Reproductive genetic counselling in non-mosaic 47,XXY patients: implications for preimplantation or prenatal diagnosis: Case report and review

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With an incidence of ~1 in 500 male newborns, the 47,XXY genotype is one of the most common sex chromosome anomalies. It is also the most frequent genetic cause of human infertility. Some non-mosaic 47,XXY patients have sperm production which allows infertility treatment to be offered by ICSI. Therefore, the risk of transmitting a chromosome anomaly to the next generation is an important problem in reproductive genetic counselling of these patients. Here, we report on a twin pregnancy where two karyotypically normal neonates 46,XX and 46,XY were born after the use of ICSI in assisted reproduction of a patient with a non-mosaic 47,XXY syndrome. To date, only 38 evolving pregnancies including the present cases, have been reported after ICSI using sperm from non-mosaic 47,XXY patients. Although these data are scarce, they suggest that the risk of chromosome anomaly in the offspring of these patients is low; hence, their reproductive genetic counselling can be reassuring, and management of the pregnancy can proceed with caution.

Key words: 47,XXY/genetic counselling/preimplantation/prenatal/reproduction

Introduction

With an incidence of 1 in 500 male newborns, the 47,XXY genotype is one of the most common sex chromosome anomalies (Smyth and Bremner, 1998). Observed in 11% of azoospermic men and 0.7% of oligozoospermic men, it is also the most frequent genetic cause of human infertility (4±6%) (De Braekeleer and Dao, 1991; Yoshida et al., 1996). The 47,XXY syndrome is classically characterized by small, firm testes with hyalinization of the seminiferous tubules, elevated gonadotrophins and azoospermia (Smyth and Bremner, 1998). Classically, no sperm are present in the adult testis, and 47,XXY patients have been considered as infertile. Nevertheless, a few cases of naturally conceived offspring of proven paternity have been reported (Laron et al., 1982; Terzoli et al., 1992). Hence, some 47,XXY patients have sperm production which allows the patient the benefit of infertility treatment. The use of ICSI allows these patients to reproduce (Bourne et al., 1997; Palermo et al., 1998). Sperm studies in mosaic 46,XY/47,XXY patients have shown that the majority of sperm analysed have normal karyotypes (Lim et al., 1999). In non-mosaic 47,XXY patients, the ploidy of the sperm used to fertilize for ICSI remains unknown. Thus, the risk of producing pregnancies with chromosome abnormalities by using sperm from non-mosaic 47,XXY men is not exactly determined. Births from non-mosaic 47,XXY men are actually rare, and the reporting of new cases is important in order to determine the chromosomal risk for those patients. Moreover, due to recent advances in reproductive therapy for men with non-mosaic 47,XXY syndrome, predictive factors of spermatogenesis such as testicular volume and testosterone levels in these patients have been recently described (Madgar et al., 2002). Therefore, the genetic risk of transmitting a chromosome anomaly to their offspring is an important problem in reproductive genetic counselling of these patients.

Here, we report on a twin pregnancy where two karyotypically normal neonates were born after the use of ICSI in assisted reproduction of a patient with non-mosaic 47,XXY syndrome. The genetic risk in the offspring of these patients, as well as the indication of preimplantation or prenatal diagnosis in embryos or fetuses conceived after ICSI of sperm from non-mosaic 47,XXY patients, are discussed.

Case report

A 27-year-old healthy man presented to our centre because of infertility. Physical examination revealed bilateral testicular atrophy with an estimated testicular volume of 6.4 ml bilaterally. Peripheral blood chromosome analysis showed a

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47,XXY karyotype in 50 metaphases analysed. Dual colour fluorescence in-situ hybridization (FISH) analysis with X and Y probes showed an absence of mosaicism for X and Y chromosomes in 700 peripheral blood lymphocytes studied. The patient’s twin brother also had a 47,XXY karyotype. The results of laboratory tests were as follows: FSH 37 IU/l, LH 14.5 IU/l and testosterone 5 ng/ml. The patient’s wife was a 28-year-old healthy woman who had a normal karyotype 46,XX.

