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Case Report

High Incidence of Chromosomal Abnormalities in Large-Headed and Multiple-Tailed Spermatozoa

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Case Report

To date, several studies of aneuploidy rate in spermatozoa from infertile men have been published. Most of them found an increase incidence of aneuploid spermatozoa in infertile patients compared with normal donors (Moosani et al, 1995; Finkelstein et al, 1998; McInnes et al, 1998; Pang et al, 1999; Rives et al, 1999; Vegetti et al, 2000). However, in other studies, no differences in aneuploidy rates between fertile and infertile men were reported (Miharu et al, 1994; Guttenbach et al, 1997). The evaluated patients often showed different types of sperm parameter impairments, including oligo-, astheno-, oligoastheno-, oligoastenoterato-, asthenoteratozoospermia, and unexplained infertility, and this may be responsible, at least in part, for the discrepancies observed in the results.

Studies taking into account an abnormality for an isolated sperm defect have shown an inverse correlation between sperm aneuploidy and concentration (Bernardini et al, 1998, 2000; Pang et al, 1999; Pfeffer et al, 1999; Rives et al, 1999; Nishikawa et al, 2000; Ushijima et al, 2000; Vegetti et al, 2000; Calogero et al, 2001a; Ohashi et al, 2001; Rubio et al, 2001; Martin et al, 2003). Furthermore, some authors have reported an association between the aneuploidy rate and the presence of abnormal head morphology and particularly with enlarged heads and multiple tails (Yurov et al, 1996; In't Veld et al, 1997; Bernardini et al, 1998; Viville et al, 2000; Devillard et al, 2002).

Because intracytoplasmic sperm injection (ICSI) is widely acknowledged to be the most effective

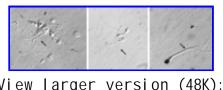
therapeutic approach for severe male-factor infertility, including teratozoospermia, it is important to counsel the couple about the risk of aneuploidy in their offspring.

We describe the case of a patient with a high incidence of sperm chromosomal abnormalities associated with morphology with large-headed and multiple-tailed spermatozoa.

Patient— We studied a 38-year-old infertile man with a clinical background of left varicocelectomy 4 years ago. Blood karyotype of the patient, as well as molecular analysis of Y chromosome microdeletions, were normal. He worked in a petroleum refinery and he was exposed daily to chemical agents such as naphtha, sulfhidric acid, asphalt, and ammonium. Four semen analyses during 10 months (Table 1) revealed a moderate oligozoospermia, severe asthenozoospermia, and total teratozoospermia according to the World Health Organization (WHO, 1999) criteria. Large-headed and multiple-tailed spermatozoa were observed in most of them (the Figure). The female partner was 37 years old and without any infertility problem.

View this table:
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View this table: Table 1. Results of patient's spermiograms



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Arrows indicate multiple tails.

The couple underwent a previous ICSI cycle in which the microinjection was technically difficult because of the size of the sperm heads and the absence of spermatozoa with normal morphology. In this cycle, 15 oocytes were microinjected, 11 fertilized, but embryo quality was impaired on day 3 of development (3 of them were blocked and the remaining embryos were of poor quality according to Alikani et al, 1999). A total of 4 embryos were transferred after assisted hatching and fragment removal and pregnancy was not achieved. Due to the poor embryo quality, the couple was offered to undergo a sperm analysis by fluorescence in situ hybridization (FISH).

