

Reproductive Failure in Patients With Various Percentages of Macronuclear Spermatozoa: High Level of Aneuploid and Polyploid Spermatozoa

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ABSTRACT: The aim of this study was to describe the association between various percentages of macronuclear spermatozoa (MNSs), sperm chromosomal abnormalities, and reproductive failure in 4 patients. One patient had a familial history of perinatal deaths. Patients were selected according to the coexistence of normal-sized spermatozoa and MNSs (19%, 22%, 29.5%, and 49.7%). Fluorescent in situ hybridization (FISH) on spermatozoa and semiautomated analysis of nuclear surface were assessed. All patients were characterized by an oligoasthenozoospermia. Three patients had a prevalence of irregular MNSs and prevalence of nondisjunction at the first meiotic division. One patient had a prevalence of regular MNSs and a prevalence of nondisjunction at the second meiotic division. FISH also showed a high rate of polyploidy and various rates of aneuploid sperm. The percentage of sperm with abnormal chromosome complements (25.6%, 43.6%, 51.4%, 71.7% with 3-color FISH) was higher than the percentage of MNSs. A population

of apparently normal-sized spermatozoa that could be used for intracytoplasmic sperm injection (ICSI) was aneuploid. Sperm nuclear surface analysis revealed either a shift toward elevated values or distinguished 2 sperm subpopulations: normal and macronuclear. Patients underwent 7 ICSI cycles. The fertilization rate was low for 3 patients (50%, 40%, 50%) and normal for 1 patient (83.3%). Pregnancy rate per transfer was low (14.3%). The present study shows that the macronuclear phenotype can manifest a variety of clinical aspects. It is also shown that mild rates of MNSs impair fertility and constitute a risk of chromosomal abnormality for the embryos and a risk of perinatal death. We suggest conducting FISH on spermatozoa and genetic counseling for a couple when the percentage of MNSs reaches 20% in at least 1 spermogram.

Key words: Chromosomal abnormalities, meiosis, FISH, nuclear surface.

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Typical human macronuclear spermatozoa (MNSs) (also termed large-headed or macrocephalic spermatozoa) are characterized by a fourfold increase of the nuclear volume, irregular head shape, and 4 flagella (Nistal et al, 1977; Escalier, 1983; In't Veld et al, 1997; Pieters et al, 1998; Kahraman et al, 1999; Benzacken et al, 2001; Devillard et al, 2002; Lewis-Jones et al, 2003). This phenotype is associated with severe male infertility. Yakin and Kahraman (2001) emphasize the genetic risks in the developing conceptus when these spermatozoa are present in the ejaculate of patients who were referred for intracytoplasmic sperm injection (ICSI). Indeed, low rates of fertilization, implantation, and pregnancy were obtained when ICSI was performed with sperm containing MNSs (Kahraman et al, 1999), and preimplantation genetic diagnosis (PGD) detected chromosomal abnormalities in 46.4% of the embryos analyzed (Kahraman

et al, 2004). These reproduction failures could mainly result from the high incidence of chromosomal abnormalities in the spermatozoa. Fluorescent in situ hybridization (FISH) reveals a failure of 1 or the 2 meiotic divisions giving rise to diploid spermatozoa (Yurov et al, 1996) or tetraploid spermatozoa (Pieters et al, 1998; Devillard et al, 2002). In all cases, polyploidy is associated with aneuploidy of the sperm cells (Yakin and Kahraman, 2001; Devillard et al, 2002; Vicari et al, 2003), which could be explained by the fact that some spermatocytes can perform 1 or 2 meiotic divisions but the meiotic division spindles are only partially functional.