Semen analysis showed normal semen volume and rare spermatozoa. Ten oocytes were obtained at oocyte retrieval. Nine matured oocytes were injected, and five of these were fertilized and cleaved. Two embryos were frozen and two morphologically good embryos were transferred. This transfer led to the establishment of a twin pregnancy. Maternal serum markers and fetal ultrasound examination were normal and the pregnancy progressed uneventfully. Two healthy male and female neonates were delivered at 38 weeks gestation, with birth weights of 2760 g and 2580 g. Post-natal cytogenetic diagnosis showed normal female (46,XX) and male (46,XY) karyotypes.

Discussion and review

Sperm from non-mosaic 47,XXY have been used successfully in assisted reproduction. However, the origin of the meiotic products of non-mosaic 47,XXY patients remains unclear. First, mosaicism cannot be excluded in non-mosaic 47,XXY patients (Tournaye et al., 1996). Indeed, the presence of a normal XY germ cell line in the testis could explain the production of normal haploid sperm in these apparently non-mosaic patients. In the present case, the absence of mosaicism was established on peripheral blood cells using karyotyping and FISH studies with X and Y probes. Nevertheless, it is well known that lymphocyte karyotyping neither predicts the chromosomal constitution of the testis cells nor the presence or absence of spermatogenesis (Westlander et al., 2001).

Some authors have hypothesized that 47,XXY germ lines are unable to proceed through meiosis (Levron et al., 2000; Blanco et al., 2001), while others have proposed that 47,XXY germ cells are able to undergo meiosis to produce 24,XY and 24,XX sperm cells (Guttenbach et al., 1997; Lim et al., 1999). The increase of sex chromosome aneuploidy in sperm of non-mosaic 47,XXY patients could be explained in two ways: either XY germ cells are able to complete meiosis and produce mature sperm; or XY germ cells are present in the testis of non-mosaic 47,XXY patients and abnormal sperm result from meiotic non-disjunction due to perturbation of the testis cellular or hormonal environment.

Some evidence exists to support the first hypothesis. For example, among 24 ejaculated sperm from a non-mosaic 47,XXY patient, six spermatozoa were observed with an XY/18 complement (Estop et al., 1998). Others (Foresta et al., 1998) have observed a 24,XY or 24,XX karyotypes in 13 of 34 spermatozids and in 10 of 45 spermatozids from testicular biopsies of two non-mosaic 47,XXY patients. Another group (Yamamoto et al., 2002) showed an increased incidence of 24,XY and 24,XX round spermatozids in testicular biopsies of non-mosaic 47,XXY patients. Finally, chromosome analyses performed in sperm from mosaic 46,XY/47,XXY patients showed an increased incidence of hyperhaploid 24,XY sperm cells (Chevre et al., 1996; Martini et al., 1996). Thus, although the majority of the testicular round spermatids (>93%) from non-mosaic 47,XXY patients have a normal 23,X or 23,Y karyotype, these results suggest that 47,XXY germ cells can produce mature hyperhaploid sperm (Yamamoto et al., 2002). By contrast, others (Levron et al., 2000) suggested that sperm cells from non-mosaic 47,XXY patients are the products of normal germ lines and that the occurrence of aneuploidies is due to environmental factors commonly observed in patients with gonadal failures. Indeed, the rate of sex chromosome aneuploidies in sperm from non-mosaic 47,XXY patients is increased, but it remains lower than expected if 47,XXY spermatogonia underwent meiosis (Levron et al., 2001). As pointed out (Blanco et al., 2001), XYX cells are unable to enter meiosis because all pachytene cells examined were exclusively XY resulting presumably from a premeiotic loss of one chromosome X. Recently, spermatogenesis was observed only in non-mosaic 47,XXY patients with a mosaicism XXX/XY confined to testis (Bergère et al., 2002), thereby supporting the hypothesis that only 46,XY cells can undergo meiosis. Similar findings have been observed in XXX mice, where the absence of germ cells is due to a progressive loss in early post-natal life (Hunt et al., 1998; Lue et al., 2001). The surviving germ cells observed in the adult XXX testis are exclusively XY and result presumably from rare mitotic non-disjunction in the fetal testis (Mroz et al., 1999a). Therefore, the meiotic abnormalities observed in sperm from XXX mice are due to segregation errors taking place in XY germ cells rather than in surviving XXX germ cells in the testis (Mroz et al., 1999b). This strongly suggests that the abnormal testis environment could affect meiosis, leading to an increased rate of non-disjunction. Similarly, it has been shown recently that spermatids from infertile mice had an increased rate of aneuploidy compared with wild-type animals (Oppedisano et al., 2002).

