Triple genetic identities for the complete hydatidiform mole, placenta and co-existing fetus after transfer of a single in vitro fertilized oocyte: Case report and possible mechanisms

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We found different genotypes for the complete hydatidiform mole (CHM), placenta and co-existing fetus derived from a single in vitro fertilized human oocyte by the analysis of short tandem repeat (STR) DNA markers. The molar tissue was found to be heterozygously androgenetic. The fetus and placenta contained identical maternal, but different paternal genomes. Two models were proposed to account for the identification of triple genetic identities in a single fertilized oocyte. In the first model, the oocyte was fertilized by a diploid sperm, resulting in diandric triploidy. Premature cytokinesis resulted in early splitting of a cytoplasmic fragment with one copy of the replicated sperm chromosome, which developed into a heterozygous CHM. The bipolar spindle in syngamy pulled the other copy of sperm chromosomes and replicated oocyte chromosomes to form two blastomeres, which develop into the fetus and placenta, respectively. In the second model, the oocyte was fertilized by two haploid sperms, followed by tripolar spindle formation. Whatever is the mechanism, this case provides direct evidence that CHM can be derived from an oocyte containing an intact maternal genome.

Keywords: complete hydatidiform mole; co-existing fetus; in vitro fertilization; post-zygotic diploidization of triploid

Introduction
The hydatidiform mole (HM) can be divided into two separate syndromes based on morphologic, genetic and clinical factors (Szulman, 1995). The complete hydatidiform mole (CHM) is a diploid androgenetic conceptus with generalized villous trophoblastic hyperplasia and hydatidiform villous swelling in the absence of an ascertainable embryo/fetus. The partial HM (PHM) is a diandric triploid conceptus with focal trophoblastic hyperplasia and focal hydatidiform villous swelling, and with a demonstrable embryo/fetus. In the Western hemisphere, CHMs occur in ~1 in every 1500 pregnancies. The incidence is higher in Latin America, Southeast Asia and the Middle East (Lindor et al., 1992).

In mammalian species, both paternal and maternal genomes are required for embryonic development. In the mouse, profuse placental trophoblastic proliferation was found in androgenetic embryos consisting only of the paternal genomes, and devoid of the maternal genomes (Surani et al., 1984). In humans, the analysis of polymorphic markers indicated that most CHM cases are homozygous, suggesting these cases arise from fertilization of an enucleated oocyte by a single haploid sperm which then duplicates (Jacobs et al., 1977; Lawler et al., 1982). Approximately 20–25% of CHM are heterozygous, suggesting these cases arise from fertilization of an enucleated oocyte by two haploid sperms or a diploid sperm (Ohama et al., 1981; Fisher et al., 1989; Kovaks et al., 1991). Post-zygotic diploidization of triploidy (PDT) has recently been proposed to explain the developmental mechanism of HM (Golubovsky, 2003), as convincing evidence of the loss of the maternal genome in the oocyte does not really exist. Herein, we report a case with different genotypes for the CHM, placenta and co-existing fetus derived from a single in vitro fertilized human oocyte. Our case provides direct evidence that CHM can be derived from an oocyte containing an intact maternal genome.

Case report
A 31-year-old Taiwanese woman underwent in vitro fertilization (IVF) treatment due to primary infertility for 8 years. Her body mass index was 34.3 kg/m². She had undertaken six cycles of intrauterine insemination treatment. Laparoscopic diathermy of the ovaries had been performed for polycystic ovarian
syndrome 1 year before the IVF treatment. Her menstrual cycle became regular after the operation. The husband’s semen showed a concentration of $10 \times 10^6$ per milliliter with moderate motility.

