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No intraindividual variation of disomy rate in sperm samples

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Abstract We assessed possible inter- and intraindividual variations in the frequency of disomy in sperm cells from three men with abnormal sperm analysis parameters. Mono- and dual-color fluorescence in situ hybridization (FISH) was applied to sperm cells from different samples of the men. Four men with a normal sperm profile were used as controls. The samples were taken separately over a period of 6 months. FISH probes used for the disomy rate analysis were clones from the satellite region of chromosomes 8, 18, X, and Y. The study group showed a significantly higher disomy rate compared with the control group, whereas there was no significant difference in the disomy rate between three different samples from the same individuals. These results suggest that the sampling time has no importance in assessing the rate of nondisjunction in sperm cells.

Key words Disomy rate · Intraindividual variation · Sperm cell

Introduction

Aneuploidy is one of the most significant genetic hazards in humans. Most genetically imbalanced embryos are lost at an early stage of pregnancy, with an incidence of 80% or higher (Edmonds et al. 1982, 1986; Burgoyne et al. 1991). Aneuploidy also affects 1 of 300 live births (Jacobs 1992), and can be caused by fertilization by an abnormal sperm cell. Therefore, it seems reasonable to study the incidence of aneuploidy in gametes, so that those lost prior to implantation are not overlooked (Holmes and Martin 1993). Cur-

rently, the estimated incidence of aneuploidy in human gametes is based mainly on studies of spermatozoa because a sufficient number of oocytes is difficult to obtain. Several sperm-analysis parameters are associated with increased incidences of chromosome abnormalities in sperm cells. Men with oligo-astheno-teratozoospermia (OATS) produce sperm cells with an increased frequency of aneuploidy when compared with fertile men.

The rate of aneuploidy in sperm nuclei was evaluated by using fluorescence in situ hybridization (FISH) with chromosome-specific DNA probes (Burgoyne et al. 1991; Holmes and Martin 1993; Chevret et al. 1994; Martin et al. 1996; Egozcue et al. 1997; Finkelstein et al. 1998; Morel et al. 1998; Shi and Martin 2000). In most studies of infertile men with OATS, an increased rate of disomy in various chromosomes has been observed (Lahdetie et al. 1997; Bernardini et al. 1997; McInnes et al. 1998; Pang et al. 1999; Ushijima et al. 2000; Vegetti et al. 2000). Egozcue et al. (1997) applied FISH for chromosomes X and Y to sperm from couples who underwent in vitro fertilization (IVF) and found a higher proportion of X+Y-bearing spermatozoa in semen (1.26%) capable of fertilizing oocytes only by intracytoplasmic sperm injection (ICSI) than in that by conventional IVF (0.37%, $P < 0.001$). The incidence of XX- and YY-bearing sperm nuclei was also significantly higher in the ICSI group (0.75%) than in the IVF group (0.22%, $P < 0.02$) (McInnes et al. 1998). Interindividual variations in the frequency of disomy in sperm cells has already been described (Chevret et al. 1994; Morel et al. 1998; Shi and Martin 2000; Martin et al. 1996). In addition, possible intraindividual variations were described in patients with Hodgkin disease who were treated with chemotherapy. A higher rate of aneuploidy was observed following chemotherapy, declining to pretreatment levels within approximately 100 days after the completion of the therapy (Robbins et al. 1997). However, intraindividual variations in nontreated men have not been described previously.

In the present study, we analyzed inter- and intraindividual variations in three men with abnormal sperm analysis parameters, and compared them to a group of four men with a normal sperm profile.

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Subjects and methods

Individuals studied

We evaluated a possible variation in the frequency of disomy for chromosomes 8, 18, X, and Y, using chromosome-specific FISH probes. The study group consisted of three infertile men. They included a 35-year-old karyotypically normal man with astheno-teratozoospermia (only 3% normal sperm cells with poor motility); a 49-year-old man with normo-zoospermia (only 10% fertilization in IVF-ICSI) associated with a balanced Robertsonian translocation [45,XY,t(13;21)]; and a 38-year-old karyotypically normal man with severe astheno-teratospermia. Three samples were taken at 3-month intervals from each of the first two individuals, and only two samples were taken from the third individual. Four normal fertile men served as a control group. Their sperm was taken only once.

