CASE REPORT

Dizygotic twin boys born after ICSI with maternal meiosis I-derived free trisomy 21 in the first and multiple congenital anomalies in the second: chance or common aetiology?

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We report on a pair of dizygotic twin boys born after ICSI. One twin was affected with maternal meiosis I-derived free trisomy 21. The other had multiple congenital malformations including a complex heart defect and oesophageal atresia. The advanced maternal age of 37 years predisposed for chromosome 21 meiosis I non-disjunction in twin A. Each of the multiple congenital anomalies in twin B has been described in trisomy 21. However, due to dizygosity demonstrated by a panel of molecular markers mapped on chromosome 21 as well as the results of investigations with 16 short tandem repeat markers localized on various other chromosomes, low level mosaicism or chimerism for this aneuploidy in twin B is unlikely. In addition, the twinning process, which by itself is associated with an increased rate of congenital malformations particularly affecting heart and oesophagus, might be responsible for the multiple congenital anomalies in twin B. Thus, in agreement with the results of several population-based studies from the literature, it appears unlikely that the micromanipulation of ICSI is causally responsible for the different anomalies found in these two boys.

Key words: oesophageal atresia/ICSI/multiple congenital anomalies/trisomy 21/twins

Introduction

Since the first description of IVF in 1978 (Steptoe and Edwards, 1978) and ICSI in 1992 (Palermo et al., 1992), the question of an increased incidence of chromosomal anomalies and/or multiple congenital anomalies has been discussed. However, so far no significantly increased risk for congenital malformations after IVF or ICSI has been reported (Bergh et al., 1999; Bonduelle et al., 1999; Ericson and Källen, 2001). In a recent survey on worldwide results of congenital anomalies in children born after ICSI, no difference in perinatal outcome of these children from that after IVF or natural conception was found (Tarlazzis and Bili, 2000). The figures were only affected by the higher rate of multiple pregnancies. Similarly, Wennerholm et al. determined a not significant odds ratio of 1.19 (95% confidence interval 0.79–1.81) after stratification for singletons/twins when comparing children born after ICSI with all births from the Swedish Medical Birth Registry (Wennerholm et al., 2000).

Here, we report on dizygotic twins born after ICSI with maternally-derived trisomy 21 in twin A and multiple congenital anomalies, including oesophageal atresia and a complex heart defect, in twin B.

Case report

A 37-year-old woman and her non-consanguineous 39-year-old husband were referred for genetic counselling because of abnormal results in a prenatal ultrasound. Both were of Iraqi origin and healthy. Karyotypes of the parents were normal (46,XX and 46,XY). Screening for the most frequent 29 mutations in the cystic fibrosis transmembrane conductance regulator gene in both parents prior to ICSI revealed no mutation (INNO LIPA™ system). The couple already had a 9-year-old healthy daughter.

The actual pregnancy was induced by ICSI due to secondary sterility, for which the exact reason was unknown. The twin pregnancy was uneventful up to week 20 of gestation. At this time, prenatal ultrasound revealed bilateral pyelectasia in twin
A and a large ventricular septum defect with coarctation of the aorta in twin B. Amniocentesis was offered, but the parents refused any invasive procedures. After 35 weeks gestation, two boys were delivered by emergency Caesarean section due to bradycardia and transverse presentation of twin A.

Birth measurements of twin A were at the 25th percentile for length (45 cm) and weight (2210 g) and between the 10th and 3rd percentile for occipito–frontal head circumference (30 cm). Apgar score after 1, 5 and 10 min was 8, 9 and 10 respectively. Ultrasound investigations after delivery revealed mild pyelectasia. The pattern of dysmorphic features was suggestive for trisomy 21, which was confirmed by chromosome analysis from peripheral lymphocytes (karyotype 47,XY,+21). Aneuploidy was found in all metaphases (n = 20) investigated by conventional Giemsa- and Quinacrine-banding (500 bands). Post-natal adaptation was good and the boy left the hospital after 17 days.

