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Recurrent triploidy of maternal origin

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We report the occurrence of triploid preimplantation embryos following *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in a woman with two previously-identified triploid conceptuses which spontaneously underwent fetal demise at 10 and 23 weeks' gestation. An error in maternal meiosis II is proposed as the most likely cause. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: preimplantation genetic diagnosis; fluorescent *in situ* hybridization

INTRODUCTION

Triploidy, a complete extra set of chromosomes, is estimated to occur in approximately 2% of conceptuses. In most instances, the extra set of chromosomes is paternal in origin, with 66% attributed to fertilization by two sperm (dispermy), 24% due to fertilization with a diploid sperm, and 10% the result of fertilization of a diploid oocyte (Jacobs *et al*., 1978). Older maternal age has not been a factor in triploidy. There are no data to indicate an increased recurrence risk of triploidy, in contrast to chromosome disorders due to non-disjunction. We describe a case of recurrent triploidy apparently as a consequence of maternal non-disjunction during oogenesis.

CASE REPORT

A 28-year-old white female, Gravida 2 Para 0 was referred for IVF and ICSI because of two previous triploid pregnancies. In her first pregnancy at 26 years of age, maternal serum multiple marker screening at 16 weeks' gestation indicated an increased risk for trisomy 18 greater than 1 : 10 (initial sample: alpha fetoprotein (AFP), 0.91 MoM; unconjugated oestriol (μE_3), 0.14 MoM; human chorionic gonadotrophin (hCG), 0.32 MoM; and, repeat sample: AFP, 1.59 MoM; μE_3, 0.17 MoM; hCG, 0.09 MoM). Ultrasound evaluation revealed oligohydramnios, possible lumbrosacral defect and lobar holoprosencephaly. Chromosome analysis of cultured amniotic fluid cells demonstrated a 69,XXX complement. The patient declined intervention and the pregnancy progressed until 23 weeks and 4 days when a fetal demise was recognized. The pregnancy was then terminated by prostaglandin induction.

The patient returned for obstetrical care after five months with a second, singleton pregnancy noted to be 8 weeks and 3 days by ultrasound measurement of crown–rump length. At 10 weeks, a repeat ultrasound evaluation demonstrated a fetal demise with biometry consistent with dates. Dilatation and evacuation was performed and chromosome analysis of the products of conception revealed a karyotype of 69,XXY.

In both pregnancies, the size and gross appearance as well as the microscopic examination of the placenta were unremarkable.

The patient and her husband received genetic counselling and were subsequently referred to the Northwestern IVF programme for ICSI on the presumption that the most likely cause of recurrent triploidy was dispermy.

MATERIALS AND METHODS

IVF

The patient was prepared for IVF using GnRHa (gonadotrophin-releasing hormone agonist) suppression followed by daily r-FSH/hMG (recombinant follicle-stimulating hormone/human menopausal gonadotrophin) ovarian stimulation (total: 225 IU/day). Twenty-two oocytes were aspirated, of which four were immature and two were atretic. Twenty oocytes were selected for ICSI, of which 16 were fertilized. On day three, 13 of the original 16 zygotes reached the eight-cell stage. All 13 embryos were biopsied and a single blastomere removed from each embryo for analysis by fluorescent *in situ* hybridization (FISH).

FISH

Individual blastomeres were transferred to 1–3 ml of hypotonic solution (6% BSA, 1% sodium citrate) and after 3–5 minutes placed on a precleaned slide with a small amount of hypotonic solution. Each blastomere

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was observed until drying was almost complete; just prior to crystallization of the hypotonic solution, fixative (Carnoy, 3 : 1, methanol : acetic acid) was dropped onto the cell and allowed to evaporate completely. Fixative treatment was repeated several times until the cytoplasm dissolved, leaving only the nucleus. The blastomere was then passed though an ethanol series (70%, 85%, 100% for one minute in each solution) and the slide allowed to dry. The Vysis MultiVysion PGT Assay, consisting of directly labelled probes LST™ 13 (13q14) Spectrum Red™, CEP® 18 (alpha-satellite D18Z1) Spectrum Aqua™, LSI 21 (21q22.12–21q22.2) Spectrum Green™, CEP® X (alpha satellite DXZ1) Spectrum Blue™, CEP Y (alpha-satellite DYZ3) Spectrum Orange™, was applied according to the manufacturer's instructions in conjunction with the HYBrite system (Vysis, Inc., Downers' Grove, IL, USA). After four hours of hybridization, each blastomere was washed in 2X SSC/0.3% NP-40, pH 7.4 for 5 minutes, followed by a 5 minute wash in 2X SSC/0.1% NP-40. After drying, 3 µl of Antifade II (Vysis, Inc.) was applied over each blastomere and a coverslip (12 mm) was placed over the target area.

