Analysis of Spermatozoa from Seven ICSI Males with Constitutional Sex Chromosomal Abnormalities by Fluorescent In Situ Hybridization

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Submitted: July 22, 1999

Accepted: November 19, 1999

Purpose: The objective was to estimate the risk for subfertile males with a constitutional sex chromosomal abnormality of transmitting such a chromosome abnormality to their children, conceived by intracytoplasmic sperm injection (ICSI).

Methods: Semen samples were obtained from seven severely oligospermic ICSI candidates. Six of them had a numerical sex chromosomal abnormality, including mosaic 45,X/46,XY, mosaic 46,XY/47,XXY, 47,XXY (Klinefelter's syndrome), and 47,XYY. One male had a structural abnormality, namely, an inversion of the Y chromosome. The semen was studied by three-color fluorescent in situ hybridization (FISH) with probes specific for chromosomes 18,X, and Y.

Results: Chromosomal aneuploidy rates of any of the three chromosomes were significantly higher than the aneuploidy rates observed in three control samples but comparable to the rates observed in 10 ICSI candidates with oligoasthenoteratozoospermia (OAT) and a normal constitutional karyotype.

Conclusions: Our data indicate that males with (mosaic) sex chromosomal abnormalities have no higher risk of producing offspring with a sex chromosomal abnormality by ICSI than OAT males with a normal karyotype.

KEY WORDS: Genetic risk; intracytoplasmic sperm injection (ICSI); infertility; Klinefelter's syndrome; oligoasthenoteratozoospermia (OAT); sex chromosomal abnormalities.

INTRODUCTION

Since the introduction of intracytoplasmic sperm injection (ICSI), several reports were published on the

potential genetic risks of this technique (1–5). Although the number of reports on follow-up studies of ICSI children is small, it is assumed that the only documented genetic risk for these children so far is an increase of approximately 1% of sex chromosomal abnormalities (6). Other follow-up studies on psychosocial development in these patients show controversial results (7–11), although most of them report normal development.

It is not clear yet how the small increase of sex chromosomal abnormalities in ICSI children should be explained. One of the possible explanations is that a small number of ICSI males have an increased proportion of spermatozoa with a sex chromosomal aneuploidy due to an undetected low level of constitutional and/or gonadal sex chromosomal mosaicism. Indeed, recently a 47,XXY fetus was reported after ICSI in a man with an elevated frequency of 24,XY spermatozoa (12). Accordingly, one would expect that at least patients with proven (mosaic) constitutional sex chromosomal abnormalities have an increased number of sex chromosomally aneuploid sperm cells. However, to the best of our knowledge only karyotypically normal ICSI pregnancies from Klinefelter or 47,XXY males have been reported so far (13–17).

In the present study we collected sperm samples of six ICSI males with a numerical and one with a structural sex chromosomal abnormality and studied the sperm cells by fluorescent in situ hybridization (FISH).

MATERIALS AND METHODS

Patients

All patients had very severe sperm parameters and all were candidates for ICSI treatment. Sperm parameters and lymphocyte karyotypes are shown in Table 1.

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Table I. Sperm Parameters and Karyotypes^a

Patient karyotype	<i>n</i>	18,X (%)	18,Y (%)	Total aneuploidy (%)	X/Y aneusomy (%)	VCM ^b (×10 ⁶)	Morph ^c (%)
47,XXY	209	97 (46.4)	100 (47.8)	12 (5.7)	8 (3.8)	<0.1	10
46,XY[4]/47,XXY[44]/48,XXXY[2]	207	103 (49.7)	96 (46.3)	8 (3.8)	8 (3.8)	<0.1	NA ^d
46,XY[4]/47,XXY[30]	255	121 (47.4)	122 (47.8)	12 (4.7)	11 (4.3)	<0.1	NA
46,XY[30]/47,XXY[2]	461	227 (49.2)	187 (40.6)	47 (10.2)	15 (3.3)	<0.1	0
45,X[16]/46,XY[25]	357	167 (46.7)	181 (50.7)	9 (2.5)	8 (2.2)	4.5	11
47,XXY	1000	471 (47.1)	457 (45.7)	72 (7.2)	48 (4.8)	0.5	9
46,X,inv(Y)	1000	514 (51.4)	474 (47.4)	12 (1.2)	9 (0.9)	1.7	0
Total	3489	1700 (48.7)	1617 (46.3)	172 (4.9)	107 (3.0)		
OAT males ^e (<i>n</i> = 10)	116–626	60–295 (42.2–51.7)	53–292 (41.4–50.0)	3–46 (2.6–11.0)	0–37 (0–9.5)		
Total	2810	1339 (47.7)	1264 (45.0)	207 (7.4)	132 (4.7)	0.1–4.6	0–15
Controls ^f (<i>n</i> = 3)	1000	480–505 (48.0–50.5)	483–501 (48.3–50.1)	8–19 (0.8–1.9)	6–10 (0.6–1.0)		
Total	3000	1489 (47.9)	1472 (49.1)	39 (1.3)	24 (0.8)	63–200	30–47

