

CASE REPORT

Two cases of Robertsonian translocations in oligozoospermic males and their consequences for pregnancies induced by intracytoplasmic sperm injection

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Two case histories are presented documenting structural chromosome abnormalities in infertile males. The abnormalities were detected only after application of intracytoplasmic sperm injection (ICSI) was repeatedly unsuccessful or resulted in an abnormal pregnancy. A mosaic Robertsonian translocation 45,XY,der(13;13)(q10;q10)/46,XY,t(13;13)(p10;p10), der(13p;13p) incompatible with normal offspring was found in a male with extreme oligozoospermia after three subsequent ICSI treatments were unsuccessful and one had resulted in a spontaneous abortion. A second case involved a Robertsonian translocation 45,XY,der(13;14)(q10;q10) which was detected in a male with extreme oligozoospermia after ultrasound abnormalities were found in an ICSI-induced twin pregnancy. Amniocentesis showed an unbalanced 46,XY,+13,der(13;14)(q10;q10) karyotype in one twin and a Robertsonian 45,XX,der(13;14)(q10;q10) karyotype in the other twin. Chromosome analysis of males with abnormal sperm characteristics is advised prior to ICSI.

Key words: infertility/intracytoplasmic sperm injection/oligozoospermia/Robertsonian translocation

Introduction

Intracytoplasmic sperm injection (ICSI) with sperm cells from males with severe oligozoospermia has allowed the reproduction of couples that would have remained without offspring in the past. In 6–7% of the patients the oligozoospermia is associated with the presence of a chromosome abnormality, which is 10–20-fold higher than the population incidence. Abnormalities include both sex chromosome aneuploidy, such as 47,XXY (Klinefelter syndrome) or 47,XYY, and structural rearrangements such as Robertsonian translocations, balanced reciprocal translocations, inversions and additional marker chromosomes (Kjessler, 1966; for a review see De

Braekeleer and Dao, 1991). Carriership of a chromosome anomaly confers a risk for an unbalanced chromosome complement in the offspring, with the size of the risk being dependent on the type of inherited aberration, the parent of origin, the chromosomes involved and the method of ascertainment (Boué and Gallano, 1984). The present paper outlines two cases of a paternal Robertsonian translocation and the consequences for the initiation of pregnancy by ICSI.

Case reports

Case 1

A couple with a 10 year history of infertility was treated with ICSI because of oligoasthenozoospermia. The ages of the male and his female partner were 41 and 36 years, respectively. Sperm analysis according to World Health Organization (1987) guidelines showed 2×10^6 sperm cells/ml, a progressive motility of 15% and normal morphology in 6%. Serum hormone concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were 0.8 U/l (normal range 2–7 U/l) and 0.3 U/l (normal range 1.5–8 U/l) respectively, serum testosterone concentration (24.5 nmol/l) was normal. ICSI was performed four times over a 1 year period with nine to 17 oocytes injected in the metaphase II stage, two to seven oocytes fertilized normally, and two to three embryos transferred per treatment cycle. The first ICSI procedure resulted in a clinical pregnancy that ended in spontaneous abortion at 8 weeks of gestation. Chromosome analysis of cultured chorionic villi gave a normal 46,XX karyotype, but retrospective DNA analysis demonstrated that the material was of maternal origin. Three ICSI procedures did not result in a clinical pregnancy.

Chromosome analysis of peripheral blood cells from the male after the fourth ICSI treatment showed a mosaic 45,XY,der(13;13)(q10;q10)/46,XY,t(13;13)(p10;p10) karyotype in 40 and 25 cells respectively. Fluorescence in-situ hybridization with the 13,21 centromeric probe L1.26 (Devilee *et al.*, 1986) demonstrated a single centromeric signal in both derivative chromosomes. The karyotype of the female partner was normal. The couple was counselled to refrain from future pregnancies as the rare homologous Robertsonian translocation in the male would preclude normal offspring and would result in either monosomy 13, which is not viable, or translocation trisomy 13 (Patau syndrome).

Case 2

A 32 year old woman was referred for prenatal diagnosis at 26 weeks of gestation because of ultrasound abnormalities

(intrauterine growth retardation; microcephaly; bilateral cheiloschizis) in one fetus of a dizygotic twin pregnancy. The pregnancy was established by ICSI because of an extreme oligoasthenoteratozoospermia in the father showing 1×10^5 sperm cells/ml, a progressive motility of 17% and 0% normal morphology. Serum FSH (13.7U/l) was elevated, while LH (2.7 U/l) and testosterone (14.8 nmol/l) concentrations were in the normal range. Bilateral cryptorchism was diagnosed at the age of 12 years and treated with Pregnyl. Seventeen oocytes in metaphase II were injected and 15 were normally fertilized. Three embryos were replaced at the 4–6-cell stage. Amniocentesis at week 26 of gestation revealed a 45,XX,der(13;14)(q10;q10) karyotype in fetus I and a 46,XY,+13,der(13;14)(q10;q10) karyotype in fetus II. The male fetus with the translocation trisomy 13 presented with an intrauterine fetal death at 34 weeks, while the female fetus with the Robertsonian translocation was born at 36 weeks without congenital malformations and a birthweight of 2725 g.

Chromosome analysis of both parents revealed in a normal 46,XX karyotype in the mother and a Robertsonian translocation 45,XY,der(13;14)(q10;q10) in the father.

Discussion

The present paper deals with two cases of Robertsonian translocations in oligozoospermic males. This type of translocation is a relatively frequent finding in oligozoospermia (1.9%) when compared to the general population (0.12%) (Retief *et al.*, 1984; Bourrouillou *et al.*, 1985; Nielsen and Wohlert, 1991). Chromosome analysis of males whose spermatozoa were used for ICSI, and whose sperm parameters were abnormal, confirm these findings: with 2.3% (Testart *et al.*, 1996) and 3.1% (Baschat *et al.*, 1996) of males carrying a Robertsonian translocation.

