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Complete hydatidiform mole and normal live birth following intracytoplasmic sperm injection

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Abstract A twin pregnancy with complete hydatidiform mole (HM) and preterm birth of a normal female infant after intracytoplasmic sperm injection (ICSI) conception was experienced. ICSI due to severe oligozoospermia was performed on three ova, and three embryos with confirmed two pronuclei (2PN) were subsequently transferred to the uterus. At 7 weeks of gestation, molar pregnancy as well as a viable fetus was recognized. At 33 weeks, the pregnancy was terminated due to preterm labor. Dichorionic pregnancy consisting of a normal fetus and placenta in one chorionic membrane and complete HM in the other was recognized. Cytomolecular analysis indicated that the complete HM genome was derived from duplication of a single sperm, and a normal neonate was from biparental genomes. It should be noted that ICSI can avoid incomplete HM (mostly triploid) due to multi-sperm

fertilization but might not be able to avoid complete HM (paternal diploid) although such a risk is very low. This is the second report of this condition and is accompanied by the first well-described molecular analysis.

Keywords Complete mole · ICSI · Normal fetus · Paternal diploid · Twin

Introduction

Hydatidiform mole (HM) with a coexisting fetus is a rare event, with a natural incidence of one in 22,000–100,000 pregnancies (Jones and Lauersen 1975; McDonald and Ruffolo 1983). Two conditions are possible: (1) a twin pregnancy with a complete HM and a normal fetus and placenta; (2) a fetus with a localized placental hydatidiform change (partial HM). Complete HM is diploid in nature, with the genetic material being totally derived from the paternal genome (Kajii and Ohama 1977). Partial HM is mostly triploid in nature, with a combination of diandric (two paternal) haploid sets and one maternal set (Jacobs et al. 1982). The abnormal triploid fetus coexisting with partial HM tends to die in the first trimester, while the fetus coexisting with complete HM does have a chance to survive, as has been previously described (Matsui et al. 2000).

Among recent assisted reproductive technologies (ART), intracytoplasmic sperm injection (ICSI) has the advantage of achieving the successful fertilization of retrieved oocytes regardless of severe male factors by using micromanipulation techniques, which also enable the direct evaluation of zygotes. In vitro fertilization (IVF) may increase partial HM due to multi-sperm fertilization (Pal et al. 1996), but ICSI may possibly avoid such abnormal conditions as only a single sperm enters an ovum.

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We encountered a twin pregnancy with complete HM and a preterm live birth following ICSI. In this article, we report our comprehensive genetic analysis of the products and discuss a possible mechanism by which this condition can occur.

Materials and methods

Case report

A 40-year-old G2P0 woman was referred to our hospital at 16 weeks of gestation (Fig. 1). She had received one cycle of ovarian stimulation and ICSI due to severe oligozoospermia in her husband and subsequent embryo-transfer at a private clinic. ICSI using a single spermatozoon had been performed in each of three metaphase II oocytes. Two pronuclei (2PN) as well as two polar bodies had been recognized in all three embryos the following day. All three embryos – a nine-cell (grade 1–2), a six-cell (grade 1–2), and a three-cell (grade 1–2) – were then transferred into the uterine cavity immediately after a laser-assisted-hatching method. At 6 weeks of gestation, a clinical pregnancy with a heart-beaten fetus (CRL: 7.3 mm) was confirmed. She began to have vaginal bleeding at 7 weeks, and a pregnancy with HM as well as a coexisting fetus was suspected by ultrasound examinations. The pregnancy was continued and the woman was hospitalized at 17 weeks of gestation because of a threatened abortion. At 33 weeks of gestation, her pregnancy was terminated for inevitable preterm labor. A 1544 g (–1.0 SD) female baby was delivered through cesarean section, followed by a total hysterectomy for the prevention of persistent

gestational trophoblastic tumor development as the risk of the latter has been reported to be much higher in patients with complete HM and coexisting fetus than in those with single complete HM (Matsui et al. 2000; Ruiz-Casares et al. 2001). The existence of one normal placenta together with the baby in one chorionic sac and apparent molar tissues in the other indicated dichorionic placenta. The molar pregnancy was diagnosed as complete HM macroscopically, and trophoblastic invasion of the myometrium or a malignant tumor of trophoblastic epithelium was not recognized histopathologically, which was consistent with complete HM. The neonate showed a normal growth without any congenital anomalies. To date, 3 months after the delivery, the mother shows no clinical signs of persistent gestational trophoblastic disease.