^a The percentages of chromosomally normal and abnormal sperm cells observed in semen samples of 7 ICSI candidates with constitutional sex chromosomal abnormalities and OAT, 10 males with OAT with a normal constitutional karyotype, and 3 normospermic controls, as detected by FISH using DNA probes specific for chromosomes 18, X and Y.

^b Total number of motile sperm cells in the ejaculate used: volume × concentration × fraction motile sperm cells.

^c The percentage morphologically normal sperm cells in the ejaculate used.

^d NA, Not analyzed: sperm count too low for reliable analysis of sperm morphology.

^e Compiled data of 10 OAT males (36).

^f Compiled data of 3 normospermic donors.

Cytogenetic Investigation on Lymphocytes

Metaphase spreads of phytohemagglutinin-stimulated peripheral lymphocytes were prepared according to standard procedures. Chromosomes were analyzed from G-banded preparations.

Analysis of Spermatozoa by FISH

Preparation of sperm samples and FISH on spermatozoa were performed as described (18). In short, the patient's semen samples that were used for FISH were prepared by the same procedure as the samples that were used for ICSI and stored at -20°C until used for further experiments. Three directly labeled probes were used, obtained from: a spectrum green-labeled centromere 18 probe (CEP 18), which served as a control probe, a spectrum orange-labeled centromere X probe (CEP X), and an aqua-blue-labeled Yq heterochromatin probe (DYZ III). Using phase contrast bright-field microscopy (Zeiss Axioskop), only apparently normal spermatozoa were selected for scoring. Sperm morphology was regarded as normal if the size and shape of the head were normal, if it had distinct boundaries, and if the sperm cell had an intact tail.

After selection of normal spermatozoa, the same cells were scored by FISH by switching to fluorescence illumination with a multiple filter set containing both a combination filter for simultaneous visualization of green, red, and blue signals and single-band-pass filters. A sperm cell was scored as having two fluorescent spots for a particular probe if the spots were bright and separated by at least one spot diameter. Only those slides in which at least 97% of the sperm cells showed one or more hybridization spots were used for scoring. There was no apparent difference between any of the patients studied and the controls with regard to hybridization efficiency. The cells without signals were excluded from the scoring. Cells were scored either as normal (18,X or 18,Y) or as abnormal. Distinction was made between cells with a sex chromosome aneuploidy (X/Y aneusomy, Table I) and cells with any abnormality (total aneuploidy).

If the slides contained sufficient morphologically normal spermatozoa after the FISH procedure, 1000 cells were scored. In most patients, however, only very small numbers of morphologically normal cells were observed after FISH, either due to the very low sperm count or to the loss of sperm cells during density

gradient centrifugation, or to both. In those patients we were not able to score 1000 cells, so we scored as many spermatozoa as possible (Table I).

RESULTS

The aneuploidy rates of the patients and controls are shown in Table I. In the samples of the patients with the numerical sex chromosomal abnormalities 2.5 to 10.2% of the spermatozoa were aneuploid for one or more of the probes used. The aneuploidy rates for chromosome 18 or either one of the sex chromosomes were not significantly different. FISH results from a previous study on ten males with oligoasthenoteratozoospermia (OAT) were included for comparison (Table I). No prevalence of any form of aneuploidy was observed in these patients (data not shown). These results were similar to the results obtained from the six males with the numerical sex chromosomal abnormalities ($\chi^2 = 1.88$, not significantly different). In semen samples of three normal fertile controls, significantly lower aneuploidy rates for any of the probes were observed, that is, 0.8 to 1.9% ($\chi^2 = 102$, $P < 10^{-10}$ for males with numerical sex chromosomal abnormalities vs. controls; $\chi^2 = 131$, $P < 10^{-10}$ for OAT males vs. controls). Again no differences in aneuploidy rates for either one of the sex chromosomes or chromosome 18 were observed. The aneuploidy rate in the patient with the inversion of the Y-chromosome (1.2%) was similar to that of normal controls.