Pretreatment of sperm nuclei

Sperm was placed on glass slides and the slides were incubated in 100 μ l freshly diluted 10 mM dithiothreitol (Sigma, St. Louis, MO, USA) in 0.05 M Tris buffer at -4°C for 25–50 min. The slides were then washed twice with $2 \times \text{SSC}$ for 10 min, passed through a cold ethanol series (70%, 80%, 100%), air-dried, and observed under a phase-contrast microscope.

Mono- and dual-color FISH

Random sperm disomy was assessed by mono- and dual-color FISH in the three infertile and four fertile men. Probes used for FISH were CEP 8 (α satellite D8Z2, spectrum orange, Vysis) for chromosome 8, CEP 9 (α satellite DNA, spectrum orange) for chromosome 9; CEP 18 (α satellite DNA/D18Z1, spectrum green) for chromosome 18; CEP X (α satellite DXZ1, spectrum green) for X chromosome; and CEP Y (α satellite DYZ3, spectrum orange) for Y chromosome. Mono-color FISH was performed for chromosomes 8, 9, and 18 on sperm and leukocyte nuclei to prevent any overlapping signals between different probes due to the small size of sperm cells, which were taken from samples left over from the infertility laboratory. To find out whether nondisjunction occurred and in which meiotic divisions, we performed dual-color FISH using probes for X and Y chromosomes. In addition, to rule out sperm diploidy, we performed dual-color FISH using probes for chromosomes 9 and 18, as reported previously (Spriggs et al. 1995). Slides were first denatured in 70% formamide, $2 \times \text{SSC}$, at 73°C for 2 min. Prior to hybridization, the labeled probes in a hybridization mixture were heated to 75°C and put on ice. The hybridization mixture (10 μ l) was applied to each slide under a coverslip and hybridization proceeded overnight at 37°C . The slides were washed at 42°C in 70% formamide, $2 \times \text{SSC}$, for 20 min; then in $2 \times \text{SSC}$ for 10 min; and finally in $4 \times \text{SSC}$, 0.1% Tween-20, for 5 min.

FISH signals were observed under an Olympus BX50 epifluorescence microscope (Olympus, Tokyo, Japan) equipped with a triple band pass filter for DAPI/Texas red and Fluorescein isothiocyanate.

A total of 55055 sperm cells from the study group and 30080 from the control group were analyzed. Diagnosis of disomy in sperm cells was based on the presence of two signals with the same intensity that were separated from each other at a distance greater than the diameter of the signal itself. Nullisomic cells were not directly scored, because their frequency was expected to be equivalent to that of disomic cells. Each sample underwent an expanded semen evaluation that included routine light microscopic sperm observations, biochemical evaluation of seminal plasma markers, and quantitative ultramorphological analysis of subcellular organelles in the sperm cell.

Statistical analysis

The two-sample *t* test and nonparametric test were applied for testing differences between the two groups for quantitative parameters. A multiple comparison test (Duncan's method) was applied for testing quantitative parameters between the groups. All tests applied were two-tailed, and a *P* value of 5% or less was considered statistically significant. The data were analyzed using SAS software (SAS Institute, Cary, NC, USA).

Results

The hybridization efficacy was approximately 95%. The rate of disomy in sperm cells analyzed with all probes was significantly higher in the three infertile men than in the controls ($P < 0.01$) (Tables 1 and 2). The mean disomy rates detected with X and Y chromosome probes among three infertile men were compared. More disomies were observed with the X or Y chromosome probe alone than with those detected with both probes together, suggesting a higher rate of nondisjunction in the second meiotic division than in the first meiotic division.

We found no significant difference in the rate of disomy between the three samples, which were taken from the same individual over a period of 6 months. (Table 2). It seems that disomy occurs at random, because the rate was similar among different chromosomes.