Birth measurements of twin B were between the 25th and 50th percentile for length (46 cm) and weight (2330 g) and between the 10th and 3rd percentile for occipito–frontal head circumference (32 cm). Apgar score after 1, 5 and 10 min was 5, 7 and 8 respectively. Oesophageal atresia characterized by a proximal blind-loop and a distal oesophagotracheal fistula (type IIIb) was surgically corrected immediately after birth. Muscular hypotonia was noted. Cardiological investigations indicated a complex heart defect including dextroposition, malposition of the large arteries, hypoplastic pulmonary vessels, persistent ductus arteriosus, double-outlet right ventricle, atrial septal defect type II (ostium secundum defect) and a right-sided aortic arch. In addition, atrioventricular arrest grade III was noted. Minor facial dysmorphisms included a high forehead, broad nasal root, upturned nose, broad mouth and high-arched palate. Screening for metabolic and haematological disorders gave normal results. Abdominal ultrasound investigations showed slightly dilated pyelons. Chromosomes of twin B were normal 46,XY evaluated in 50 metaphases by Giemsa- and Quinacrine-banding (850 bands). In addition, 100 nuclei from lymphocytes were investigated by interphase fluorescence in-situ hybridization (FISH) with the locus-specific probes D21S259, D21S341 and D21S342. No metaphase or interphase nuclei with three chromosome 21 signals were detected. A microdeletion 22q11 was excluded by FISH (TUPLE, Vyxis®, Inc., Downers Grove, IL, USA).

DNA was extracted from peripheral lymphocytes of both twins and their parents using standard protocols. Screening for the most frequent 29 cystic fibrosis mutations was performed by standard procedures (INNO LIPA™ Inno- genetics, Heiden, Germany) and showed no mutation in either twin boy.

Dizygosity was confirmed using the GenePrint® PowerPlex™16-system (Promega, Madison, USA) run on an automated sequencer (ABI 377, Perkin-Elmer Applied Biosystems Inc., Applera Deutschland, Weiterstadt, Germany), which showed differences in 10 out of the 16 short tandem repeat markers tested. According to the manufacturer’s information, low level mosaicism and/or chimerism (<10%) would have been detected by this method.

To determine the parental origin and the mechanism of formation of the extra chromosome 21 in twin A, three highly polymorphic microsatellite markers located on chromosome 21 were used (Table I). Primers were selected from the genome database and obtained from BioTeZ® (Berlin, Germany). PCR amplification was performed according to standard methods. Products were analysed by automated sequencing on an AMI 377 (Perkin-Elmer). Two maternal and one paternal allele in marker D21S1411 and D21S1413 in twin A indicated maternal origin. In addition, these results were considered to point towards a maternal meiosis I non-disjunction. In twin B, no third allele was seen, making mosaicism or chimerism also unlikely.

Discussion
To the best of our knowledge so far, parental origin and mechanisms of formation of aneuploidy in children born after ICSI have been investigated in only 10 cases. In one case with trisomy 18, maternal origin was demonstrated (Van Opstal et al., 1997). For trisomy 21, maternal as well as paternal origin was confirmed in each case (Van Opstal et al., 1997; Bartels et al., 1998). In the case with monosomy 21, only the paternal chromosome was present (Ma et al., 2001). Sex chromosomal aneuploidy was paternal in all six cases (Van Opstal et al., 1997). Particularly, the latter finding was discussed with respect to the apparently increased incidence of sex chromosome aneuploidy, which was reported in former studies to be present in children born after ICSI (Liebaers et al., 1995). However, as mentioned before, in several large studies published recently, no significantly increased rate of congenital malformations was found (Bergh et al., 1999; Bonduelle et al., 1999; Tarlatzis and Bili, 2000; Ericson and Källen, 2001). Therefore, the increased rate of sex chromosome aneuploidy might not be a direct consequence of ICSI, but an ascertainment bias due to an over-representation of patients with sex chromosome aneuploidy in fertility clinics and the increased rate of diploid spermatozoa in these patients (Guttenbach et al., 1997).