The IVF cycle was preceded with oral pills and GnRH-a nasal spray following our routine protocol (Hsu et al., 2005). The hormone profile on Day 3 of the IVF cycle revealed FSH 8.62 mIU/ml, LH 1.36 mIU/ml, estradiol 8.98 pg/ml and progesterone 0.34 ng/ml. Recombinant follicle-stimulating hormone 150 IU/day (Gonal-F, Serono, Switzerland) was given subcutaneously for 9 days. On the day of the human chorionic gonadotrophin (hCG) injection, the leading follicle size was 20 mm, followed by 14, 14, 12, 12 mm follicles. The oocyte retrieval and insemination procedures were performed according to the established method (Hsu and Hsu, 2006). Five oocytes were obtained and in vitro fertilization using washed sperm was performed. Only one 2 pronucleus (PN) stage pre-embryo was observed at 16 h after insemination. An advanced cleavage status of that embryo as 6-cells at 42 h and 12-cells at 66 h after insemination, both of best grading, was noted. The 12-cell embryo was transferred back to the uterus on Day 3 of insemination. The couple maintained abstinence during the entire IVF treatment serum β-hCG was 357 mIU/ml 12 days after embryo transfer (ET). The intrauterine gestational sac was visible 19 days after ET, and the fetal heartbeat was detectable 1 week later. A bulging portion of placenta with an echo-lucent vesicle pattern was noted beginning at the gestational age of 9 weeks (Supplementary Fig. S1), and progressing with the growth of the fetus. HM with a co-existing fetus was highly suspected. Intrauterine fetal demise occurred at the gestational age of 14 weeks, and the gestational products were delivered for investigations. A grossly normal female fetus was delivered with a body weight of 260 g. The placenta weighed 380 g with a normal gross appearance. The molar tissue was clearly separated from the normal placental tissue and there was no intermixed portion of normal placenta and HM (Supplementary Fig. S2). Histological examination of the placenta showed areas of morphologically normal second trimester chorionic villi. The molar tissue showed marked trophoblastic hyperplasia and villous hydrops with central cistern formation (Supplementary Fig. S3). The serum β-hCG level was regularly followed and became undetectable 6 months after termination of pregnancy.

**Genetic studies**

Informed consent for genetic investigations was obtained from the couple. The chromosomal constitutions of the umbilical cord, the gross vesicle of molar tissue and the normal-appearing placenta were analyzed using established methods (Hsu et al., 1993). DNA was extracted from the couples’ peripheral blood lymphocytes, molar tissue, umbilical cord and normal-appearing chorionic villi and analyzed using STR markers (AmpF/STR-Plus™ zygosity determination system, Applied Biosystems, Foster City, CA, USA). The profiler kit includes 15 STR markers on the autosomal chromosomes and DNA markers specific for each of the sex chromosomes (Amelogenin X and Y).

**Results**

The karyotype was 46,XX for the cord, the placenta and the molar tissue. The analysis of Amelogenin X/Y genes showed only Amelogenin X gene in the cord, placenta and molar tissue. The original data of all markers are shown in the Supplementary Table S1, whereas the results of STR assay after data transformation by the software is shown in Table I. Of 15 STR markers, six were heterozygously androgenetic and five were homozygously androgenetic in the molar tissue. The zygosity of molar tissues in the remaining four markers could not be determined because they were homozygous in the paternal genome (Fig. 1). The STR patterns in the cord and the placenta were consistent with biparental inheritance. Interestingly, they shared the same maternal alleles in all informative markers analyzed. However, different patterns for six paternal alleles between the cord and the placenta were noted. For example, the cord tissue inherited allele 32.2 and the placenta inherited allele 30 from paternal alleles 30/32.2 in locus D21S11 (Fig. 1, Table I). These six alleles happened to be the alleles which were heterozygous in the molar tissue (Fig. 2).

**Discussion**

In the present case, the karyotype is 46,XX in all three tissues, making it impossible to cytotogenetically distinguish their genetic components. By the analysis of STR markers, we found the presence of three different genotypes for three different tissues arising from a single in vitro fertilized oocyte. Identical maternal alleles from the umbilical cord and placenta in 11 informative markers confirmed the origin of a single oocyte. Normal tissues of umbilical cord and placenta showed a single maternal contribution but two distinct paternal contributions. They differed for all markers heterozygous in the CHM, corresponding to six different chromosomes and suggesting separate paternal contributions to these tissues. These findings infer that both paternal and maternal genome contributed to the placenta and fetus while the molar tissue contained both copies of paternal genome.