Aside from patients exhibiting 100% MNSs, infertile patients exhibiting both large-headed spermatozoa (32% to 64%) and various nuclear sizes have been reported (Yurov et al, 1996). In all cases, total teratozoospermia was always detected. Until now, patients with mild percentages of MNSs have been poorly explored. Viville et al (2000) and Vicari et al (2003) reported that the presence of MNSs is associated with an increased incidence of chromosomal abnormalities. Here, we report a detailed semen analysis of 4 infertile human males undergoing an ICSI program and showing various percentages of sperm abnormality. One of them

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Table 1. *Reproductive and semen characteristics of the patients studied**

Patients	Concentration (10 ⁶ /mL)	Total No. of Sperm	Motility (%)†	Typical Forms (%)	Macronuclear Spermatozoa (%)	No. of ICSIs	No. of Pregnancies After ICSI
4	8.2	23.7	7–25–15	30	29.5	2	1
5	6.2	23.6	3–30–25	31	22	1	0
6	1.3	2	0–7–12	8.5	49.7	2	0
11	1.7	3.9	2–7–10	4	19	3	0

* All patients presented with primary infertility of the couple and the man. For semen characteristics, mean values are shown. Patients 4, 5, and 11 had 2 spermograms, and patient 6 had 4 spermograms.

† Rapid progressive motility–slow progressive motility–nonprogressive motility.

had a predominance of regular large-headed spermatozoa and a family history of reproductive failure. The spermatozoa of all patients were investigated by FISH and semiautomated analysis of nuclear surface. ICSI was performed with selected normal-sized and typical spermatozoa.

Materials and Methods

Patients

Study participants were selected from 5556 patients who presented to our reproductive center with issues of infertility between November 1993 and July 2003. A total of 7292 spermograms were obtained from these 5556 patients, and the mean percentage of MNSs per spermogram was determined to be 1.26. Of the 5556 patients, 15 (2.7/1000) displayed 20% or more MNSs in the first spermogram. In a first group of 3 patients (0.5/1000), 100% of spermatozoa presented with an MNS phenotype. More frequently (12 patients, 2.15/1000), various percentages of MNSs were found (20% to 62%). Four of these patients agreed to undergo cytogenetic analysis of spermatozoa by FISH. At that time, new semen analysis and the percentage of MNSs were reevaluated. The patients were included in this study if the second spermogram confirmed the presence of MNSs even if the percentage was less than 20%, which is the case for patients 5 and 11, who had 19% and 17% MNSs in the second analysis, respectively. Unlike In't Veld et al (1997), we were unable to observe large variations in the percentage of MNSs.

Patients 4, 5, 6, and 11 were 27, 36, 36, and 37 years old, respectively. In all cases, the men presented with problems of infertility (Table 1). No direct consanguinity was observed between the relatives of the men. Analysis of the pedigree of patient 11 showed that 5 siblings died during the first days of life from unknown causes. Interestingly, a brother of the patient also suffered from primary infertility but refused to submit to semen analysis. Three patients (patients 4, 5, 6) had a normal karyotype. For patient 11, 50 mitoses were analysed, 2 had the karyotype 47,XY,+21, 1 mitosis was 47,XXY, and 1 mitosis was 45,X. Complementary study of 20 mitosis showed chromosomal loss on 4 mitoses (10-, 15-, 18-, 21-). Interphase FISH with specific probes for chromosomes X, 8, 13, 18, and 21 showed that 5% of the nuclei presented with either a monosomy for chromosome 18 or a trisomy for

chromosome 21. This percentage was considered nonsignificantly increased by cytogeneticists, and a constitutional mosaicism was eliminated.

All women had normal menstrual cycles, hysterosalpingography, and hormonal assessment. The women's ages were 24, 37, 38, and 35 for patients 4, 5, 6, and 11, respectively.

Semen Analysis

Sperm concentration and motility were evaluated according to the World Health Organization guidelines (1999). One hundred spermatozoa were evaluated for each patient according to strict criteria described by Kruger et al (1986).

Semiautomated Analysis of Sperm Nuclear Surface and its Statistical Treatment

Analysis of sperm nuclear surface was performed for the patients, 3 healthy controls, and a control patient with 100% MNSs (control 100%). Healthy controls were selected among fertile men showing normal spermograms. Papanicolaou-stained sperm were analysed using a Zeiss Axioplan microscope (63× magnification; Zeiss, Oberkochen, Germany). Images were digitized with a Sony 3CCD XC077 camera (Sony, Tokyo, Japan) and analyzed with the IPS 32 software (Samba Technologies, Grenoble, France). A specific macro was designed: a constant greytone threshold distinguished sperm heads from background, and the operator controlled the limit between head base and flagellum. The nuclear surface was automatically calculated in μm^2 . Measures were analyzed with an analytical software (STAT 2005; Samba) to fit the maximum amount of measurements in a normal distribution. Results for this operation are given as mean, SD, and percentage of measures that fit in the model.