Our first case involves a homologous 13;13 translocation, which, in analogy to the 21;21 translocation, mainly originates *de novo* as carriers are not expected to have normal offspring (Jacobs *et al.*, 1987; Shaffer *et al.*, 1994). Male gametes will either be devoid of chromosome 13 or will carry two copies of 13q resulting in fertilized oocytes with either a monosomy 13, which is non-viable, or in a trisomy 13 (Patau syndrome). In the present case the 13;13 q-arm translocation chromosome was accompanied by the corresponding 13;13 p-arm derivative in some cells, which is a rare finding in carriers of a Robertsonian translocation (Palmer *et al.*, 1969). The presence of both p- and q-arm material suggests a reciprocal translocation resulting in two monocentric derivative chromosomes and subsequent loss of the small der(13;13)(p10;p10) chromosome in some cells. The couple had a 10 year history of infertility and had gone through an extensive series of fertility treatments and attempts at assisted fertilization. The cytogenetic result makes it evident that these medical interventions could only have resulted in offspring with trisomy 13.

Our second case involves a 13;14 translocation in an ICSI father and represents the most common chromosome defect found in oligozoospermic males (1.6% versus 0.1% in the general population; Retief *et al.*, 1984; Bourrouillou *et al.*, 1985; Nielsen and Wohlert, 1991). Interestingly, not all male

carriers of a Robertsonian translocation are infertile. Most carriers appear to be fertile, and within the same family fertile and infertile male carriers can be found (Palmer *et al.*, 1973; Plymate *et al.*, 1976). Whether carriership is accompanied by a general trend towards abnormal semen analysis, with the infertile oligozoospermic males representing the lower end of a distribution curve, or whether structural differences exist between the translocation chromosomes in fertile and infertile males, is not known (Marmor *et al.*, 1980). The association between carriership of a structural chromosome abnormality and male infertility has been recognized for more than 30 years (Kjessler, 1966; Chandley *et al.*, 1972). Observations in experimental animals and electron microscopic examination of meiotic profiles in human male carriers have suggested that the trivalents formed at the pachytene stage of meiosis by the Robertsonian translocation chromosome and the two single D or G group chromosomes, may interact with the X/Y bivalent, resulting in spermatogenic impairment (Johannison, 1993; Everett *et al.*, 1996).

Carriership of a Robertsonian 13;14 translocation is empirically found to carry a low risk for unbalanced offspring with translocation trisomy 13, as no unbalanced offspring has been encountered during prenatal diagnosis in 230 pregnancies in which one of the parents was a carrier for the translocation (Boue and Gallano, 1984). The finding of a translocation trisomy 13 in the offspring of an oligozoospermic ICSI male is therefore of potential interest and poses the question whether such imbalances are more frequent in this group. Normal offspring from three ICSI males carrying a balanced 13;14 translocation has been reported (Testart *et al.*, 1996) but larger sample sizes will be necessary to address the issue. Prolonged follow-up studies will be necessary to establish whether male offspring carrying the same structural chromosome abnormally inherited from their oligozoospermic infertile father will themselves be oligozoospermic and infertile.

ICSI has been applied in The Netherlands since 1994 in two strictly defined indication groups, namely in cases of extreme oligozoospermia ($<1 \times 10^6$ motile sperm cells/ml) and in cases of total fertilization failure (two consecutive IVF treatments which do not lead to fertilization). Chromosome analysis of idiopathic oligozoospermic males prior to ICSI was formally advised in 1996 and most centres performing ICSI have now implemented chromosome studies systematically as part of the routine pre-ICSI workup. The decision to offer chromosome studies to oligozoospermic males prior to ICSI was based on published data in which incidences of constitutional chromosome abnormalities were reported at 6–7% in males with $<10 \times 10^6$ sperm cells/ml (Retief *et al.*, 1984; Bourrouillou *et al.*, 1985) and on the observation of an inverse correlation between the sperm count and the amount of constitutional chromosome abnormalities (Chandley *et al.*, 1984). Although inclusion criteria for ICSI treatment differ markedly between various centres worldwide, initial results suggest that oligozoospermic males selected for ICSI display abnormalities in 4–7% of cases (Baschat *et al.*, 1996; Testart *et al.*, 1996). It cannot be excluded that such percentages may be higher in centres with stricter inclusion criteria or in

subgroups of patients with extremely low sperm counts, such as in males with cryptozoospermia.

The two cases described above date from before the introduction of systematic chromosome analysis in the two ICSI centres involved. Each case in itself forms a strong argument in favour of a chromosome analysis programme prior to ICSI. Ideally, chromosome analysis should be offered as early as possible, preferably at the stage when the diagnosis of male infertility due to abnormal sperm characteristics is made. Carriers of structural chromosome abnormalities may thus be identified and offered genetic counselling. In addition, identification of carriership will also allow the testing of any family members of the proband who may be normally fertile but are carriers of the same abnormality and are thus at an enhanced risk for abnormal offspring. A question that remains to be answered is whether chromosome analysis should be limited to oligozoospermic males, or whether all subfertile couples should be karyotyped prior to ICSI. Until more data are available on the incidence of chromosome abnormalities in the various subgroups, many centres will continue to opt for an approach in which chromosome analysis of the oligozoospermic male prior to ICSI is complemented by an offer of prenatal cytogenetic analysis by amniocentesis or chorionic villus sampling to all couples having ICSI treatment, including couples in which the male displays normal sperm characteristics.

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