Intracytoplasmic sperm injection combined with preimplantation genetic diagnosis for the prevention of recurrent gestational trophoblastic disease

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Introduction

Patients who have experienced an episode of gestational trophoblastic disease (GTD) are at an increased risk of developing a molar pregnancy in subsequent conceptions. After one molar pregnancy, the incidence of repeated GTD in a future gestation is ~1% (Berkowitz et al., 1994). After two trophoblastic disease episodes, the risk rises to 20–28% (Sand et al., 1984; Berkowitz et al., 1994). Furthermore, the risk of persistent GTD is higher among patients with recurrent molar conceptions, and increases with subsequent events (Parazzini et al., 1988; Berkowitz et al., 1994). Nevertheless, after two or more episodes of GTD, a delivery of a viable infant may still be anticipated in 44–66% of future conceptions (Lurain et al., 1982; Sand et al., 1984; Berkowitz et al., 1994; Yapor et al., 1994).

Current knowledge regarding the genetic composition and pathogenesis of molar pregnancies may aid in the prevention of an additional event in patients with repeated GTD. Genetic studies have established that complete moles are most often diploid and androgenic having two paternal chromosome sets (Kajii and Ohama, 1977). The majority are genetically homozygous and seem to arise from the impregnation of an inactive oocyte by a haploid X-bearing spermatozoon, which subsequently duplicates its own chromosomes to provide a diploid 46,XX complement. Alternatively, 16% of complete moles originate from a dispermic fertilization, and are therefore diploid (46,XX or 46,XY) heterozygous (Wake et al., 1987; Lawler et al., 1991). The karyotype of partial mole is typically triploid (69,XXX, 69,XXY, or 69,XYY) with usually one maternal and two paternal haploid complements (Berkowitz et al., 1994), arising mostly from dispermic fertilization of a haploid ovum (Lawler et al., 1991).

The prevention of recurrent molar pregnancy was initially attempted by Edwards and his colleagues based on detailed morphological analysis of in-vitro fertilization (IVF) and the selection for transfer of embryos presumed to be growing a molar pregnancy in subsequent conceptions. After one further IVF cycle was attempted at the patient’s request. Five embryos were transferred. Monospermic fertilization was confirmed in four. After transfer, persistently high β-human chorionic gonadotrophin (HCG) concentrations which did not resolve following endometrial curettage and were successfully treated with methotrexate.

After a follow-up of 18 months with repeated measurements of negative serum β-HCG concentrations, a second IVF/embryo transfer cycle was attempted at the patient’s request. Five embryos were transferred. Monospermic fertilization was confirmed in four. After transfer, persistently high β-HCG concentrations were measured again; this time, the persistent β-HCG levels did not respond to methotrexate chemotherapy. The sustained β-HCG concentrations dropped to <10 mIU/ml only after combined chemotherapy with etoposide, methotrexate, actinomycin D, cyclophosphamide and oncovine. The course of these persistent GTD episodes was previously reported in detail (Tanos et al., 1994).

Materials and methods

Patient details

A 32 year old nullipara was admitted to our IVF unit 8 years ago due to bilateral tubal occlusion. The husband was 34 years old with normal semen indices. In her first IVF/embryo transfer cycle, a single embryo which was presumed to result from monospermic fertilization, as confirmed by the observation of two pronuclei, was transferred. Subsequently, the patient developed persistently high serum β-HCG concentrations which did not resolve or more episodes of GTD, a delivery of a viable infant may still be anticipated in 44–66% of future conceptions (Lurain et al., 1982; Sand et al., 1984; Berkowitz et al., 1994; Yapor et al., 1994).

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The couple approached our IVF unit for further treatment after a follow-up of 24 months with repeated measurements of negative serum β-HCG. They were counselled regarding the high risk of a repeated episode of GTD which has an increased probability of being persistent. They were determined to proceed with IVF treatments and take this risk. Since an abnormal regulation of polar body and pronucleus formation in the ovum have been implicated as the fundamental cause of the abnormal fertilization leading to molar pregnancies (Edwards et al., 1990), oocyte donation was suggested as an alternative that may reduce the risk. The couple rejected this approach due to religious restraints. The combination of ICSI and preimplantation genetic diagnosis (PGD) was then offered to prevent an additional event of molar pregnancy. They accepted this approach, being fully aware that it may prevent complete or partial mole but of each of the directly-labelled CEP probes (40–50 ng/µl) fluorescent haptens (Vysis, Naperville, IL, USA) were used. The hybridization mixture included 1 µl of each of the directly-labelled CEP probes (40–50 ng/µl) and 7 µl of hybridization mix II solution (Vysis). It was applied to the slide under a 22×22 mm coverslip and the slide was placed on a slide warmer at 80°C for 3 min. After sealing with rubber cement, hybridization was performed at 37°C in a dark moist chamber for 45 min. Post-hybridization washings included: 5 min in 50% formamide, 2× sodium citrate/sodium chloride (SSC, pH 7.6), at 42°C; 5 min in 2× SSC, pH 7.0, at 42°C; 5 min in 2× SSC, NP-40 0.1%, pH 7, at 42°C and PBD (Na2HPO4 0.1 M, NaH2PO4 0.1 M, NP-40 0.1%, pH 8.0) at room temperature for 2 min. The slides were then counterstained with 4',6-diamidino-2-phenylindole (DAPI) in antifade solution which was followed by signal analysis.