Genetic analysis

Standard chromosomal analysis was performed using blood leukocytes of the mother, father, and newborn, and tissues from a normal placenta (three different sites), umbilical cord, and molar tissues. When the chromosomal analysis was not successful, fluorescence in situ hybridization (FISH) analysis using the probes specific for the X and Y chromosomes on uncultured cells was performed. All procedures were carried out after obtaining the written informed consent of the parents.

A QuickGene DNA whole blood kit (Fujifilm, Tokyo, Japan) was used to isolate genomic DNA from peripheral blood leukocytes of the mother and her husband and from umbilical cord blood leukocytes, and DNeasy Tissue kits (Qiagen, Valencia, Calif.) were used to isolate genomic DNA from tissues of the umbilical cord, normal placenta at three different portions, and molar tissues. Microsatellite analysis was carried out using ABI Prism linkage mapping set ver. 2.5, MD10 (Applied Biosystems, Foster City, Calif.) (Table 1). A total of 14 probes that mapped close to the centromere at *D6S422*, *D7S493*, *D8S285*, *D9S161*, *D10S208*, *D11S987*, *D12S345*, *D16S503*, *D17S1868*, *D17S921*, *D18S53*, *D19S220*, *D20S196*, and *DXS990* were used for this study. The PCR analysis was carried out on 10 μ l aliquots of 10 ng genomic DNA, 0.5 μ M of each primer, 0.2 mM each dNTP, 10 $\times$ PCR buffer (Takara, Ohtsu, Japan), and 0.25 U of Takara Ex Taq DNA polymerase (Takara) under the following conditions: 40 cycles of 30 s at 94°C, 30 s at 55°C, 30 s, at 72°C. The PCR products were analyzed on an ABI 3100 Genetic Analyzer (Applied Biosystems), and their sizes were determined using the GENEMAPPER software ver 3.5 (Applied Biosystems).

Fig. 1 Ultrasound image at 16 weeks of gestation. The presence of a normal placenta (white arrow) as well as of a fetus and separated cystic parts suggesting molar tissues (black arrow) are noted

Results and discussion

The karyotypes of the blood leukocytes from the father, mother, and newborn, and from the molar tissues and

Table 1 Microsatellite analysis

Markers ^a	Genotype											
	Father		Mother		Mole		Neonate		Cord		Placenta	
D6S422	1	2	3	2	1	1	3	2	3	2	3	2
D7S493	1	2	1	3	2	2	1	2	1	2	1	2
D8S285	1	2	3	3	1	1	3	1	3	1	3	1
D9S161	1	2	1	1	2	2	1	1	1	1	1	1
D10S208	1	2	2	2	2	2	2	2	2	2	2	2
D11S987	1	2	1	1	2	2	1	1	1	1	1	1
D12S345	1	2	1	2	1	1	1	2	1	2	1	2
D16S503	1	1	1	2	1	1	1	2	1	2	1	2
D17S1868	1	1	1	1	1	1	1	1	1	1	1	1
D17S921	1	2	3	2	1	1	1	2	1	2	1	2
D18S53	1	2	3	1	2	2	3	2	3	2	3	2
D19S220	1	2	2	2	2	2	1	2	1	2	1	2
D20S196	1	2	3	2	2	2	1	2	1	2	1	2
DXS990	1		2	3	1	1	1	3	1	3	1	3

^aDifferent alleles in each marker are numbered arbitrarily. Paternal isodisomy was recognized at *D6S422*, *D7S493*, *D8S285*, *D9S161*, *D11S987*, *D17S921*, and *D18S53*

an umbilical cord tissue were 46,XY, 46,XX, 46,XX, 46,XX, and 46,XX, respectively. The chromosomal analysis of three sites of normal placental tissues was not successful due to bacterial contaminations. Subsequent FISH analysis of 50 uncultured cells of placental tissues using probes of the X and Y chromosomes only showed two signals of X chromosomes in all cells, indicating that the karyotype of the placenta was 46,XX (data not shown).