In humans, a significantly higher rate of sex chromosome abnormalities has been reported among infertile men undergoing ICSI (Aran et al., 1999). For example, one group (Ohashi et al., 2001) showed that severe oligozoospermic men with normal karyotype have a higher incidence of XY disomy in sperm. Also, epididymal and testicular sperm cells from non-obstructive azoospermic men showed a higher incidence of chromosomal anomalies, and of sex chromosome aneuploidy in particular (Palermo et al., 2002). This suggests that, as in infertile mice, the rate of sex chromosome aneuploidy is comparable in 47,XXY patients and other male infertility groups (Rives et al., 2000; Lezon et al., 2001). Non-disjunction could be due to a compromised testicular environment related to an increase in FSH concentrations (Egozcue et al., 2000; Blanco et al., 2001) and may also affect sex chromosomes and autosomes (Lim et al., 1999; Rives et al., 2000; Hennebicq et al., 2001). Other causes of increasing XY non-disjunction in sperm have been recently demonstrated such as paternal age (Lowe et al., 2001) and lack of recombination in the pseudoautosomal region (Shi et al., 2001).
ICSI per se is also associated with an increased risk of producing a chromosome anomaly in offspring (Aboulghar et al., 2001). Recent reports have shown that IVF is associated with an increased risk for de-novo chromosomal aberrations, especially those involving the sex chromosomes (Van Opstal et al., 1997; Bonduelle et al., 1998). This risk of ICSI might be due to the fact that sex chromosomes are localized preferentially in the subacrosomal region of sperm (Sbracia et al., 2002). Thus, by using ICSI, the introduction of an intact sperm perinuclear theca into the oocyte cytoplasm may lead to an impaired decondensation of chromatin located in the subacrosomal region, leading in turn to an increased rate of sex chromosomal anomalies (Terada et al., 2000).

With respect to the risk of chromosome anomaly actually observed in the offspring, to our knowledge, only 36 evolving pregnancies have been reported after ICSI using sperm from non-mosaic 47,XXY patients (Bourne et al., 1997; Hinney et al., 1997; Palermo et al., 1998; Reubinoff et al., 1998; Nodar et al., 1999; Ron-El et al., 1999, 2000a,b; Kitamura et al., 2000; Revlon et al., 2000; Crüger et al., 2001; Greco et al., 2001; Poulakis et al., 2001; Bergère et al., 2002; Rosenlund et al., 2002; Yamamoto et al., 2002). These 36 pregnancies produced 32 karyotypically normal neonates, two karyotypically normal pregnancy losses, one healthy unkaryotyped neonate, and one 47,XXY prenatally diagnosed fetus which led to an interruption of the pregnancy. Unfortunately, in the last case the parental origin of the supernumerary X chromosome was not determined. Indeed, two-thirds of cases of 47,XXY are of maternal origin (Eskenazi et al., 2002). Clearly, these data are too scarce to establish an empiric risk.

Thus, the reproductive genetic counselling of 47,XXY patients remains difficult. Some authors have recommended preimplantation or prenatal diagnosis (PND) after ICSI using sperm from 47,XXY patients (Staessen et al., 1996; Estop et al., 1998; Reubinoff et al., 1998; Rosenlund et al., 2002).