FISH on Sperm

Written informed consents were obtained for each patient before analysis. Cryopreserved semen samples from the patients were thawed and washed twice in 0.01 mol/L Tris, pH 8, for 10 minutes at 600 × g. The in vitro decondensation procedure was performed as previously described (Yurov et al, 1996). In brief, the sperm preparations were treated with 2N NaOH for 2 minutes at room temperature to induce sperm nuclear decondensation.

Three-color and dual-color FISH experiments were performed using Qbiogen classical satellite probe (Qbiogen, Illkirch, France). Three-color FISH experiments were per-

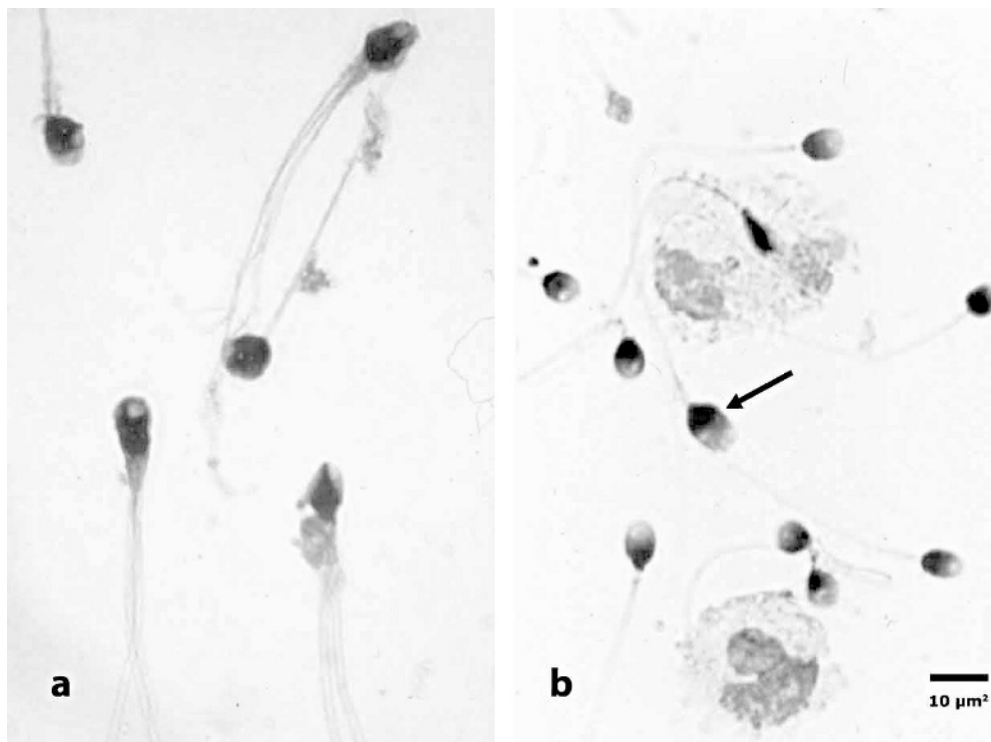


Figure 1. Light microscopic analysis of macronuclear spermatozoa (MNSs) after Papanicolaou staining. (A) Irregularly shaped MNSs with multiple flagella. (B) Regular macronuclear spermatozoa (arrow).

formed with specific probes for chromosomes 9, X, and Y (Chromosome X Alpha Satellite DXZ1 Probe/Red, Chromosome Y Classical Satellite DYZ1 Probe/Green, Chromosome 9 Classical Satellite Probe/Green and Red; Abbott Molecular Inc, Des Plaines, Ill). Dual-color FISH experiments were performed with specific probes for chromosomes 8 and 18 (Chromosome 8 Alpha Satellite D8Z2 Probe/Green, Chromosome 18 Alpha Satellite D18Z1 Probe/Red; Abbott). FISH was performed according to the protocol recommended by the manufacturer. Preparations were counterstained with 4,6-diamino-2-phenylindole (DAPI). Slides were screened using a 100 \times objective on a Zeiss Axioskop microscope equipped with a fluorescein isothiocyanate (FITC)/rhodamine/DAPI triple band-pass filter. Signals of a specific color were considered multiple only when separated by at least 1 signal diameter. The efficiency of hybridization was evaluated from the DAPI-stained spermatozoa without FISH signals.