Discussion

In the present study, we tested whether intraindividual variations of the disomy rate exist in sperm cells. The intraindividual variation has not been assessed previously, and only a comparison of the disomy rate between infertile and normal men has been described. In contrast to previous reports of a higher rate of disomy in sperm cells for sex chromosomes than for autosomes in infertile men undergo-

Table 1. Disomy rate detected by FISH analysis in sperm cells of the control group

Case	Probes for chromosome									
	8		18		X		Y		XY	
	NDC	TNCO	NDC	TNCO	NDC	TNCO	NDC	TNCO	NDC	TNCO
1	11	2000	6	2004	3	1057	4	943	3	2003
2	13	2005	11	2017	5	1033	4	947	2	2002
3	4	2009	7	2006	4	1042	5	960	5	2007
4	7	2001	10	1999	6	1017	3	988	6	2011
Mean	9	2004	8	2006	4	1037	4	959	4	2006

FISH, Fluorescence in situ hybridization; NDC, number of disomic cells; TNCO, total number of cells observed

Table 2. Disomy rate detected by FISH analysis in different sperm samples from three infertile men (A, B, and C)

Sample	Probes for chromosome									
	8		18		X		Y		XY	
	NDC	TNCO	NDC	TNCO	NDC	TNCO	NDC	TNCO	NDC	TNCO
A1	107 (6)	1893	122	1878	64	1103	53	715	65	1818
A2	106 (6)	1894	80 (4)	1920	33 (3)	1094	25 (3)	795	53 (3)	1889
A3	92 (5)	1908	89 (5)	1911	51 (4)	1227	14 (2)	660	48 (3)	1887
B1	78 (4)	1922	55 (3)	1945	50 (4)	1155	40 (6)	715	40 (2)	1870
B2	81 (4)	1919	56 (3)	1944	35 (3)	1279	40 (6)	607	39 (2)	1886
B3	75 (4)	1925	72 (4)	1928	27 (3)	1025	44 (5)	880	24 (1)	1905
C1	90 (5)	1910	81 (4)	1919	41 (3)	1221	44 (6)	665	29 (2)	1886
C2	71 (4)	1921	72 (4)	1928	37 (3)	1232	29 (4)	681	21 (1)	1913

The number in parentheses is proportion of disomic cells

FISH, Fluorescence in situ hybridization; NDC, number of disomic cells; TNCO, total number of cells observed

ing ICSI (Pang et al. 1999; Ushijima et al. 2000; Vegetti et al. 2000), we did not find such a higher rate for sex chromosome disomy. Vegetti et al. (2000) evaluated patients with OATS and reported that the aneuploidy rate in the gametes does not show any consistent patterns of autosomal gain or loss. Similarly, we found an erratic picture for all chromosomes analyzed.

It has been suggested that at least 10000 spermatozoa have to be analyzed to assess the aneuploidy rate (Jacobs 1992; Spriggs et al. 1995; Martin et al. 1996; Lahdetie et al. 1997; Egozcue et al. 1997; Bernardini et al. 1997; McInnes et al. 1998; Pang et al. 1999; Ushijima et al. 2000; Vegetti et al. 2000; Shi and Martin 2000; Egozcue et al. 2000). However, it may be difficult to obtain sufficient numbers of spermatozoa from oligospermic men. In recent studies, the analysis of less than 10^4 spermatozoa achieved a high statistical significance and substantial conclusions (Vegetti et al. 2000). Our results of 2000 sperm cells from infertile men showed a highly significant disomy rate compared with the control individuals. In addition, the difference between samples from the same individuals did not reach statistical significance. These findings are of clinical importance for three reasons. First, when attempting to assess the nondisjunction rate in the gametes of men, sampling time is not important unless the patient is receiving certain drugs or therapy. Second, the scoring of some 2000 sperm cells per person and per probe set is sufficient. Third, FISH in human spermatozoa is an accurate and reliable method for deter-

mining the frequency of aneuploidy, as indicated by Egozcue et al. (2000).

The observed elevated frequency of autosomal non-disjunction in sperm cells, as already described in eggs, may not have a marked impact on the presentation of autosomal abnormalities in newborns, because all resulting autosomal monosomies and most trisomies are lethal and are miscarried. The contribution of aneuploid spermatozoa to fertilization and early spontaneous abortions is unclear. However, frequent aneuploidy in the spermatozoa of OATS men in whom there is a high miscarriage rate suggests that they are at increased risk for transmitting abnormal genetic material to their offspring. Further larger studies are required to support this hypothesis.

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