Arguments from authors who propose a preimplantation genetic diagnosis (PGD) or PND is the increased risk of producing sex chromosomal-abnormal offspring (Hinney et al., 1997; Palermo et al., 1998; Ron-El et al., 2000a,b; Kitamura et al., 2000; Revlon et al., 2000; Crüger et al., 2001; Rosenlund et al., 2002). Nevertheless, a high proportion of couples did not accept PGD or PND (Table I). With regard to the risk of a higher incidence of sex chromosome aneuploidies in non-mosaic 47,XXY patients, the unbalanced offspring would be 47,XXX or 47,XXY. The more important theoretical genetic risk for sex chromosomes is the 47,XXY karyotype which, nevertheless, represents the same karyotype of the father. Hence, the diagnosis of a fetus with a sex chromosome aneuploidy may be more acceptable to patients carrying a sex chromosome anomaly (Meschede et al., 1998). The other gonosomal risk is 47,XXX, which is not associated with major phenotypic anomalies (Linden et al., 1996), and the discovery in PND does not change the management of the pregnancy. In PGD, the limitations of the technique—which is performed on few available embryos—include the possibility of chromosomal mosaicism in embryos, false FISH results and FISH failure (ESHRE PGD Consortium, 2002). Six normal embryos and two chaotic embryos were reported using PGD after ICSI of sperm from 47,XXY patients (Staessen et al., 1996; Reubinoff et al., 1998). In PND, the infertile couples do not wish to risk abortion by using an invasive procedure (Crüger et al., 2001). Indeed, the risk of fetal loss in PND is estimated to be 0.5–2% (Jauiaux et al., 2000). Moreover, there are no medical reasons for these couples to terminate the pregnancy, even if a 47,XXY or 47,XXX karyotype were to be diagnosed. In PND of a 47,XXY fetus, the information given to the couple would be that the baby would have an IQ 10 or 15 points lower than his brother, and that he would be infertile (Abramsky and Chapple, 1997). Most males born with a 47,XXY pattern go through life without being karyotyped, and the most common indication for a 47,XXY patient to be karyotyped will be hypogonadism and/or infertility (Abramsky and Chapple, 1997). Reports on outcomes of prenatally diagnosed 47,XXY show variabilities for the continuation or termination of the pregnancy, and depend in particular on the genetic counselling made. After a non-directive genetic counselling, a low rate of pregnancy termination (17.4%) was reported in 23 prenatally

### Table 1. Pregnancy reports after ICSI of sperm from nonmosaic 47,XXY patients. Karyotypes and the use or not of either preimplantation genetic diagnosis (PGD) or prenatal diagnosis (PND) are indicated

<table>
<thead>
<tr>
<th>Reference</th>
<th>Karyotypes</th>
<th>PGD</th>
<th>PND</th>
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<tbody>
<tr>
<td>Bourne et al. (1997)</td>
<td>46,XY</td>
<td>NI</td>
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<td></td>
<td>46,XX</td>
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<td>Hinney et al. (1997)</td>
<td>46,XXa</td>
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<td>Palermo et al. (1998)</td>
<td>46,XY</td>
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<td>46,XX</td>
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<td>46,XY</td>
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<td>NI</td>
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<td>Ron-El et al. (1999)</td>
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<td>Ron-El et al. (2000b)</td>
<td>46,XX</td>
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<td>47,XXYa</td>
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<tr>
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<td>Bergère et al. (2002)</td>
<td>NR</td>
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<tr>
<td>Present report</td>
<td>46,XX</td>
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<td>46,XY</td>
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aAbortion.
btermination of pregnancy.

*PND accepted before reduction of one embryo in the triplet pregnancy.

A = accepted by the couple; NA = non-accepted by the couple; NI = non-indicated in the report; NR = non-realised.
diagnosed non-mosaic 47,XXY fetuses (Meschede et al., 1998). Prenatal cytogenetic analysis of 71 fetuses conceived by ICSI resulted in the detection of two cases of 47,XXY with the continuation of the pregnancies (Van Opstal et al., 1997). The overall termination rate in PND of 47,XXY fetuses was 44% in a recent European study (DADA study group, 2002). This study showed an association between the termination rates and the speciality of the health professional providing counselling after the diagnosis. In particular, women were less likely to terminate affected pregnancies when counselling involved only a genetic specialist (DADA study group, 2002). Other recent data also indicate that genetic counselling of sex chromosome aneuploidy should be reassuring (Linden and Bender, 2002). Nevertheless, with respect to aneuploidy involving the autosomes, non-invasive management of the pregnancy using maternal serum markers and fetal ultrasound examinations should always be proposed to the couples.

In conclusion, the genetic risk in the offspring of 47,XXY patients remains unknown, but is presumably low. This risk concerns sex chromosomal as well as autosomal aneuploidy. Genetic counselling should be reassuring, and management of the pregnancy should proceed with caution. Further empiric data are needed and the parental origin of all chromosome anomalies observed in the offspring of 47,XXY patient should be established.

References


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