The fact that three different tissues with distinct genotypes arose from a single in vitro fertilized oocyte seems to favor a mechanism where the triploid zygote divides into three cells at the first cleavage. Two models could be used to explain the developmental mechanism for this case. In the first model, an oocyte was fertilized by a diploid ‘Xp1Xp2’ sperm (Fig. 3A) to form a diandric triploid zygote. As replication of the sperm chromosomes occurred between 5 and 8 h after sperm penetration (Bradford, 1982) which was followed by premature cytokinesis, a cytoplasmic fragment containing one copy of the replicated sperm chromosomes ‘Xp1Xp2’ may develop into heterozygous CHM (Edwards and Brody, 1995). The bipolar spindle in syngamy then pulled the other copy of sperm chromosomes (Xp1Xp2) and replicated oocyte chromosomes (XmXm) to form two blastomeres (Xp1Xm and Xp2Xm). These two blastomeres later develop into the fetus and placenta, respectively.

The second model is shown in Fig. 3B. In this scenario, a triploid zygote was formed by dispermic fertilization with 3 PN
Tripolar spindle formation occurred after the replication of the sperm and oocyte chromosomes and the zygote further cleaved into three separate 2n cells (Xp1Xp2, Xp1Xm and Xp2Xm), which formed the heterozygous CHM, the fetus and placenta, respectively (Edwards and Brody, 1995; Golubovsky, 2003). Although only a 2 PN zygote was observed at the standard time point in this case, 3 PN formation may still escape detection (Sharpe-Timms and Zimmer, 2000). Thus, the second scenario remains as one of the possible mechanisms.

Table I. Results of informative microsatellite markers, and their cytogenetic locations.

<table>
<thead>
<tr>
<th>STR Loci</th>
<th>Paternal</th>
<th>Maternal</th>
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<th>Mole</th>
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</table>
| Alleles are numbered arbitrarily.

Figure 1: STS analysis for DNA from each of the tissue samples and parental blood samples.

Samples are identified as follows: paternal blood (Pa), molar tissue (M), umbilical cord (C), placenta (P) and maternal blood (Ma). Of 15 STR markers, six were heterozygously androgenetic (upper column) and four were homozygously androgenetic (middle column) in the molar tissue. The remaining four markers were homozygous in the paternal genome and we could not tell whether they were heterozygously or homozygously androgenetic in the molar tissue (lower column). DNA markers from cord and normal placenta show biparental inheritance and share the same maternal alleles but different paternal alleles.

Figure 2: Pedigree of the case. The upper part shows alleles for sperm and oocytes numbered arbitrarily. The lower part of the figure shows derivation of three genetic identities in the cord placenta and mole tissues.
PDT has been proposed to explain the mechanism for the fetuses co-existing with CHM or PHM. In the literature, diploidization of triploid might have occurred during the first cleavage in either diplospermic or dispermic zygote to form two cell lines. The outcomes include: (i) derivatives of 2n/3n heteroploidy which may develop into PHM (3n) with a co-existing fetus (2n); (2) diploidy which may develop into a fetus (2n), CHM (2n) or a fetus with co-existing CHM and (3) 1n/2n heteroploidy with subsequent endomitosis of the 1n cells to form CHM (Golubovsky, 2003). This 2n/3n or 2n/1n segregation at first cleavage of the triploid zygotes has been widely used to explain the mechanism for the fetuses co-existing with CHM or PHM and recent reports on unusual cases with 2n/3n mosaic molar complexes (Ikeda et al., 1996; Zhang et al., 2000). In contrast to examples of fetuses co-existing with CHM or PHM in the previous reports, our case contained three different tissues with distinct genotypes and cannot easily be explained by PDT as put forward by Golubovsky in 2003, where triploids divide into two daughter cells with loss of at least one complete chromosome complement in at least one of the daughter cells. Should PDT play some roles in this case, it would have occurred simultaneously in both 3n daughter cells of the diandric triploid zygote which had undergone cytokinesis: one daughter cell developed into the CHM by exclusion of the complete maternal genome and the other developed into the fetus and placenta, respectively, by exclusion of each set of paternal genomes. This seems highly speculative.

