Hydatidiform moles associated with multiple gestations after assisted reproduction: diagnosis by analysis of DNA fingerprint

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In spite of the widespread use of assisted reproductive technology, there have been, to our knowledge, only two reported cases of molar pregnancies after gamete intra-Fallopian transfer and five reported cases after in-vitro fertilization and embryo transfer. We report here a case of a complete hydatidiform mole in a twin pregnancy after gamete intra-Fallopian transfer, as well as a case of a complete hydatidiform mole in a triplet pregnancy after in-vitro fertilization and embryo transfer. The genetic constitution of each conceptus was determined by examination of the restriction fragment length polymorphism of the DNA with four different single-locus probes. This analysis revealed that both hydatidiform moles were of androgenetic origin and probably of monospermic origin. Moreover, the analysis confirmed that the pregnancies were dizygotic and trizygotic pregnancies respectively. The diagnostic utility of the analysis of DNA polymorphism is discussed in cases of a molar pregnancy with coexisting fetuses.

Key words: assisted reproductive technology/coexistent fetus/hydatidiform mole/multiple pregnancy/restriction enzyme length polymorphism

Introduction

There are several possible types of molar placental tissue that can coexist with a living fetus: (i) a triploid partial hydatidiform mole in a singleton pregnancy; (ii) a complete hydatidiform mole (androgenetic, diploid) coexisting with a normal fetus in a multiple pregnancy; (iii) tissues with focal partial hydropic changes in the non-molar placenta of a chromosomally normal fetus in a singleton pregnancy; and (iv) a blighted ovum with diffuse hydropic changes coexisting with a normal fetus in a multiple pregnancy (Vejerslev, 1991; Osada et al., 1995). Types (i) and (iii) can also be found in one conceptus in cases of multiple gestation.

It is important to distinguish a complete hydatidiform mole from other types because a complete hydatidiform mole has a greater propensity to develop into persistent trophoblastic disease. This is especially true in multiple pregnancies because the incidence of gestational trophoblastic disease after a complete hydatidiform mole is three to four times higher with a multiple pregnancy than with a singleton pregnancy (Steller et al., 1994).

In the past, discrimination of a complete hydatidiform mole from a partial hydatidiform mole has been made mainly on the basis of histological and macroscopic observations, namely, absence of a fetus, absence of nucleated erythrocytes and absence of fetal membranes (Vassilakos et al., 1977). In a multiple pregnancy, however, such a morphological distinction between a complete mole and a partial mole is not applicable because a living fetus can be found in both (Vejerslev, 1991). Recently, genetic analysis of DNA polymorphism (DNA fingerprinting) has been used to make the diagnosis of hydatidiform mole (Fukuyama et al., 1991; Kovacs et al., 1991; Fisher et al., 1993; Hsu et al., 1993). This method provides accurate information on the genetic constitution of each conceptus and, thus, a complete mole (homozygotic, androgenetic and diploid) can be distinguished from a partial mole (heterozygotic and triploid).

We report here two cases of a complete hydatidiform mole with coexistent fetuses after assisted reproduction. In these cases, analysis of DNA polymorphism was useful for the diagnosis of moles that originated from one conceptus in a multiple pregnancy.

Case 1

A 31 year old Japanese woman with primary infertility, secondary to oligozoospermia (15×10^5 ml) for 4 years, underwent an initial course of gamete intra-Fallopian transfer (GIFT). Under pituitary suppression with buserelin acetate, 1050 IU of human menopausal gonadotrophin (HMG, Humegon®; Organon, Tokyo, Japan) and 10 000 IU of human chorionic gonadotrophin (HCG, HCG-Mochida®; Mochida, Tokyo, Japan) were administered. At 35 h after injection of HCG, five oocytes were retrieved transvaginally and transferred transabdominally into the Fallopian tubes together with 300 000 spermatozoa. Oral luteal support (dedroxyprogesterone, 30 mg/day) was continued for 16 days after the retrieval of oocytes.