ICSI Cycles

In vitro fertilization cycles with the ICSI procedure were performed as previously described (Porcu et al, 2003).

Statistical Analysis

Sperm Head Surface Analysis—To determine whether multiple sperm subpopulations (according to nuclear size) coexisted within an ejaculate, a maximum threshold of 20 μm^2 was applied and the remaining population was tested for normal distribution by Shapiro-Wilk W, Anderson-Darling, Martinez-Iglewicz, and Kolmogorov-Smirnov tests. Whenever the

remaining population showed a normal distribution, the existence of 2 sperm populations was assumed.

FISH on Spermatozoa—To compare the difference between sex chromosome and autosome nondisjunction, a χ^2 test was used.

Results

Sperm Analysis

Oligoasthenozoospermia is a general feature of these patients (Table 1). The viability of spermatozoa was normal (more than 50%) for patients 4, 5, and 6. A low viability was found for 2 ejaculates (15% and 6%) of patient 11. Regular analysis of sperm morphology after Papanicolaou staining showed that the mean percentages of MNSs varied from 19% to 49.7%. Two patients (patients 4 and 5) had a normal mean percentage of typical forms whereas it was low for patients 6 and 11. Considering MNSs alone, patients 4, 5, and 6 presented with a prevalence of irregular nuclear shapes (86%, 67%, and 76%) whereas patient 11 presented with a prevalence of regular nuclear shapes (52%) (Figure 1A and B).

Sperm Head Surfaces Analysis

Characteristics of measured spermatozoal surfaces for each control/patient are presented in Table 2. Means of

Table 2. *Characteristics of the sperm nuclear surfaces obtained by semiautomated analysis for 3 healthy controls (1, 2, and 3); patients 4, 5, 6, and 11 of the present study; and a control patient showing 100% macronuclear spermatozoa (control 100%)**

	No. of Sperm Analyzed	Mean Surface (μm^2)	SD (μm^2)	Range (μm^2)
Control 1	366	13.5	2.8	5.8–31
Control 2	416	11.9	1.9	7.1–21.8
Control 3	277	13.4	1.9	7.7–18.6
Control 100%	216	30.4	9.8	7–66
Patient 4	220	16	5.9	7–54.4
Patient 5	171	18.4	6.1	9.5–41.7
Patient 6	108	18.7	6.3	7–37.3
Patient 11	946	11.8	4.2	4.3–42.1

* The population is poorly spread for normal controls (SD, 1.9 to 2.8), widely spread for control 100% (SD, 9.8), and intermediate for patients 4, 5, and 6 (SD, 4.2 to 6.3).

healthy controls (1, 2, and 3) were 13.5, 11.9, and 13.4 μm^2 with low dispersion (ie, SDs were 2.8, 1.9, and 1.9 μm^2 , respectively). Measurements for control 100% had an elevated mean (30.4 μm^2) and a widely spread distribution (SD, 9.8 μm^2). We observed that the distributions of the measured surfaces for the 4 patients