DISCUSSION

Many papers were published on meiotic studies in males with either 47,XXY Klinefelter's syndrome (19–21), mosaic Klinefelter's syndrome (22–25), a 47,XYY karyotype (23,24,26–30) or 45,X/46,XY mosaicism (31). The aneuploidy rates of sex chromosomes in spermatozoa from the patients described in these reports, the majority of which were case reports, show a considerable range from 0 to 20% in patients with (mosaic) Klinefelter's syndrome and from 1.1 to 15% in patients with (mosaic) 47,XYY. It should be noted that along with this variety of results, there is a variety in sample treatments and techniques that were used to study the spermatozoa.

Similarly, Guttenbach *et al.* (32), who reviewed the literature on the analysis of structural and numerical chromosomal abnormalities in sperm of normal men and carriers of constitutional chromosome aberrations,

concluded that differences in techniques used by different investigators made it difficult to directly compare all data obtained from different studies. Moreover, the distribution of sperm karyotypes in patients with apparently identical constitutional karyotypes does not always need to be the same. Hence, the available literature does not provide us with clear figures on the risk of producing offspring with a sex chromosomal abnormality after ICSI for men with a constitutional sex chromosomal abnormality.

Since in our ICSI program constitutional sex chromosomal abnormalities are diagnosed in a small percentage of OAT men (33), we designed a study to try to obtain such risk figures. We therefore chose to do FISH studies on spermatozoa treated the same as if they were used for ICSI, as much as possible. Thus, the results obtained only give an indication of the risk in ICSI and do not provide information as to the distribution of chromosomal abnormalities in the native sperm sample.

Sperm aneuploidy rates in males with a numerical constitutional sex chromosomal abnormality were slightly increased (mean 4.9%) as compared to normal fertile controls (mean 1.3%). Aneuploidy rates in the patient with the inversion of the Y chromosome were similar to those in fertile controls. This illustrates the interindividual variability and suggests that structural abnormalities of the Y chromosome have a different meiotic effect compared to numerical abnormalities.

Aneuploidy rates in the group of OAT males (mean 7.4%) are in agreement with those reported in a recent paper by Pang *et al.* (34) in which semen samples of nine OAT males were studied by FISH with a large number of probes. These authors reported aneuploidy rates for chromosomes 18,X and Y from 7.0 to 9.8%

Interestingly, our results also are in agreement with the results of recent meiotic studies on testicular biopsies from Klinefelter mice that showed a general increase of chromosomal aneuploidy in the few gametes that were produced and not particularly for X and Y, indicating that the gametogenesis in these mice is probably impaired due to a mechanism common to many infertile (mice) males and not specific for Klinefelter's syndrome (35).

There is an apparent discrepancy between the sex chromosomal aneuploidy rates in the ICSI males (4.7%) and the fertile controls (0.8%) on one hand and pregnancy outcome on the other hand in that there is "only" about 1% increase of sex chromosomal aneuploidy in ICSI offspring. This may be explained by an embryonic arrest or pregnancy loss in the majority of those conceptions where an aneuploid spermatozoon

was involved. Alternatively, the observed 1% increase may be an underestimation of the real increase, since this figure was derived from an ICSI population in which oligospermia was not very well defined and patients with quite substantial levels of sperm cells were included in the analysis.

Thus, our data indicate that the risk of producing offspring with a sex chromosomal abnormality for ICSI males with a constitutional numerical sex chromosomal abnormality is similar to the risk for ICSI males with a normal constitutional karyotype. Therefore, we believe that the results of our study do not provide us with strong arguments to advise these men to refrain from ICSI treatment as long as the couples choose to have prenatal diagnosis, realizing that in individual situations the risk may be different.

ACKNOWLEDGMENTS

We thank Dr. Lodewijk Sandkuyl for help in the statistical analysis of our data and for critically reading of the manuscript.

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