concentration of 3868 pg/mL on the day of human chorionic gonadotropin (HCG) administration. Ovulation was induced with 10,000 IU human chorionic gonadotropin (HCG) and a total of 16 oocytes were retrieved transvaginally 35 h after HCG injection.

ICSI using a single spermatozoon was performed on three metaphase II oocytes. Two pronuclei (2PN) and two polar bodies were recognized in five embryos the following day. Four D4 embryos—two morulas, one 10-cell (grade 2) and one 11-cell (grade 3)—were transferred into the uterine cavity immediately following a laser-assisted hatching. At 6-week gestation, a clinical pregnancy with a fetal heartbeat (crown-rump length, 7.3 mm) was confirmed. At 12-week gestation, during a visit for chorionic villus sampling (CVS), ultrasound examination revealed a normal placenta with a simultaneously coexisting PM. The pregnancy was terminated at 14 weeks gestation because of the diagnosis of a PM. A gross image of the incomplete mole fetus was made at 14 weeks gestation, after termination (Fig. 1). All procedures were carried out after obtaining written informed consent from the parents.

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Genetic analysis

Standard chromosomal analysis was performed using the parents' blood leukocytes and molar placental tissue obtained through CVS. Fluorescence in situ hybridization (FISH)

Fig. 1 A gross image of the incomplete mole fetus was made at 14 weeks gestation, after termination

analysis was performed using probes specific for X and Y chromosomes on uncultured cells.

A DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) was used to isolate genomic DNA from the molar placental tissue and parents' peripheral blood leukocytes. Microsatellite analysis was conducted using GenePhile G-Plex PCR Amplification Kit (GenePhile Bioscience Co., Ltd., Taipei, Taiwan) (Table 1). Multiplex polymerase chain reaction for each sample was performed with 15 primer pairs (loci D3S1744, D4S2366, D8S1110, D10S2325, D12S1090, D13S765, D14S608, Penta E, D17S1294, D18S536, D18S1270, D20S470, D21S1437, Penta D and D22S683). Genotype was analyzed using GeneMapper ID software (Applied Biosystems) through comparison with the allelic ladder and reference DNA control samples 9947A (female; Applied Biosystems). The genotype of the molar villi tissue was compared with that of the parents to identify the origin of chromosomes in the molar tissue.

Flow cytometry analysis of spermatozoa DNA content

Fresh spermatozoa were fixed in 75% ethanol and preserved at -30°C before analysis. Fixed spermatozoa were washed twice with phosphate-buffered saline and stained with BD Pharmingen™ PI/RNase staining buffer (BD Pharmingen, San Diego, CA) following the manufacturer's instructions.

Spermatozoa were analyzed via BD FACSCalibur™ and DNA content was analyzed using CellQuest software.

Results and discussion

Histopathological review conducted at Taipei Medical University Hospital revealed typical PM features, including hydropic chorionic villi with focal trophoblastic hyperplasia, irregular scalloped outlines and trophoblastic inclusions. For all 15 informative short tandem repeats used in the first panel, chorionic villus tissue was found to be trisomic with three alleles of equal dosage, confirming triploidy (Table 1). Paternal contribution was dominant for each marker and a diandric triploid conceptus of PM was confirmed. Finally, fetal sex was determined as female as the chorionic villus tissue was shown to have DNA from all of the X chromosome.

Semen analysis of the father revealed oligozoospermia with a volume of 5.6 mL, concentration of 4 million/mL, and 10% motility of which 30% were progressively motile. Spermatozoa morphology was above the lower limit of the 2015 World Health Organization standards. Flow cytometry analysis revealed multiple populations including haploid and diploid spermatozoa (Fig. 2).