Thirty-five days after the retrieval of oocytes, ultrasonography revealed an intrauterine gestational sac with a fetal heartbeat. Additionally, an irregular multicystic lesion (2×2×3 cm^3) was noted within the uterine cavity adjacent to...
the gestational sac, which was ascribed to hydropic changes of a blighted ovum. Follow-up ultrasound performed at 12 weeks and 3 days showed a normal-appearing 12.5-week fetus and no abnormal cystic lesion.

The pregnancy evolved uneventfully until 14 weeks and 4 days when vaginal bleeding occurred. Ultrasound examination at 15 weeks demonstrated a viable fetus and coexisting molar tissues that occupied 80% of the uterine cavity, and the urinary HCG concentration was as high as 2,000,000 IU/l. Because of the rapid increase in the volume of molar tissue and uncontrollable vaginal bleeding, the pregnancy was terminated electively at 16 weeks and 2 days. A karyotype of 46, XX was determined in both the molar tissue and the fetus. Urinary HCG decreased to a non-pregnant concentration 3 weeks after termination of the pregnancy and remained low during a 1 year follow-up.

To determine the genetic constitution of molar tissues, we examined the DNA polymorphism of molar tissues, fetal lymphocytes, placental tissues and the parents’ lymphocytes by restriction fragment length polymorphism (RFLP) analysis. Variable numbers of tandem repeats of DNA fragments were detected with four single-locus probes of high heterozygosity more than 96%: MS1, MS43a, MS31 and g3 (Wong et al., 1987; Jeffreys, 1990). These probes hybridize to specific sites on chromosomes 1p, 12q, 7p and 7q, respectively and, therefore, two bands of different mobility are usually generated by the fetal genome: one of maternal origin and one of paternal origin. Figure 1 shows the results of RFLP analysis and Table I summarizes the sizes of detected fragments. Fetal and placental samples yielded two bands with each probe: one corresponding to the maternal band and one corresponding to the paternal band. The 4.3 kb fragment detected with the MS1 probe in the fetal sample appeared to be 40 kb shorter than the corresponding fragment from the father. This difference might have been due to a spontaneous new mutation at this locus. By contrast, the molar tissue yielded only one band of paternal origin for each probe. This result confirmed the androgenetic homozygotic origin of the molar tissue. Moreover, the molar tissues had MS43a and MS31 alleles that differed from those of the fetus and the placenta. This result enabled us to exclude the possibility that the molar tissue and the fetus were the conceptus of the same sperm. In other words, the molar tissue and fetus were not identical twins. Thus, the pregnancy was diagnosed as a monospermic complete mole of one dizygotic twin.

### Table I. Summary of fragments detected by RFLP analysis in Case 1

<table>
<thead>
<tr>
<th>Probe (chromosome number)</th>
<th>MS1 (1p)</th>
<th>MS43a (12q)</th>
<th>MS31 (7p)</th>
<th>g3 (7q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>5.5/7.5</td>
<td>4.5/10.0</td>
<td>4.0/7.3</td>
<td>5.7/8.0</td>
</tr>
<tr>
<td>Fetus</td>
<td>5.5/4.3</td>
<td>4.5/15.0</td>
<td>4.0/7.1</td>
<td>8.0/5.7</td>
</tr>
<tr>
<td>Placenta</td>
<td>5.5/4.3</td>
<td>4.5/15.0</td>
<td>4.0/7.1</td>
<td>8.0/5.7</td>
</tr>
<tr>
<td>Mole</td>
<td>4.3</td>
<td>5.4</td>
<td>5.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Father</td>
<td>4.0/4.3</td>
<td>15.0/5.4</td>
<td>5.0/7.1</td>
<td>1.7/5.7</td>
</tr>
</tbody>
</table>

*Sizes of fragments (kb).

bThe band of 4.3 kb appeared to be approximately 40 bp smaller in the fetus and placenta than in the father and mole.