FISH— The sperm sample was fixed with methanol: acetic acid (Merck, Darmstadt, Germany) and spread in superfrost/plus slides (0. Kindler, GmBh, Freiburg, Germany). For FISH analysis, sperm nuclei were decondensed by slide incubation for 5 to 7 minutes at 37° C in 5 mmol/L 1.4-Dithiothreitol (DTT, Roche Diagnostics, GmbH Mannheim, Germany) and 1% Triton X-100. DNA was denatured for 5 minutes at 73° C \pm 1° C in a water bath in 70% formamide (Roche Diagnostics GmbH, Mannheim, Germany). Triple FISH was performed for chromosomes 18 (locus D18Z1, CEP 18 Spectrum Aqua; Vysis Inc Downers Grove, III), X (locus DXZ1, CEP X Spectrum Green; Vysis Inc), and Y (locus DYZ1, CEP Y Spectrum Orange; Vysis Inc). Double FISH was performed for chromosomes 13 (locus RB, LSI 13 Spectrum Green; Vysis Inc) and chromosome 21 (loci D21S259, D21S341, D21S342, LSI 21 Spectrum Orange; Vysis Inc) on a different slide. FISH incubation and detection were performed according to the manufacturer's instructions.

Analysis was carried out using an Olympus AX70 epifluorescence microscope equipped with a triple band-pass filter for 4'6-diamidino-2-phenylindole (DAPI)/Texas Red/fluorescein isothiocyanate (FITC) and single band-pass filters for FITC, Texas Red, and Aqua Blue.

Ejaculated spermatozoa from 5 normozoospermic fertile donors, classified according to WHO (1999) criteria and processed in the same manner as the samples of the patient were used as control group (Rodrigo et al., 2004).

Statistical Analysis— The incidence of disomy and diploidy for the analyzed chromosomes in the patient and in a control group of normozoospermic individuals were compared using x^2 test (with Yates correction when necessary) and Fisher's exact test (Graphpad Instat v. 2.05a, Graphpad Software, San Diego, Calif).

Results

The results of triple- and double-color FISH are reported in Tables 2 and 3. <u>Table 2</u> shows the distribution of the different types of abnormalities for chromosomes X, Y, and 18. A total of 215 sperm heads were scored for these chromosomes and 196 were abnormal (91.2%). In our control group, only 179 out of 50 404 (0.36%) were abnormal (<u>Rodrigo et al, 2004</u>), showing clear statistical differences (P < .0001).

View this table: Table 2. FISH results for chromosomes X, Y, and 18 [in this window]
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View this table: <u>[in this window]</u> <u>[in a new window]</u>

View this table: Table 3. FISH results for chromosomes 13 and 21

<u>Table 3</u> shows the distribution of the different types of abnormalities for chromosomes 13 and 21. In this analysis, a total of 102 sperm heads were evaluated and 96 were abnormal (94.1%). In the control group, 50 373 spermatozoa were analyzed and 186 were abnormal (0.37%), again with marked statistical differences (P < .0001).

Discussion

The available literature on FISH analysis of human sperm confirms higher rates of sperm aneuploidy in infertile men as compared with fertile men, despite a normal blood karyotype (Moosani et al, 1995; Lähdetie et al, 1997; Bernardini et al, 1998; Arán et al, 1999; Pang et al, 1999; Pfeffer et al, 1999; Rives et al, 1999; Ushijima et al, 2000; Vegetti et al, 2000; Martin et al, 2003). An inverse correlation between sperm quality and sperm aneuploidy rates has been reported (Bernardini et al, 2000; Nishikawa et al, 2000; Ushijima et al, 2000; Vegetti et al, 2000; Rubio et al, 2001), but contradictory results have been published concerning the relationship of sperm aneuploidy with specific sperm defects.

An inverse relationship has been reported between sperm aneuploidy and sperm concentration (<u>Pang et al, 1999</u>; <u>Vegetti et al, 2000</u>; <u>Calogero et al, 2001b</u>; <u>Rubio et al, 2001</u>) and between sperm

aneuploidy and sperm motility (<u>Vegetti et al, 2000</u>). With respect to the sperm morphology, there are conflicting results, mainly due to the variability of sperm morphological types between patients and within the same sperm sample.