had overall characteristics that were intermediate between those of normal and 100% macronuclear controls. The means were 16, 18.4, 18.7, and 11.8 μm^2 for patients 4, 5, 6, and 11, respectively, with SDs of 5.9, 6.1, 6.3, and 4.2 μm^2 . The observation of the frequency histograms for each surface (Figure 2) shows a shift toward elevated values for patients 5 and 11 but not for healthy controls. Indeed, the percentages of spermatozoa whose surfaces were higher than 20 μm^2 represented only 2.7% (10/366), 0.5% (2/416), and 0% (0/277) of the total for controls 1, 2, and 3 whereas they accounted for 20% (44/220), 32.2% (55/171), 35.2% (38/108), and 4.4% (42/946) in patients 4, 5, 6, and 11, respectively. The presence of a subpopulation of MNSs for 2 patients was assessed in the following manner. Measures for controls showed a normal distribution whereas measures for the patients did not (the normality tests used are listed in “Material and Methods”). However, after suppressing values above 20 μm^2 , the remaining populations showed normal distribution for patients 4 and 5, with means of $13.7 \pm 3 \mu\text{m}^2$ and $15 \pm 2.5 \mu\text{m}^2$. This was not observed in patients 6 and 11 because of the low number of measures above the threshold. Therefore, we concluded

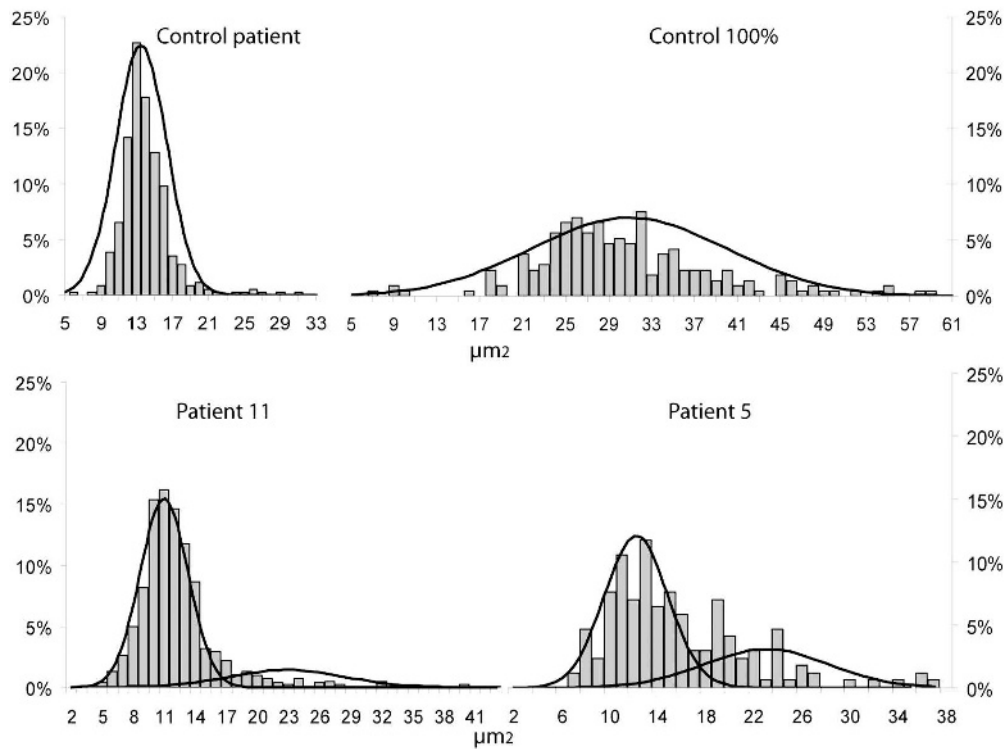


Figure 2. Semiautomated analysis of sperm nuclear surfaces by optical microscopy (μm^2) for a control patient, a patient showing 100% macrocephalic sperm (control 100%), and patients 5 and 11 (n = 366, 216, 171, and 946 sperm, respectively). Histograms show the distribution in percentages. For each population, the STAT 2005 software fitted a maximum of cases within normally distributed populations. All of the cases were fitted for control (mean, $13.5 \pm 2.8 \mu\text{m}^2$) and control 100% (mean, $30.4 \pm 9.8 \mu\text{m}^2$). Two subpopulations were observed for patients 5 and 11.

that, for patients 4 and 5, 2 populations of spermatozoa coexisted (normal and oversized).

FISH on Sperm

The mean efficiency of hybridization was 99.2% (range, 96.4% to 100%). The percentage of chromosomal abnormalities was first evaluated on all spermatozoa despite their size and then on apparently normal-sized spermatozoa (nonmacronuclear). Totals of 1430, 2811, 1955, and 3981 sperm nuclei were scored from patients 4, 5, 6, and 11, respectively.