RESULTS

Table 1 lists the results of the FISH analysis on the 13 embryos biopsied on day three as well as the status on day four and day five of embryos determined to have a normal chromosome number for the five applied FISH probes. Seven embryos were disomic for chromosomes 13, 18, 21, X and Y; three embryos were trisomic for chromosome 18; one embryo was monosomic for chromosome 21; and, two embryos were trisomic for the three autosomal chromosomes with an XXX sex chromosome constitution in both. Efficiency of probe hybridization, previously established in control lymphocytes, amniocytes and chorionic villus cells, ranged from 97% (CEP 18 Spectrum Aqua) to 99% (LSI 13 Spectrum Red and LSI 21 Spectrum Green).

Table 1—The application of FISH for chromosomes 13, 18, 21, X and Y to preimplantation embryos on day three in a case of recurrent triploidy

Embryo	FISH analysis	Day 4 stage	Day 5 stage
1	Normal	Morula	Blastocyst
2	Normal	8–10 cells	Blastocyst
3	Trisomy 18		
4	Monosomy 21		
5	Triploid		
6	Normal	Morula	
7	Normal	Morula	Blastocyst
8	Normal	Morula	Morula
9	Trisomy 18		
10	Normal	Morula	Blastocyst
11	Triploid		
12	Normal	8–10 cells	
13	Trisomy 18		

On day five, two embryos (1 and 7) were transferred. A singleton pregnancy occurred and the patient is currently in her 35th week of gestation.

DISCUSSION

Preimplantation diagnosis by FISH analysis was performed on day three in a 28-year-old woman because of her previous history of two triploid fetuses. Characteristically, the presence of triploidy of paternal origin results in spontaneous abortion before 16 weeks' gestation. Triploidy of maternal origin is suggested in the present case because the first triploid pregnancy completed 23 weeks' gestation before a spontaneous fetal demise (Miny *et al*., 1995; Dietzsch *et al*., 1995). In this third pregnancy, the presence of two preimplantation embryos with triploidy following IVF by a single sperm injection (ICSI) (Table 1) confirms the maternal origin of triploidy. Furthermore, since ICSI was applied after the formation of the first polar body, this implies that triploidy occurred as a consequence of the failure of the second meiotic division. To account for this failure occurring at least four times in this patient (two previous pregnancies and two preimplantation embryos), it is proposed that a genetically-determined defect in the second meiotic division mechanism is responsible for producing diploid oocytes. Since triploidy of maternal origin occurs in approximately 10% of triploid embryos, and since triploidy comprises 2% of all conceptuses, it is estimated that 1 in every 500 oocytes (0.2%) has a failure of maternal meiosis. In the present case, there were 4 triploid embryos in 15 accountable zygotes.

Alternative causes for the triploidy in the present case include (1) endoreduplication in a normal 23,X oocyte and fertilization by a normal 23,Y spermatozoon (Rosenbusch *et al*., 1997); (2) formation of an additional female pronucleus due to irregular chromosome distribution during the second meiotic division (Rosenbusch *et al*., 1997); and, (3) formation of an empty first polar body following a failure of disjunction during the first meiotic division. Should this patient undertake another pregnancy by means of *in vitro* fertilization, the timing of failure of disjunction, either during meiosis I or meiosis II, could be investigated by FISH analyses of the first polar body and blastomere of each preimplantation embryo and confirmed by microsatellite analysis in the case of any triploid embryos.

Although the recurrence risk in the case of triploidy is considered to be low, preimplantation genetic diagnosis in conjunction with IVF and ICSI appears to be a viable approach when there are clinical or genetic indications of repeated dispermy, unreduced (diploid) spermatozoa or unreduced (diploid) oocytes.

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