Molecular analysis showed that genomic DNA of the newborn, a cord tissue, and placental tissues showed a uniform pattern in which biparental genomes contributed in a Mendelian fashion. However, the molar tissue indicated paternal isodisomy at seven markers, *D6S422*, *D7S493*, *D8S285*, *D9S161*, *D11S987*, *D17S921*, and *D18S53*, suggesting that the molar tissues resulted from a doubling of a haploid paternal genome following monospermic fertilization of an inactivated oocyte. Indeed, most cases of complete HM have been described as being paternally uniparental 46,XX (homozygous constitution derived from the father), with only rare cases of the dispermic 46,XX or 46,XY conditions. In addition, the 46,YY constitution has never been described, probably because of early death of the conceptus (Reubinoff et al. 1997; Fisher et al. 2000). In this case, dispermic complete HM had been prevented by ICSI using a single spermatozoon. It should be noted that complete HM occurred irregardless of the presence of a morphologically normal embryo. There seems to be no way to differentiate HM from a normal embryo at the pre-transfer stage, although such a risk is very rare. Furthermore, the management of HM and a coexisting fetus is complicated. It is important to avoid a risk of a multiple pregnancy in ART by performing a single-embryo transfer.

In conclusion, we experienced a twin pregnancy with complete HM and a coexisting fetus following ICSI conception. To our best knowledge, this is the

second report of this condition following ICSI (Petignat et al. 2001), but the first comprehensive genetic analysis.

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References

- Fisher RA, Khatoon R, Paradinas FJ, Roberts AP, Newlands ES (2000) Repetitive complete hydatidiform mole can be biparental in origin and either male or female. *Hum Reprod* 15:594–598
- Jacobs PA, Szulman AE, Funkhouser J, Matsuura JS, Wilson CC (1982) Human triploidy: relationship between parental origin of the additional haploid complement and development of partial hydatidiform mole. *Ann Hum Genet* 46:223–231
- Jones WB, Lauersen NH (1975) Hydatidiform mole with coexistent fetus. *Am J Obstet Gynecol* 122:267–272
- Kajii T, Ohama K (1977) Androgenetic origin of hydatidiform mole. *Nature* 268:633–634
- Matsui H, Sekiya S, Hando T, Wake N, Tomoda Y (2000) Hydatidiform mole coexistent with a twin live fetus: a national collaborative study in Japan. *Hum Reprod* 15:608–611
- McDonald TW, Ruffolo EH (1983) Modern management of gestational trophoblastic disease. *Obstet Gynecol Surv* 38:67–83
- Pal L, Toth TL, Leykin L, Isaacson KB (1996) High incidence of triploidy in in-vitro fertilized oocytes from a patient with a previous history of recurrent gestational trophoblastic disease. *Hum Reprod* 11:1529–1532
- Petignat P, Senn A, Hohlfield P, Blant SA, Laurini R, Germond M (2001) Molar pregnancy with a coexistent fetus after intracytoplasmic sperm injection. A case report. *J Reprod Med* 46:270–274
- Reubinoff BE, Lewin A, Verner M, Safran A, Schenker JG, Abelliovich D (1997) Intracytoplasmic sperm injection combined with preimplantation genetic diagnosis for the prevention of recurrent gestational trophoblastic disease. *Hum Reprod* 12:805–808
- Ruiz-Casares E, Henriques-Gil N, Opera M, Fernandez-Pacheco RP, Aguaron A (2001) Molecular analysis of a gestation consisting of a complete hydatidiform mole and normal dizygotic twin. *J Reprod Med* 46:1041–1045