Three-Color FISH—High percentages of chromosomal abnormalities were found in the 4 patients (51.4%, 43.6%, 71.7%, and 25.6% in patients 4, 5, 6, and 11, respectively) (Table 3). These values are greater than the percentages observed in 3 controls (total abnormalities, 0.9%, 0.3%, and 0.5%, respectively). A great heterogeneity in the distribution of the chromosomes was found in the 4 patients. Nevertheless, diploidy was the prevalent abnormality in patient 11 (64.5% of the chromosomal abnormalities vs 33.2%, 23.6%, and 25.3%, for patients 4, 5, and 6, respectively) whereas complex aneuploidies prevailed in other patients.

Various types of diploidies were present with respect to the sex chromosomes (ie, either 99XY, 99XX, or 99YY). Nevertheless, for patients 4, 5, and 6, nondisjunction at the first meiotic division (99XY) (12.4%, 7.3%, and 14.1% for patients 4, 5, and 6, respectively) prevailed over nondisjunction at the second meiotic division (99XX or 99YY) (4.7%, 2.9%, and 4.0% for patients 4, 5, and 6, respectively). Concerning patient 11, nondisjunction was prevalent at the second meiotic division (9.9% vs 6.6% of nondisjunction at the first meiotic division).

In the 4 patients, the total percentage of chromosome abnormalities with probes specific for chromosomes X,Y and 9 was higher than the mean percentage of MNSs (Tables 1 and 4): 51.4% vs 29.5%, 43.6% vs 22%, 71.7% vs 49.7%, and 25.6% vs 19% for patients 4, 5, 6, and 11, respectively. The mean percentage of aneuploid normal-sized spermatozoa in patients was significantly higher than those observed in the controls (3.5% vs 0.5%, $P < .001$); patient 11 had particularly few aneuploid normal-sized spermatozoa (1.1% in patient 11 vs 12.3%, 4.5%, and 3.7% in patients 4, 5, and 6, respectively).

Dual-color FISH—High percentages of chromosomal abnormalities were found (54.5%, 37.6%, 61.8%, and 11.1% for patients 4, 5, 6, and 11, respectively) (Table 4). These values were greater than the percentages observed in the 3 controls (0.7%, 0.5%, and 0.8%, respectively). As with 3-color FISH, few spermatozoa had more than 46 chromosomes in patient 11 compared

with the 3 others. The mean rate of aneuploid normal-sized spermatozoa was significantly higher than those observed in the controls (2.8% vs 0.7%, $P < .001$); this rate is normal in patient 11 (0.4% vs 5.3%, 3.1%, and 2.4% in patients 4, 5, and 6, respectively).

ICSI Cycles

Seven ICSIs were performed for these 4 patients. Thirty-two metaphase II oocytes were retrieved and injected, and 21 diploid zygotes were obtained. The mean fertilization rate (diploid zygotes/metaphase II oocytes) was not different from the normal values obtained in our laboratory (65.6% vs 36%). Notably, however, this rate was lower for patients 4, 5, and 6 (50%, 40%, and 50%, respectively) than for patient 11 (83.3%). The cleavage and transfer rates (diploid embryos/diploid zygotes) were 90.5% (19/21) and 100% (7/7), respectively. The mean number of transferred embryos per transfer was not different from usual values of our laboratory (1.7 [12/7] vs 1.8). One clinical pregnancy was obtained for patient 4, leading to the delivery of a healthy girl. The clinical pregnancy rate per transfer was lower (14.3%) compared with the mean rate of our center (30%). Nevertheless, the number of patients is too limited for a statistical analysis.