In a further case, recurrent triploidy was reported in two preimplantation embryos (Pergament et al., 2000). A maternal meiosis II error was discussed, because a single sperm was

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location</th>
<th>Mother</th>
<th>Father</th>
<th>Twin A (trisomy 21)</th>
<th>Twin B</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>D21S11</td>
<td>21q21</td>
<td>aa</td>
<td>ab</td>
<td>aaaa</td>
<td>aa</td>
<td>Non-informative</td>
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<tr>
<td>D21S1413</td>
<td>21q22.1</td>
<td>ab</td>
<td>cc</td>
<td>abc</td>
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<td>Maternal origin of trisomy 21 in twin A</td>
</tr>
<tr>
<td>D21S1411</td>
<td>21q22.3</td>
<td>ab</td>
<td>cd</td>
<td>abd</td>
<td>ac</td>
<td>Maternal origin of trisomy 21 in twin A</td>
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injected by ICSI after formation of the first polar body. In summary, knowledge of parental origin and mechanisms of formation of chromosomal aberrations in children born after ICSI is still scarce.

Trisomy 21 is the most common aneuploidy in human live births, occurring in ~1:700 live-borns (Antonarakis, 1998). Free trisomy 21 constitutes ~95% of all cases with 68% of them originating from maternal meiosis I errors, ~20% from maternal meiosis II errors, 6–7% from paternal meiosis errors and 5–6% from mitotic errors (Antonarakis, 1998). Mosaicism is found in ~2% of all cases.

The spectrum of associated congenital malformations in trisomy 21 is broad; in particular, heart defects were reported in 26% of cases with trisomy 21 (Källen et al., 1996). In another study, trisomy 21 was found in 12 out of 670 patients (1.8%) with oesophageal atresia (Beasley et al., 1997). Within a cohort of 309 cases with oesophageal atresia and a normal karyotype, cardiac malformations were present in 23.3% (Rokitsky et al., 1994). The incidence of oesophageal atresia is 1.3–4000 and it is associated with structural anomalies in ~60% (Spary and Robson, 2000). Oesophageal atresia has been reported more often in twins than in the general population (Orford et al., 2000). However, based on the molecular results of dizygosity in the twins presented here, there is no indication for a clinically relevant mosaic trisomy 21 in twin B. The same is true for chimerism, which has been described recently in a newborn with true hermaphroditism (Strain et al., 1998). Low level mosaicism or chimerism remains possible, but this cannot be excluded by any method. Thus, the malformation spectrum in twin B can be considered to be unrelated to the chromosomal aneuploidy of the dizygotic co-twin A.

The association of a complex heart defect with oesophageal atresia is also part of the VACTERL spectrum, which is considered a non-random and causally heterogeneous association of three or more of either vertebral, anal, cardiac, tracheo–oesophageal, renal or limb anomalies (Rittler et al., 1996). Because in twin B only two of the traits were present, by definition, VACTERL association cannot be diagnosed.

The main question remains whether there is any relationship between maternally-derived trisomy 21 in twin A and/or the multiple congenital malformations in twin B and/or ICSI. However, in the family presented here the basic risk for trisomy 21 due to advanced maternal age was increased (~0.45%). Furthermore, there is no indication of better stimulation of hyperhaploid oocytes by the standard pre-ICSI treatment, and no increased incidence of trisomy 21 in children born after ICSI has been reported so far. On the other hand, the risk of congenital malformations, particularly of the heart and the oesophagus, is slightly increased in dizygotic twin pregnancies with normal karyotypes. Mastroiacovo reported a relative risk of 2.56 for oesophageal atresia and of a variously increased relative risk for almost all heart defects (Mastroiacovo, 1999). Therefore, we consider that both the trisomy 21 in twin A and the multiple congenital malformations in twin B are unrelated to ICSI and are not likely to be due to a common aetiology. For twin B, it appears more likely that the twinning process might have triggered the complex heart defect and oesophageal atresia.

This observation is in accordance with the suggestion to strongly encourage efforts to reduce the number of embryos transferred in order to minimize the number of multiple gestations. This could further reduce the number of children born with congenital defects based on the problems of twinning and might thus also help the discussion on the true rate of malformations in children born after ICSI.

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References


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