Although early splitting of cytoplasmic fragment containing one set of paternal genome is only speculative, we still think that the first model might be the most plausible one considering 2 PN zygote was observed with subsequent development of three different 2n cell lines. Our second model describes a situation in which 3 PN and tripolar spindle forms following dispermic penetration, followed by even segregation of chromosomes. This would be a very rare exception considering the general rule of chaotic cell division and gross aneuploidy of embryonic development in this scenario (Golubovsky, 2003). In the present case, 6- and 12-cell pre-embryos were noted at the routine observation time for the 4- and 8-cell stages. The cleavage patterns of triploid zygotes were different with those of diploid zygotes. The diploid zygotes usually divide

![Figure 3](image_url)

**Figure 3:** Proposed mechanisms for the development of this case.

The genomic structures of the triploid zygote and its derivatives to three cell lines of heterozygous CHM, the fetus and the placenta are indicated, where Xm, maternal, and Xp1 and Xp2, two diverse paternal genomes. Circles inside the oocytes are PN; maternal genomes are represented in closed circles, whereas paternal genomes are represented in open circle. (A) The oocyte was fertilized by a diploid sperm followed by early splitting of a copy of replicated paternal genomes into cytoplasmic fragment before syngamy. The cytoplasmic fragment later developed into heterozygous CHM accompanied by a zygote with regular bipolar spindle in the first cleavage. (B) The oocyte was fertilized by two haploid sperms followed by tripolar spindle formation and appearance of a 3-cell embryo after the first cleavage division.
first into two and then four cells, whereas most triploid zygotes divide first into three, followed by six cells (Kola et al., 1987). Though pre-embryo morphology may be affected by intrinsic factors such as developmental and genetic errors, and/or extrinsic factors such as the stimulation protocol or suboptimal culture conditions (Sharpe-Timms and Zimmer, 2000), the accelerated cleavage pattern of our case could reflect the distinct developmental potential of the triploid zygote derived from diandric triploidy. For both the first and the second models mentioned above, the cleavage patterns would follow the triploid zygotes to be three cells followed by six cells. However, for zygotes undergoing PDT with heteroploidy, cleavage to two cells and followed by four cells would be expected in most conditions (Edwards and Brody, 1995; Golubovsky, 2003).

Confined placental mosaicism with diffuse change of CHM in placenta co-existing with a phenotypically normal live birth in a natural conception has been reported as an infrequent event (Makrydimas et al., 2002). In their case, microsatellite analyses showed that the fetus, the placenta and the molar tissues were derived from a single fertilized conception. The fetus and the placenta had the same genetic composition and the mole was homozygously androgenetic. The differences between their case and our case are quite distinct. In our case, the fetus and the placenta had different genetic components, and the molar tissue was heterozygously androgenetic. PDT might have occurred in the early post-fertilization period in both cases, although in our case, the possibility of PDT seems far remote. It was evident that only one oocyte was conceived and a single embryo was transferred in our case. However, there was no solid evidence to show that a single oocyte was conceived for Makrydimas’s case.

Recent studies indicated at least 8.3% of diandric triploids originated from diploid sperm (Zaragoza et al., 2000). In oligospermic men, most triploid embryos develop from diplopermy, whereas in a normozoospermic male, triploid embryos most likely develop from fertilization of two sperms (Macas et al., 2001; Egozcue et al., 2002). A study on the chromosomal constitution of paternal PN in zygotes obtained by ICSI indicated that 33.3% of the abnormal male PN were diploid (Macas et al., 2001). In cases of dispermic fertilization of the oocyte, tripolar spindles derived from two pairs of active centrioles in a single fertilized oocyte will inevitably lead to chaotic chromosomal distributions and gross aneuploidy (Kola et al., 1987; Golubovsky, 2003). In diplopermy fertilization, as in the first model raised, relatively regular synangy and zygotic division regulated/orchestrated by a single paternal centrosome could be expected. This phenomenon may account for the observation that paternal genomes were precisely segregated into two different cell lineages, the fetus and the placenta.

To our knowledge, triple genetic identities resulting from a single fertilized oocyte have not been identified before. Occurrence of dual or triple identities after IVF or in natural conception could be more prevalent than suspected, as many of those pregnancies might have been missed. The present case provides evidence for the following hypotheses which have been proposed previously: (i) CHM could be derived from an oocyte containing the intact maternal genome instead of an ‘enucleated oocyte’ and (ii) PDT fertilization. This case also provides evidence to support the hypothesis that CHM and PHM may result from same mechanism, i.e. the embryogenesis of a diandric triploid zygote with or without undergoing the process of PDT.

**Supplementary Data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

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