Discussion

MNSs were rarely observed in fertile patients with normal sperm parameters (0.9% [Bujan et al, 1988] and 1.6% [Schwartz et al, 1984]) and in infertile patients (1.26% [our personal data]). Inversely, a high level (ie, about 100%) of irregular and multitailed MNSs is associated with severe male infertility. It appears that the macronuclear phenotype can manifest a variety of clinical aspects. Indeed, Escalier (2002) has already described 3 types of this syndrome, and a new type with perinatal death was described by Benzacken et al (2001). Then, patients with frequencies of 64% and 54% of MNSs and total teratozoospermia were reported by Viville et al (2000) and Vicari et al (2003) whereas Yurov et al (1996) described a patient with 40% spermatozoa displaying MNSs, all of which were of the regular form. A new situation appears in the present study in which 4 patients had mild mean percentage of MNSs (19%, 22%, 29.5%, and 49.7%) (Table 1) associated with typical spermatozoa. Three of these patients had a predominance of irregular MNSs whereas patient 11 showed a predominance of regular forms (52%), which is a rare phenotype only previously described by Yurov et al (1996). This patient, whose familial history revealed that 5 siblings died during the perinatal period, was also

Table 3. Chromosomal constitution frequencies of sperm from the 4 patients studied detected by FISH with probes directed against chromosomes 9, X, and Y*

	Patient			
	4	5	6	11
	Nuclei, No. (%)	Nuclei, No. (%)	Nuclei, No. (%)	Nuclei, No. (%)
9/X	186 (23.2)	460 (29.6)	72 (12.7)	542 (37.1)
9/Y	203 (25.3)	416 (26.8)	89 (15.7)	542 (37.1)
9/9/X/Y	99 (12.4)	114 (7.3)	80 (14.1)	97 (6.6)
9/9/X/X	16 (2)	24 (1.5)	8 (1.4)	67 (4.6)
9/9/Y/Y	22 (2.7)	22 (1.4)	15 (2.6)	78 (5.3)
9/9/9/X/X/Y/Y	24 (3)	30 (1.9)	72 (12.7)	1 (0.07)
Aneuploidies	251 (31.3)	486 (31.3)	232 (40.8)	130 (8.9)
T abnormalities	412 (51.4)	676 (43.6)	407 (71.7)	373 (25.6)
Total	801	1552	568	1457

* Analysis of diploid sperm showed a prevalence of first meiotic division in the patients 4, 5, and 6 (12.4%, 7.3%, and 14.1% vs 4.7%, 2.9%, and 4%, respectively, for the second meiotic division). In patient 11, nondisjunction was prevalent at the second meiotic division (9.9% vs 6.6%). High percentages of total chromosomal abnormalities were found in the 4 patients (51.4%, 43.6%, 71.7%, and 25.6% for patients 4, 5, 6, and 11, respectively). The percentages of aneuploid sperm for patients 4, 5, and 6 (31.3%, 31.3%, and 40.8%, respectively) were higher than for patient 11 (8.9%). There is a prevalence of complex aneuploidies in patients 4, 5, and 6 whereas diploidies prevail in patient 11.

distinct in that FISH showed a different profile of sperm chromosomal abnormalities (see “Results”) and a prevalence of nondisjunction during the second meiotic division (Table 3). This last characteristic was never described until now and could be correlated with the perinatal deaths in the proband’s sibling. Indeed, whereas the first division is specific to meiosis, the mechanisms used in meiotic division II are similar to those used in normal mitosis. Thus, the factor involved in both spermatogenic defect and perinatal deaths in the case of patient 11 could be a cell cycle–regulating factor expressed both in somatic cells and in division II meiotic cells. Nevertheless, it is too early to say if preferential involvement of the second meiotic division in the MNSs predisposes the embryos to subsequent perinatal deaths. It is still also too early to propose a preferential relationship between a particular sperm phenotype and a clinical phenotype.