Fluorescence in-situ hybridization
Our FISH protocol followed the guidelines of previous publications (Munné et al., 1993; Harper et al., 1994) with slight modifications. Slides were treated with pepsin (100 µg/ml) in 0.01 N HCl for 20 min at 37°C, rinsed in bi-distilled water followed by PBS and fixed for 10 min in 1% paraformaldehyde at 4°C. Slides were then rinsed in PBS and twice in bi-distilled water followed by dehydration through an ethanol series. The hybridization mixture included 1 µl of a phosphate-buffered saline (PBS) without calcium and magnesium bodies, was observed in six. On the third day, five... from each embryo (Table I). FISH analysis of chromosomes... from each embryo (Table I). FISH analysis of chromosomes

Results
A total of 11 oocytes were retrieved, of which nine were in metaphase II and two were degenerative. After sperm microinjection to the nine matured oocytes, normal fertilization, confirmed by the presence of two pronuclei and two polar bodies, was observed in six. On the third day, five zygotes cleaved to the 4–8-cell stage and 1–2 blastomeres were biopsied from each embryo (Table I). FISH analysis of chromosomes X, Y and 18 demonstrated two male and three female embryos. Four of the embryos had the expected two signals of chromosome 18. An 18 monosomy was demonstrated in the fifth embryo which was a female; however, this diagnosis was uncertain since it was made on a binucleated blastomere (Munné and Cohen, 1993). The two male embryos were transferred to the uterus but pregnancy was not achieved. Whole embryo FISH analysis was available for two of the female embryos while the third was lost due to a technical error. All blastomeres in both embryos were disomic for chromosome X and 18 and the initial preimplantation diagnosis of 18 monosomy in embryo no. 6 was not confirmed.

Discussion
This is the first attempt to avoid repeated molar pregnancy by combining the techniques of ICSI and PGD, a strategy which is based on the pathogenesis of these pregnancies. In this
Prevention of GTD by ICSI and PGD

<table>
<thead>
<tr>
<th>Embryo no.</th>
<th>No. cells biopsied</th>
<th>No. cells spread</th>
<th>PGD FISH results</th>
<th>Whole embryo FISH results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/4a</td>
<td>1</td>
<td>XY[18] ×2</td>
<td>(transferred)</td>
</tr>
<tr>
<td>5</td>
<td>1/8</td>
<td>1</td>
<td>XY[18] ×2</td>
<td>(transferred)</td>
</tr>
<tr>
<td>6</td>
<td>2/4a</td>
<td>1a</td>
<td>XX[18]</td>
<td>4 cells = XX[18] ×2</td>
</tr>
<tr>
<td>8</td>
<td>1/5</td>
<td>1</td>
<td>XX[18] ×2</td>
<td>(not available)</td>
</tr>
<tr>
<td>9</td>
<td>1/4</td>
<td>1</td>
<td>XX[18] ×2</td>
<td>5 cells = XX[18] ×2</td>
</tr>
</tbody>
</table>

PGD = preimplantation genetic diagnosis.

aLysis of one cell.
bLoss of one cell during fixation.
cBinucleated cell with a normal-sized nucleus and an additional smaller nucleus. FISH results summarize both nuclei.

Table I. Details of biopsy and spreading of five embryos, and results of fluorescence in-situ hybridization (FISH)

An alternative approach to the prevention of repeated molar pregnancy may be the combination of ICSI with preimplantation determination of parental origin, by DNA typing (Edwards et al., 1990; Fisher and Newlands, 1993; Findlay et al., 1995). The advantage of DNA typing over male sex preselection by FISH is that it allows the transfer of female embryos in whom a maternal and paternal origin is demonstrated. Nevertheless, it is uncertain whether this approach would absolutely prevent the occurrence of partial moles. Although it seems that ICSI should still be conducted when DNA typing is attempted, since it guarantees a monospermic fertilization. Prevention of dispermic fertilization may potentially increase the cohort of normal embryos in women with recurrent GTD.

The performance of IVF, which is an invasive procedure with medical risks, to avoid repeated molar pregnancies, seems to be justified. It is supported by the high risk (20–28%) of a repeated episode of GTD after two previous ones (Sand et al., 1984; Berkowitz et al., 1994). Furthermore, the risk of persistent GTD is increased in patients with repeated moles. In these patients, persistent GTD may be anticipated after a subsequent complete or partial molar pregnancy in 50% and 20% of the cases respectively (Berkowitz et al., 1994). In addition, there is an increasing degree of invasiveness with subsequent GTD episodes (Acosta-Sison et al., 1959; Federschneider et al., 1980; Sand et al., 1984). These observations are in favour of the employment of IVF as a preventive measure of repeated moles.

In summary, we have reported for the first time an approach for the prevention of repeated molar pregnancies by using ICSI coupled with PGD by FISH. Our strategy is based on the pathogenesis of molar pregnancies and may aid in the prevention of both complete and partial moles.

References


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