There have been several reports regarding the relationship of specific morphological types to the incidence of aneuploidies. Significant differences in aneuploidy rates have not been found between normal controls and patients with shortened flagella syndrome and spermatozoa with irregular acrosomes (Viville et al, 2000). Regarding globozoospermia, there are few studies and they yield controversial results: some of them found an increased incidence of sperm chromosomal abnormalities (Carrell et al, 1999, 2004; Martin et al, 2003; Morel et al, 2004; Ditzel et al, 2005), whereas other authors did not find such an increase (Viville et al, 2000; Vicari et al, 2002). However, there is an agreement in patients with large-headed spermatozoa with significantly higher incidence of sperm chromosomal abnormalities (In't Veld et al, 1997; Viville et al, 2000; Lewis-Jones et al, 2003, Vicari et al, 2003).

Our patient had a total teratozoospermia with almost all spermatozoa showing large head and multiple tails. There was clearly identified an isolated type of morphological abnormality, and FISH results for chromosomes X, Y, and 18 showed that 91.2% of the spermatozoa had chromosomal abnormalities. A high incidence of aneuploidy for these chromosomes and mainly hyperhaploid spermatozoa were also obtained by In't Veld (1997) and more recently by Lewis-Jones et al (2003). In our study, we also evaluated chromosomes 13 and 21, and with these results, we confirmed that the predominant abnormality found in the macrocephalic spermatozoa would be hyperhaploidy with aneuploidy for all the tested chromosomes. A low number of spermatozoa were evaluated due to the difficulty to discriminate between sperm heads and immature cells and only tailed spermatozoa were taken into account. Despite this consideration, we believe our results are as conclusive as other FISH studies in severe oligozoospermic, teratozoospermic, or azoospermic patients in whom a low number of spermatozoa were also evaluated and the results were statistically analyzed in the same manner (Levron et al, 2001; Lewis-Jones et al, 2003; Mateizel et al, 2002; Gianaroli et al, 2005).

To our knowledge, only 1 study reports the effect of the exposure to the chemicals in workers of a petroleum refinery on the sperm morphology (<u>Rosemberg et al, 1985</u>). They find a lack of association between abnormal morphology and the exposure to chemicals in this environment, without any mention of sperm chromosomal abnormalities.

The clinical consequences of using sperm samples with an abnormal FISH result in ICSI programs have been evaluated by several authors. It seems that sperm chromosomal abnormalities may adversely affect ICSI outcome in oligoasthenoteratozoospermic patients and in epididymal and testicular spermatozoa from azoospermic patients, decreasing fertilization (Pfeffer et al, 1999) and pregnancy rates (Pang et al, 1999; Pfeffer et al, 1999; Bernardini et al, 2000; Calogero et al, 2001b; Rubio et al, 2001) and increasing miscarriage rates (Rubio et al, 2001), at least in some cases. Moreover, Gianaroli et al (2000) and Silber et al (2003) analyzed the incidence of chromosomal abnormalities in embryos originated from azoospermic patients participating in a preimplantation genetic diagnosis program. Embryos from these patients suffered higher rates of abnormalities than those obtained from normozoospermic or oligozoospermic patients, with high incidences of embryos with aneuploidies for sex chromosomes (Gianaroli et al, 2000) and mosaic embryos (Silber et al, 2003). Furthermore, Burrello et al (2004) have described that, in oligoasthenoteratozoospermic patients, there is an increased incidence of sperm chromosomal abnormalities in both types of spermatozoa, those with normal and abnormal head shape. Therefore, the selection of normal-shaped spermatozoa for ICSI would not prevent the risk of chromosomal abnormalities in the offspring.

Preimplantation genetic diagnosis has been offered by several groups as an alternative in patients at risk of chromosomal abnormalities in their spermatozoa (<u>Arán et al, 1999</u>; <u>Gianaroli et al, 2000</u>; <u>Silber et al, 2003</u>). However, in our patient, with nearly all spermatozoa being abnormal with only 5 out of 23 chromosomes analyzed, we discouraged a new ICSI attempt and therefore sperm donation was advised. The couple accepted and is currently in our donor insemination program.

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