FISH on sperm also revealed in all patients high frequencies of chromosomal abnormalities (Tables 3 and 4) compared with the frequencies of MNSs (Table 1), suggesting that apparently normal-sized spermatozoa presented aneuploidy. Indeed, evaluation of the aneuploidy rate of apparently normal-sized spermatozoa (see “Results”) showed that these spermatozoa, which could be used for ICSI, have significantly higher abnormal karyotype than controls but with

Table 4. Chromosomal constitution frequencies of sperm from patients 4, 5, 6, and 11 detected by FISH with specific probes for chromosomes 8 and 18*

	Patient			
	4	5	6	11
	Nuclei, No. (%)	Nuclei, No. (%)	Nuclei, No. (%)	Nuclei, No. (%)
8/18	289 (45.9)	786 (62.4)	530 (38.2)	2244 (88.9)
8/8/18/18	238 (37.8)	317 (25.2)	431 (31.1)	186 (7.3)
8/8/8/8/18/18/18/18	44 (7)	48 (3.8)	50 (3.6)	10 (0.4)
Aneuploidies	58 (9.2)	108 (8.6)	376 (27.1)	84 (3.3)
T abnormalities	340 (54.5)	473 (37.6)	857 (61.8)	280 (11.1)
Total	629	1259	1387	2524

* High percentages of total chromosomal abnormalities were found in the 4 patients (54.5%, 37.6%, 61.8%, and 11.1% for patients 4, 5, 6, and 11, respectively). The percentages of aneuploid sperm for patients 4, 5, and 6 (9.2%, 8.6%, and 27.1%, respectively) were higher than for patient 11 (3.3%). There is a prevalence of complex aneuploidies in patients 4, 5, and 6.

interindividual variations, leading to a high risk of chromosomally abnormal embryos. We compared the results of FISH with semiautomated analysis of the sperm nuclear surface. This last approach enabled the visualization of an increase of the mean nuclear sperm surface for patients 4, 5, and 6 and above all in the SD of the 4 patients with either a spreading of the histogram toward the great value (patients 6 and 11) or a second subpopulation of oversized spermatozoa (patients 4 and 5) (Figure 2). This method also shows the existence of various sizes of spermatozoa from the apparently normal-sized to fourfold increased nuclear spermatozoa (Figure 2). Consequently, several spermatozoa with moderate size increase may be classified as normal sized during the assessment of sperm morphology by light microscopy analysis or during the selection of spermatozoa for ICSI.

The present study points out that mild rates of MNSs significantly impair fertility and constitute a risk for embryos. Nevertheless, ICSI was performed on selected typical spermatozoa in the 4 patients. Although the mean fertilization rate was normal in comparison with the normal values of our center (65.6% vs 63%), only patient 11 had a normal fertilization rate (83.3%). The other 3 patients displayed relatively low fertilization rates (50%, 40%, and 50%, for patients 4, 5, and 6, respectively). This discrepancy could be related to the low rate of chromosomal abnormalities in the apparently normal-sized spermatozoa in patient 11 (1.1% and 0.4% with 3-color and dual-color FISH, respectively). Moreover, in this limited experience with mild levels of MNSs, the implantation and ongoing pregnancy rates were low (14.3%). Only 1 pregnancy was obtained, in

the couple in which the wife was the youngest (24 years old versus 35, 36, and 37 years old) (Table 1). Further studies will be necessary to confirm the low fertilization rate in these couples.

In conclusion, this study emphasizes that mild phenotypes of MNSs reduce the fertility and may have consequences on the embryos. Low fertilization and pregnancy rates may be due to the high incidence of aneuploidy in the apparently normal-sized spermatozoa that may be used for ICSI (patients 4, 5 and 6). Yet, chromosomal imbalance in the embryos could directly result from the gene mutation (patient 11). Indeed, a mutation in a gene that plays a role in the function and regulation of both mitotic and meiotic cycles could lead to the failure of chromosomal segregation in an embryo that initially has a normal karyotype, leading to embryo developmental arrest or perinatal death. Although the factor involved in this syndrome is unknown, we suggest conducting FISH on spermatozoa and genetic counseling before ICSI for infertile couples when the percentage of MNSs reaches 20% in at least 1 spermogram. The monitoring of possible pregnancies is difficult to define. We think that several ultrasound examinations must be recommended in case of ongoing pregnancy, whereas PGD is arguable. Indeed, Kahraman et al (1999, 2004) reported a possible beneficial effect of eliminating chromosomal abnormal embryos with PGD on abortion rates in patients with high percentages of MNSs whereas the success of PGD has yet to be proven in aneuploidy screening (Shahine and Cedars, 2006).

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