CASE REPORT

High incidence of triploidy in in-vitro fertilized oocytes from a patient with a previous history of recurrent gestational trophoblastic disease

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The patient described has a history of recurrent gestational trophoblastic disease following spontaneous conception. She subsequently underwent two cycles of in-vitro fertilization (IVF) for management of infertility related to tubal obstruction. IVF of the oocytes retrieved showed a significantly high incidence of abnormal fertilization resulting in the development of triploid embryos. This report explores the possible association of an oocyte defect predisposing to abnormal fertilization, resulting in a high incidence of triploid embryos. Since the development of partial hydatidiform moles is related to the origin of triploidy, this phenomenon is suggested to explain the occurrence of recurrent trophoblastic disease in this patient. We propose the use of intracytoplasmic sperm injection (ICSI) as a therapeutic option to minimize the incidence of triploidy in future IVF cycles; donor oocyte IVF would be another alternative.

Key words: fertilization/IVF would be another alternative.

Introduction

The development of partial moles is found to be strongly associated with the mechanism of origin of triploidy (Jacobs et al., 1982). Patients with hydatidiform mole, either complete or partial, are at an increased risk of developing either type of molar pregnancy in subsequent conceptions (Federschneider et al., 1980; Berkowitz et al., 1994). This increased risk persists despite a change in partners (Berkowitz et al., 1994), indicating an oocyte related problem. After two molar pregnancies, the risk of a further episode is 20% (Berkowitz et al., 1994). In the case presented, a significantly high incidence of triploid embryos was noted to result from in-vitro fertilization (IVF) of oocytes from a patient with a prior history of recurrent gestational trophoblastic disease (GTD). This association raises the question as to whether there is an intrinsic defect in these eggs in the form of altered integrity of the zona pellucida, predisposing to polyspermy, or alternatively whether these oocytes might be predisposed to defective meiosis with retention of polar body, triploidy then being the result of digyny rather than dispermy. This, to our knowledge, is the first report suggesting an association between an oocyte defect predisposing to development of triploid embryos following IVF, and a tendency to recurrent GTD.

Case report

The patient was a 30 year old gravida 3, para 1 woman undergoing IVF for tubal infertility. Her past history was remarkable for recurrent GTD, occurring twice over a period of 2 years, following spontaneous conceptions. At the time of diagnosis of the first episode of GTD in January 1988, the extent of disease was classified as stage 3 with pulmonary metastases. Histopathology of the evacuated uterine contents confirmed tissue consistent with the diagnosis of a complete mole; the tissue was not subjected to cytogenetic assessment. The patient was treated with three cycles of actinomycin-D with resolution. Serial estimation of serum concentrations of β-human chorionic gonadotrophin (β-HCG) was negative over the next 24 months. The patient presented in October of 1990 with a history of spotting per vaginum; last menstrual period was 4 weeks prior to presentation and the patient was having unprotected intercourse. The urine pregnancy test was positive. The initial serum β-HCG concentration was 11 000 mIU/ml. A transvaginal ultrasound scan performed showed an enlarged uterus with a 1 cm hyperechoic mass in the right cornual region, no clearly discernable sac or fetal pole was identified. Colour flow Doppler studies performed showed increased vascularity in the right cornual region. The patient subsequently underwent a diagnostic laparoscopy and dilatation and curettage; minimal tissue was obtained on curettage, which on histopathology showed decidual changes with no evidence of trophoblastic tissue. Laparoscopic findings were essentially negative. A repeat pelvic scan 2 days following the initial one showed evidence of enlargement of the cornual lesion and serial estimation of serum β-HCG showed progressive escalation to 17 570 mIU/ml 24 h after presentation. The clinical impression was of a cornual pregnancy with molar changes and she received six pulses of triple therapy with methotrexate, BP-16 and actinomycin-D, which resulted in a complete recovery. Repeated measurements of serum β-HCG were negative over a period of 24 months. During an infertility work-up following 1 year of unprotected intercourse with a new partner, there was evidence of bilateral proximal tubal occlusion on hysterosalpingography. This was subsequently confirmed on an attempted hysteroscopic tubal cannalization. She was therefore considered a candidate for IVF. The partner’s semen analysis showed normal criteria.
The clinically recognizable pregnancies (Jacobs et al., 1982). Incidence of triploidy has been quoted as occurring in 1% of cases (Balakier et al., 1993).

The proposed mechanisms for the extra set of haploid chromosomes include (i) dispermy, (ii) failure of first or second paternal meiotic divisions and (iii) failure of first or second maternal meiotic divisions. Dispermy is by far the commonest mechanism (Jacobs et al., 1978, 1982). Association between triploidy and the underlying cytogenetic defect in partial moles is well documented and appears to result from diandry rather than digyny; Jacobs et al. (1982) in a study of 106 triploid abortuses found a strong association between mechanism of origin of triploidy and development of partial mole; all 54 of the paternally derived triploids on cytogenetic assessment showed histological evidence of molar changes whereas only three of the 15 informative maternally derived triploids were molar. In contrast, the complete moles are always diploid and of homozygous androgenetic origin (Lawler et al., 1982).

