CASE REPORT

Study of aneuploidy in large-headed, multiple-tailed spermatozoa: case report and review of the literature

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Objective: To determine the meiotic segregation in large-headed, multiple-tailed spermatozoa.
Design: Analysis of sperm nuclei by fluorescence in situ hybridization (FISH).
Setting: University hospital.
Patient(s): A 34-year-old man with 100% morphologically abnormal spermatozoa.
Intervention(s): Dual-color FISH for chromosomes 13 and 21 and triple-color FISH for chromosomes X, Y, and 18 were performed.
Main Outcome Measure(s): Aneuploidy rates.
Result(s): More than 99% of the spermatozoa had abnormal content for chromosomes X, Y, 13, 18, and 21. Diploidy, triploidy, and tetraploidy rates were found to be 18.42%, 6.14%, and 33.99% in triple-color FISH and to be 16.09%, 16.28%, and 38.95% in dual-color FISH.
Conclusion(s): Our results and those from other investigators show that large-headed, multiple-tailed spermatozoa are associated with a high rate of polyploidy and aneuploidy. Intracytoplasmic sperm injection should not be recommended to those patients, not only because of its low success rate but also because of its high genetic risk. (Fertil Steril® 2008;90:1201.e13–e17. ©2008 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy, large-headed spermatozoa, multiple-tailed spermatozoa, meiotic segregation, male infertility, FISH

Men with azoospermia and oligoasthenoteratozoospermia (OAT) have a higher risk of carrying a constitutional chromosomal aberration than do fertile men (1, 2). Furthermore, chromosomal aneuploidies are more frequent in spermatozoa among infertile men with a normal karyotype (1, 3). Several investigators also have reported an association between chromosomal aneuploidies and morphological defects in spermatozoon heads (4–7).

In 1977, Nistal et al. (8) reported on an infertile man in whom a high number of spermatozoa showed low motility and had irregular large heads, with a variable number of tails ($\leq$4 flagella). Men presenting with teratozoospermia associated with large heads and multiple-flagella spermatozoa are now considered to have the *macrocephalic sperm head syndrome,* also known as *meiotic division deficiency* (9). This condition accounts for <1% of male infertility, its frequency being estimated at 0.27% (10).

Quantification of the DNA content in four infertile men with large-headed and multiple-tailed spermatozoa revealed a large increase in nuclear volume (11). Several infertile men with large-headed, multiple-tailed spermatozoa have now been reported, and the chromosomal content of their spermatozoa has been analyzed by fluorescent in situ hybridization (FISH). In the present study, we report on our FISH results in a patient with large-headed, multiple-tailed spermatozoa and review the literature.

MATERIALS AND METHODS

Patients

A couple (34-y-old man and 29-y-old woman) presented with a 4-year history of infertility. Both partners had a normal blood lymphocyte karyotype. An ICSI attempt had been scheduled some months earlier but was canceled because all the spermatozoa were macrocephalic and had multiple tails. Before this study, the patient was informed about the investigations and gave his consent.

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Sperm concentration and motility were evaluated according to the recommendations of the World Health Organization (12), and sperm morphology was evaluated by strict criteria (13). The characteristics of sperm examination are given in Table 1. The multiple anomalies index (the total number of anomalies divided by over the total number of abnormal spermatozoa) was 2.3 (normal value, 1.6) (14).

Analysis of Aneuploidy

The sperm sample was washed in phosphate-buffered saline, and 20 μL of sperm was dropped and fixed on a slide with Carnoy's solution (methanol–acetic acid; 3:1, vol/vol). The sperm nuclei were partially decondensed for 3 minutes by using a solution of NaOH (1 mol/L), then they were washed in 2× SSC for 10 minutes.

The sperm sample was analyzed by using dual FISH (chromosomes 13 and 21) with a specific cocktail probe of 13q14 and 21q22 (Abbott, Rungis, France) and by using triple FISH (chromosomes X, Y, and 18) with specific alphoid probes of the X chromosome (probe DXZ1, spectrum green; Abbott), Y chromosome (probe DYZ3, spectrum orange; Abbott), and chromosome 18 (D18Z1, spectrum aqua; Abbott).

Before hybridization, the sperm DNA slides were immersed in a jar of 2× SSC–0.4% NP40 solution for 30 minutes at 37°C and then were passed through an ethanol series of increasing concentrations before being allowed to air dry.

The denaturation was performed simultaneously on sperm nuclei and probes for 1 minute at 72°C. The slides were incubated overnight at 37°C. Posthybridization washes included 40 seconds in 0.4× SSC–0.3% NP40 at 72°C, followed by 15 seconds in 2× SSC–0.1% NP40 at room temperature.

The slides were counterstained with 4′,6-diamino-2-phenylindole and observed by using a Zeiss Axioplan microscope (Zeiss, Le Pecq, France), with the appropriate set of filters. Subsequent image acquisition was performed by using a CCD camera with Isis (In Situ Imaging System; MetaSystems, Altlussheim, Germany) (15–18).

TABLE 1

Light-microscope characteristics of sperm examination.

Characteristic	Data
Volume (mL)	9
Concentration (per mL)	2.8×10^6
Abnormal forms (%)	100
Vital spermatozoa (%)	40
Progressively motile (%)	0
Shaking (%)	13
Abnormal acrosome (%)	42
Macrocephalic forms (%)	62
Abnormal flagella (%)	54
Cytoplasmic droplets in midpiece (%)	22

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RESULTS

Table 2 shows the results of the meiotic segregation study. A total of 2,156 spermatozoa could be analyzed, 1,124 in triple-color FSH and 1,032 in dual-color FISH. When dual FISH (13, 21) and triple FISH (X, Y, 18) were used, only 1.07% and 0.71% of the spermatozoa were found to have normal chromosomal content, respectively.

More than 99% of the spermatozoa had abnormal content for chromosomes X, Y, 13, 18, and 21. Diploidy, triploidy,

TABLE 2

Results of sperm aneuploidy in a patient with large-headed multiple-tailed spermatozoa.

Parameter	Frequency (%)
X-18[a]	0.62
Y-18[a]	0.09
X-Y-18-18	13.17
X-X-18-18	3.11
Y-Y-18-18	2.14
X-X-Y-18-18-18	3.29
X-Y-Y-18-18-18	2.85
X-X-Y-Y-18-18-18-18	33.99
X-X-Y-Y-18-18	7.03
X-Y-18	5.69
X-X-Y-18-18	4.8
X-Y-Y-18-18	3.74
X-X-Y-18	3.47
X-Y-18-18-18	3.11
X-Y-Y-18	2.05
X-Y-18-18-18-18	1.96
X-X-Y-Y-18-18-18	1.96
X-X-Y-Y-18	1.69
X-18-18	1.16
Others	4.08
13-21[a]	1.07
13-13-21-21	16.09
13-13-13-21-21-21	16.28
13-13-13-13-21-21-21-21	38.95
13-13-13-21-21-21-21	5.62
13-13-21-21-21-21	4.55
13-13-13-13-21-21-21	3.49
13-13-21-21-21	2.71
13-13-13-21-21	2.52
13-13-13-13-21-21	2.62
13-21-21	1.07
Others	5.03

[a] Normal.

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