### Case 2

A 31 year old Japanese woman with unexplained primary infertility for 5 years underwent a third cycle of in-vitro fertilization (IVF) and embryo transfer. She had previously failed to become pregnant after a course of GIFT in which five oocytes had been transferred and after two courses of
**Table II. Summary of fragments detected by RFLP analysis in Case 2**

<table>
<thead>
<tr>
<th>Probe (chromosome number)</th>
<th>MS1 (1p)</th>
<th>MS43a (12q)</th>
<th>MS31 (7p)</th>
<th>g3 (7q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>1.5/4.9</td>
<td>5.4/6.9</td>
<td>6.5/8.6</td>
<td>6.0/6.1</td>
</tr>
<tr>
<td>Fetus</td>
<td>3.9/4.9</td>
<td>5.4/6.6</td>
<td>6.5/8.3</td>
<td>6.1/8.1</td>
</tr>
<tr>
<td>Placenta</td>
<td>1.5/3.9</td>
<td>5.4/6.6</td>
<td>3.8/8.6</td>
<td>4.4/0</td>
</tr>
<tr>
<td>Mole</td>
<td>3.9</td>
<td>4.6</td>
<td>8.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Father</td>
<td>3.9/5.8</td>
<td>4.5/4.6</td>
<td>3.8/8.3</td>
<td>4.4/8.1</td>
</tr>
</tbody>
</table>

*Sizes of fragments (kb).

IVF. During IVF, five out of six and four out of four oocytes that had been retrieved were fertilized, and five and two morphologically normal embryos were transferred to the uterus, respectively. In these previous attempts, 2550–3750 IU of HMG was required for successful retrieval of four oocytes or more, and this dose was almost double the average dose used at our institution.

In the third course of IVF, 3150 IU of HMG and 10 000 IU of HCG were administered. The patient’s serum oestradiol concentration was 1097 pg/ml at the time of injection of HCG. Eight oocytes were retrieved and inseminated with 300 000 spermatozoa 6 h later. Six oocytes had two pronuclei and two polar bodies 19 h after insemination. Five out of the six oocytes that had developed to the 4-cell stage were transferred into the endometrium via myometrial puncture 45 h after insemination. A luteal phase was achieved by daily injection of 50 mg of progesterone for 2 weeks after transfer. Four weeks after the transfer, sonographic examination confirmed the presence of two gestational sacs with fetal cardiac activity.

Vaginal bleeding occurred intermittently from about 12 weeks of gestation. Ultrasonographic examination at 12 weeks and 3 days revealed a hydatidiform mole and two living fetuses. At 15 weeks, the urinary HCG concentration was 6 400 000 IU/l. Due to increased vaginal bleeding, the pregnancy was terminated electively at 15 weeks and 4 days. The karyotype of the molar tissue and both fetuses was 46,XX. Evacuation of the uterus was repeated 1 week later. Urinary HCG concentrations decreased to 400 IU/l 3 weeks after termination and then rose to 8000 IU/l 4 weeks after termination. Ultrasonography with colour flow mapping and pelvic angiography revealed a hypervascular lesion (2×2×1.5 cm³) on the posterior uterine wall, while chest roentgenography was normal. Six courses of combined chemotherapy (125 mg of methotrexate and 0.5 mg of actinomycin D per course) was completed over 10 weeks. Serum HCG concentrations returned to non-pregnant concentrations over the next 3 weeks and never rose again during the 1 year follow-up period.

RFLP analysis was performed as in Case 1 and the results are summarized in Table II. Molar tissue yielded a single band of paternal origin with each probe. Furthermore, the combination of paternal alleles detected by the four probes differed between the molar tissue and the fetuses. Thus, the pregnancy was diagnosed as an androgenetic monospermic complete mole that originated from one trizygotic triplet.

**Discussion**

Androgenesis of a complete hydatidiform mole has been diagnosed by karyotyping (Kajii et al., 1977; Fisher et al., 1982), HLA typing (Yamashita et al., 1979) and analysis of enzyme polymorphism (Jacobs et al., 1980). Analysis of DNA polymorphisms for a variable number of tandem repeats in DNA (VNTR) has several advantages over the afore-mentioned methods: (i) it is simple and rapid; (ii) the amount of sample required is minimal and (iii) the method can be applied to frozen or formalin-fixed samples (Osada et al., 1995).