Berkowitz et al. (1994) in a study of subsequent pregnancy experience in 24 patients with GTD have clearly shown a higher propensity for a patient with one episode of GTD (complete or partial mole) to develop molar disease of either type in an ensuing pregnancy; five of these patients with a history of a complete mole developed a partial mole in a subsequent conception.

Edwards et al. (1990) have described an attempt to avoid a molar pregnancy following IVF by selecting embryos for transfer which are presumed to be growing normally in vitro in a patient with a history of four successive molar pregnancies; they found a higher incidence of single pronuclear embryos following IVF and postulated a defective oocyte meiosis with complete exclusion of the second meiotic spindle, followed by duplication of androgenic chromosomes as a mechanism most likely resulting in a complete mole; no cytogenetic evidence is available to support their theory. Interestingly, the incidence of triploid embryos in their study was 14%, higher than expected in IVF programmes. A case of recurrent GTD following IVF and embryo transfer was described earlier (Tanos et al., 1994), though the validity of the diagnosis in the absence of histological evidence is questionable. Other than the single case report of recurrent GTD following IVF–embryo transfer (Tanos et al., 1994), there is mention of three incidences of coexistent molar pregnancy and a fetus (Zella et al., 1984; Jinno Masao et al., 1994; Cheng et al., 1995). There appears to be no evidence of an increased rate of GTD following assisted reproduction or ovulation induction (Tanos et al., 1994). The reported incidence of triploidy in programmes of assisted reproduction ranges from 8–10% (Wentz and Repp, 1983; Plachot, 1985; Vanderven et al., 1985).

There seems to be a crucial relationship between the incidence of triploidy and degree of maturity of oocytes (Vanderven et al., 1985), with a higher incidence with immature oocytes. This may reflect an immaturity of the zona pellucida/cytoplasmic granules/vitelline membrane apparatus which, when intact, ensures penetration of the zona by no more than one spermatozoa. Fertilization and/or post-maturity, however, may be as commonly associated with polyspermy as immaturity, secondary

### Table 1. Incidence of abnormal fertilizations in the first and second cycles of IVF

<table>
<thead>
<tr>
<th>IVF cycle</th>
<th>Total oocytes</th>
<th>Oocyte maturation</th>
<th>Fertilization</th>
<th>Abnormal fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16</td>
<td>MII = 5/16</td>
<td>14/16</td>
<td>Triploid = 3/14 = 21%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MI = 10/16</td>
<td></td>
<td>2/3 of triploid of MII origin,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PI = 1/16</td>
<td></td>
<td>1/3 of triploid of MII origin,</td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>MII = 3/17</td>
<td>17/17</td>
<td>Tetraploid = 8/17 = 47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MI = 11/17</td>
<td></td>
<td>6/8 of triploid of MII origin,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PI = 3/17</td>
<td></td>
<td>2/8 of triploid of MII origin,</td>
</tr>
</tbody>
</table>

M = metaphase, P = prophase.

The first IVF–embryo transfer cycle was undertaken in March 1995. Ovarian stimulation 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 using vaginal ultrasound and estimation of serial serum oestradiol concentrations. Optimal ovarian stimulation was obtained with a total of eight follicles >16 mm in diameter on cycle day 12, with the two leading follicles of 18 mm diameter and serum oestradiol concentration of 3868 pg/ml on the day of HCG administration. Ovulation was induced with 10 000 IU HCG and a total of 16 oocytes were retrieved transvaginally 35 h after HCG injection. Inseminating concentration was 300 000 spermatozoa per oocyte (the standard number for normal semen parameters in our laboratory) after incubation; the length of incubation varied with the degree of oocyte maturation and was 2–3 h for metaphase 2, 6 h for metaphase I and 24 h for prophase I oocytes. Fertilization was assessed at 18 h post-insemination when 14/16 (87%) of the oocytes showed evidence of fertilization with 3/14 showing triploidy, an incidence of 21% (Table I). A total of four embryos were transferred at 48 h post-insemination, all of diploid lineage and subsequent failure was documented by a negative serum β-HCG 14 days post-transfer.

The patient underwent a second cycle of IVF–embryo transfer in June 1995 using a similar stimulation protocol to the first cycle. Ovulation was induced by 10 000 IU HCG on cycle day 11, when a total of five follicles of >16 mm were seen on vaginal scan with the two leading follicles of 18 mm diameter; serum oestradiol level on the day of HCG injection was 3860 pg/ml. A total of 17 oocytes was retrieved transvaginally 35.5 h post-HCG administration. Inseminating concentration of spermatozoa was 300 000 per oocyte; 100% fertilization was achieved. The incidence of triploidy was 47% with 8/17 showing evidence of three pronuclei at 18 h post-insemination (Table I). Five embryos were transferred with failed outcome.