PM, which is generally caused by fertilization of one ovum by two spermatozoa, rarely occurs because the formation of the zona pellucida largely preventing two spermatozoa from entering the same ovum [6–8]. Theoretically, the ICSI procedure, in which a single, selected spermatozoon is inserted into an ovum, would be absolutely protective against the development of PM. Indeed, ICSI has been suggested as a way to avoid further molar pregnancies in women with a history of two or more partial molar pregnancies [9]. Lubna et al. proposed the use of ICSI as a therapeutic option to minimize the incidence of triploidy in future IVF cycles [10]. However, while dispermy can cause diandric triploid zygotes in normozoospermic males, oligo-crypto-azoospermic males produce diandric triploid zygotes mainly through the fertilization of normal oocyte by a diploid spermatozoon [11]. Further, the ICSI procedure may cause degeneration of the oocyte pronucleus or impair embryo development [12, 13]. Similarly, we may consider that PM could be due to delayed maturity of the oocyte nucleus, which was inactivated during the ICSI procedure, combined with a divided spermatozoon. Thus, even with ICSI, occasional cases of PM proven to be triploid conceptions have been reported [14, 15]. In addition, triploidy could be a consequence of fertilization by a diploid spermatozoon with nonreduction or endoreduplication of the spermatozoa DNA [11, 14–16]. Interestingly, diploid spermatozoa, which contain two sets of chromosomes, are more common in infertile males [11]. Although flow sorting has been reported to eliminate diploid spermatozoon [17] and the hyaluronan binding assay can exclude aneuploidy and diploidy spermatozoa

Table 1 Genotyping of partial molar pregnancy using markers for centromeric loci

Locus	Chromosome location	Patient	Partial molar pregnancy	Partner
D21S1437	21q21.1	9/16	9/15/15	14/15
D22S683	22q12.3	13/13	12/12/13	12/12
D8S1110	8q11.23	23/27	27/27/27	27/28
D10S2325	10p13	7/11	7/7/11	7/12
D12S1090	12q13.3	12.3/14	12/12/14	6/12
D17S1294	7q11.2	14/15	14/16/16	13/16
PentaD	21q22.3	9/10	9/9/10	9/12
D3S1744	3q24	17/17	17/17/17	14/17
D14S608	14q12	10/13	10/10/13	9/10
D20S470	20p12.1	12/15	15/18/18	10/18
PentaE	15q26.2	18/20	16/16/20	16/20
D4S2366	4p16.1	9/9	9/9/9	9/14
D18S536	18q12.1	10/12	12/12/12	10/12
D13S765	13q14.11	9/9	8/8/9	8/8
D6S474	6q21	14/15	14/15/15	15/15

populations [18], there is no suitable method for couples with male infertility. To date, there is no practical measure for selecting a single genetically normal spermatozoon for ICSI [19].

The risk of subsequent molar pregnancy increases with each molar pregnancy [1, 20–25]. Moreover, women are at increased risk of GTN while carrying a molar pregnancy. Rawlins et al. reported on the removal of the extra pronucleus

via microsurgical enucleation in an attempt to recover the zygote [26], but with a high frequency of mosaicism and loss of chromosomes [27]. Therefore, methods to prevent subsequent molar pregnancy are worth a comprehensive discussion. First, ICSI should be used to ensure that a single spermatozoon is used to fertilize a single mature oocyte, though the procedure does not guarantee avoidance of a molar pregnancy, as emphasized by the current case. If ICSI is unavailable, the

Fig. 2 Flow cytometry analysis of DNA content in sperms. Single cells were gated for analyzing the DNA content

morphology of oocytes and spermatozoa should be carefully inspected. Premature oocytes should be excluded because spermatozoa can easily penetrate the immature zona pellucida [5]. Second, assessment of fertilization should be conducted early (15–18 h post-insemination) since triploid and diploid embryos are less clearly distinguishable beyond 40 h post-insemination and because the male pronuclei are larger than the female pronucleus, which lies closer to the polar body, early after fertilization [26]. Third, preimplantation diagnosis can play an important role. Dual FISH can be used to simultaneously detect X- and Y-specific sequences, identifying triploidy during the early embryonic stage by revealing three sex chromosomes [10]. Fourth, early pelvic sonography may reveal the typical signs of a molar pregnancy. Finally, performing CVS or amniocentesis to identify the number of chromosomes through karyotyping is the last line of defense. In conclusion, we have described a patient with recurrent molar pregnancies who experienced CM and PM through natural conception and ICSI, respectively. We suggest steps for avoiding the recurrence of molar pregnancy.

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