There are two methods for detecting VNTR: the RFLP method and the PCR method. In the PCR method, a DNA fragment containing the VNTR is amplified with specific primers and is visualized on an agarose gel after electrophoresis and staining with ethidium bromide. This method is very sensitive and has been used for the diagnosis of molar pregnancies (Fisher and Newlands, 1993). However, with amplification of DNA by PCR, there is a potential risk of misdiagnosis because of contamination of the sample by maternal DNA or by DNA from other fetuses that can be co-amplified sufficiently to be visualized. In the RFLP method, DNA fragments are subjected to Southern blotting analysis without amplification. Since DNA amplification is not required in the RFLP method, it can be more accurate than the PCR method. The RFLP method does, however, require a much larger sample and is much more time-consuming than the PCR method.

VNTR analysis using several single-locus probes also allows us to distinguish a dispermic (heterozygotic) complete hydatidiform mole from a monospermic (monozygotic) complete hydatidiform mole (Fisher and Newlands, 1993). The presence of two different alleles of paternal origin indicates dispermic androgenesis, while one allele of paternal origin is suggestive of a monospermic origin of the molar tissue but is not conclusive. Two spermatozoa involved in the formation of a dispermic complete mole might coincidentally have the same alleles for each single-locus probe. An increase in the number of single-locus probes or utilization of multi-locus probes would make the diagnosis more accurate. In the present cases, both complete hydatidiform moles had only one allele of paternal origin when we used four probes, three of which corresponded to sites on different chromosomes. Therefore, the probability of dispermic origin was estimated to be roughly 12.5% or lower. The discrimination of a dispermic mole from a monospermic mole is vital since a dispermic complete mole has a higher malignant potential (Fisher and Newlands, 1993).

The potential risk of molar pregnancy has been considered in assisted reproductive technology (Balakier et al., 1991). Handling of the oocyte, in particular cooling during follicular aspiration and laboratory manipulations, may cause disruption of the meiotic spindle and loss of maternal chromosomes. Degeneration of oocytes can also lead to fragmentation and loss of maternal chromosomes. Numerous disturbances in meiosis, leading to chromosomal abnormalities, have been found in unfertilized oocytes and abnormal zygotes after in-vitro fertilization. The incidence of molar pregnancy after assisted reproduction seems, however, to be rather lower than

The reason for this low incidence is unclear (Wiswedel, 1987; Trabetti et al., 1993).

There have been two reports of abnormal development of in-vitro fertilized eggs, which presumably developed into hydatidiform moles, prior to embryo transfer. Near-normal cleavage of unipronucleate eggs without syngamy was detected in a woman with four previous pregnancies that involved complete hydatidiform moles (Edwards et al., 1992). In addition, a high incidence of triploidy of in-vitro fertilized eggs was found in a woman with a history of recurrent gestational trophoblastic disease that included a complete mole (Pal et al., 1996). In these cases, abnormal development of embryos was identified prior to embryo transfer. Thus, IVF may reduce the potential risk of recurrent trophoblastic disease in these high-risk cases. This is, however, not always the case. In Case 2 presented here, the presence of two pronuclei and normal embryonal development were confirmed prior to embryo transfer. Three other reports of complete hydatidiform moles after IVF had also confirmed the presence of two pronuclei and normal embryonal development prior to embryo transfer (Ibrahim et al., 1989; Jinno et al., 1994; Cheng et al., 1995).

Therefore, an embryo that is destined to develop into a complete mole cannot always be identified by morphological assessment prior to embryo transfer.

We have reported the useful application of RFLP analysis for the diagnosis of androgenetic complete moles in multiple pregnancy. Discrimination of a monospermic complete mole from a dispermic complete mole is desirable since it allows minimization of the contraceptive period after a molar pregnancy. The duration of this period is problematic, especially in couples with a history of long-term infertility.

References