### Discussion

Failure of the block to polyspermy with resultant dispermic fertilization is the mechanism responsible for most human triploid embryos (Jacobs et al., 1978; Balakier et al., 1993). Incidence of triploidy has been quoted as occurring in 1% of the clinically recognizable pregnancies (Jacobs et al., 1982).
that the incidence of polyspermy is significantly increased when more than 1–1.5 × 10^6 spermatozoa are used per oocyte (Van Der Ven et al., 1985). Conversely, there appears to be an inverse relationship between the quality of spermatozoa and occurrence of polyspermy (Blerkom et al., 1984); it is postulated that delayed fertilization resulting from poor quality spermatozoa with low fertilizing ability allows ageing of the oocytes and consequently a decrease in the efficiency of the zona reaction. There has been evidence for maternal predisposition to chromosomal aneuploidy in multiple oocytes of some patients in IVF programmes (Zenzes and Casper, 1992) indicating a maternally derived disturbance in chromosomal behaviour during meiosis. This group of patients may be at risk from recurrent failure in IVF procedures. Fractures of zona pellucida caused by oocyte handling have also been put forward as a proposed mechanism contributing to polyspermy (Webster et al., 1985).

Triploid embryos rarely survive embryogenesis (Rawlins et al., 1988). The typical presentation is of spontaneous first trimester abortion, although triploid fetuses have been known to develop to term and survive up to 2 months (Rawlins et al., 1988). The potential for pathological development, i.e. of a hydatidiform mole, also dictates against uterine transfer of polyplid eggs. Early assessment of fertilization at 15–18 h post-insemination is of vital importance for detection of pronuclear stage because by >40 h after insemination, the differences between triploid and diploid embryos may not remain apparent and normal cleavage stages might be observed (Rawlins et al., 1988).

The case presented here is remarkable for a significant history of GTD complicating natural conception on two consecutive occasions. Current attempts to achieve pregnancy in this case, through assisted reproduction technique with a new partner, gave in-vitro evidence of a higher than expected number of triploid embryos. The rate of polyplidy at our laboratory has been an average of 12.5%. We have no data available on the cytogenetic composition of these triploid embryos, nor can a valid comment be made regarding the number of polar bodies per embryo. Assessment of the embryos was performed using a light microscope and ×200 magnification; ×400 magnification using an Axiovert 135, inverted microscope, was utilized in uncertain situations. There was no obvious evidence of markers for a high risk for such an outcome based on the above discussion. Thus, the timing of HCG administration as assessed by the size of dominant follicles and serum oestradiol concentrations was appropriate, the interval between HCG and oocyte retrieval was in accordance with current concepts, the oocytes were of metaphase II or I maturity and the spermatozoa employed were of adequate morphology and physiology and in acceptable numbers. The decision not to decrease the concentration of inseminating spermatozoa per oocyte in the second IVF cycle following documentation of a higher incidence of triploidy in the first attempt was based on the evidence that the incidence of polyspermy is significantly increased only if the inseminating concentration of spermatozoa is >800,000 to 1 × 10^6 spermatozoa, as shown by Van Der Ven et al. (1985).

In view of the recurrent phenomenon of a high incidence of triploidy and a previous history of GTD from a different partner, our hypothesis in the light of available data is that there might be a primary defect in the oocytes predisposing them to polyspermic penetration, dispermy thus contributing to triploid embryos. Alternatively there might be a defective mechanism of meiosis with retention of polar body, thus resulting in digyny. The origin of the extra haploid chromosomal component may be determined by subjecting the embryos to detailed morphological analysis and cytogeneric assessment, neither of which is, unfortunately, available in our case. Wenz et al., (1983) observed at the ultrastructural level the presence of a sperm tail associated with each male pronucleus in a triploid egg at 15–18 h post-insemination, thus documenting a paternal origin of the extra haploid set of chromosomes. Male pronuclei furthermore tend to be larger, whereas the female pronucleus is smaller and lies closer to the polar body (Blerkom et al., 1984; Rawlins and Binor, 1988). Triplo-nucleate zygotes with a single polar body could be digynic, though the possibility of degeneration of the first polar body exists (Balakier, 1993). Premplantation diagnosis with simultaneous detection of X- and Y-specific sequences, using dual fluorescent in-situ hybridization (FISH) (Delhanty et al., 1993), can identify triploidy successfully at the embryonic stage by demonstrating three sex chromosomes. Microsurgical enucleation of triplo-nuclear human zygotes has been described as an attempt to salvage them (Rawlins and Binor, 1988; Palermo et al., 1994). Human monospermic, digynic embryos usually become diploid after the removal of a single pronucleus indicating that the mitotic spindle is bipolar (Palermo et al., 1994), unlike dispermic embryos where abnormal mitotic spindle organization at syngamy results in a high frequency of mosaicism. This abnormality persists in the cell progeny even after removal of the extra pronucleus, and even though the number of chromosomes is reduced by one third (Palermo et al., 1994).

If the hypothesis of polyspermic origin of triploidy is correct, subjecting the oocytes retrieved from a future stimulated cycle to intracytoplasmic sperm injection (ICSI), thus ensuring delivery of a haploid number of paternal chromosomes, would result in a significant reduction in the incidence of triploidy of paternal origin. If successful, ICSI then might benefit this category of patients whose eggs have a propensity for developing polyplid embryos as well as those with a history of recurrent trophoblastic disease. Alternatively if digynic origin of GTD is considered, ovum donation might be a therapeutic modality available for such cases.

References

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