

Primary Flagellar Abnormality Is Associated With an Increased Rate of Spermatozoa Aneuploidy

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ABSTRACT: The frequencies of aneuploid and diploid spermatozoa were determined in 3 patients presenting a complete asthenozoospermia due to a primary and specific flagellar anomaly: patients 1 and 2 presented a “stump tail syndrome,” more than 50% of spermatozoa with a short flagella, patient 3 had a Kartagener syndrome including situs inversus, sinusitis, and bronchiectasis. No pregnancy was obtained after 3 intracytoplasmic sperm injection (ICSI) attempts in patients 1 and 2. A 3-color fluorescence in situ hybridization analysis was performed on their spermatozoa using centromeric probes for chromosomes X, Y, and 18 and compared with those of

8 fertile males. The frequency of disomic 18 and hyperhaploid XY spermatozoa was not significantly increased in the 3 patients when compared with controls. However, the 3 patients showed elevated frequencies of XX, YY, and diploid spermatozoa. These data add to growing evidence that systematic sperm anomalies of flagella increase the rate of spermatozoa aneuploidy and may also reduce the chances of pregnancy after intracytoplasmic sperm injection.

Key words: Chromosome abnormalities, FISH, flagellum, male infertility, sperm.

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Dysmotility of sperm flagella is a frequent cause of male infertility. Spermatozoa ultrastructural examination revealed a high incidence of flagellar anomalies: 1) nonspecific flagellar anomalies, acquired alterations affecting a variable number of spermatozoa, and 2) primary and specific flagellar anomaly affecting most spermatozoa (Chemes et al, 1998).

Primary flagellar anomaly may be associated with respiratory disease and defines the largest entity of immotile cilia syndrome (ICS). ICS is a heterogeneous disease of variable severity, described as an autosomal recessive disorder (Afzelius, 1976) and more recently renamed as primary ciliary dyskinesia (PCD, Rossman et al, 1981). Clinical manifestations of ICS/PCD are caused by impaired ciliary and flagellar functions. They are associated with upper and lower respiratory tract infections, dysmotility, or complete immotility of sperm flagella. Approximately 50% of ICS/PCD patients have alterations in the visceral rotation (situs inversus) with dextrocardia, corresponding to the Kartagener syndrome (for review see Afzelius, 1998). Cilia from upper and lower respiratory tracts and sperm flagella contain a resembling structure: core axoneme, complex of microtubules, and associated proteins (Afzelius et al, 1995). Electron microscopic ex-

amination of respiratory cilia and sperm tails from ICS/PCD patients revealed variable anomalies of cilia components, suggesting that several genes are implicated in this pathology (Afzelius et al, 1998). Furthermore, an ultrastructural anomaly of the ciliary axoneme is not always accompanied by a similar anomaly of the sperm axoneme (Jonsson et al, 1982; Walt et al, 1983; Wilton et al, 1986). The classical ultrastructural flagellar anomaly in ICS/PCD is lack of both dynein arms and normal fibrous sheaths (Afzelius et al, 1975). However, this defect is quite exceptional, and other disorders of the axoneme have been reported in ICS/PCD (Escalier et al, 1984; Chemes et al, 1998).

Short, thick, and irregular flagella also induce significant motility disorders and male infertility. These abnormalities are generally included in the so-called stump tail or “short tail syndrome” and correspond, after electron microscopic examination, to dysplasia of the fibrous sheath (DFS) (Chemes et al, 1998; for review see Chemes and Rawe, 2003). DFS spermatozoa show a redundant and haphazardly arranged fibrous sheath with variable alterations of the axoneme, from well formed to almost complete obliteration (for review see Chemes and Rawe, 2003).

Until recently, ICS/PCD and DFS severely affected male fertility prognosis. Only 1 live birth has been reported after in vitro fertilization (FIV) using spermatozoa with no progressive motility (Kay and Irvine, 2000). However, microfertilization techniques provide a rational therapy for ICS/PCD and DFS patients. Fertilization (Pa-

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Table 1. Baseline semen parameters evaluated on 3 examinations 30 min after ejaculation

Parameter	Patient 1	Patient 2	Patient 3
Volume, mL	6.5 (1.04)*	5.27 (0.76)	1.5 (0.05)
pH	7.93 (0.20)	7.83 (0.07)	8.3
Sperm concentration, $\times 10^6/\text{mL}$	38.33 (10.93)	54 (5.03)	43.5 (1.5)
Total sperm count, $\times 10^6$	228.33 (10.93)	284.87 (52.41)	58.80 (4.2)
Motility after 30 min, %			
Rapid progressive	0	0	0
Slow progressive	0	0	0
In situ	0	0	0
Absent	100	100	100
Vitality, %	70	83.33 (4.41)	80
Abnormal forms, %	80.67 (0.33)	84 (4)	29.5 (0.5)

* Data are mean (SEM) unless otherwise indicated.

padimas et al, 1997; Chemes et al, 1998; Okada et al, 1999), pregnancies (Stalf et al, 1995; Von Zumbusch et al, 1998; Kay et al, 2000), and live births (Stalf et al, 1995; Papadimas et al, 1997; Olmedo et al, 1997; Von Zumbusch et al, 1998) have been reported after intracytoplasmic sperm injection (ICSI). In 23 reported cases of ICS/PCD and DFS patients, ICSI led to satisfactory fertilization (54% and 63%, respectively) and pregnancy (45.45% and 83.33% respectively) rates, as well as to 21 live births (for review see Chemes and Rawe, 2003). Flagella anomalies do not seem to affect ICSI outcome when sperm viability is preserved. The number of pregnancies and live births remain nevertheless low in ICS/PCD and DFS.

The aim of our study was to demonstrate that structural and ultrastructural flagellar anomalies may also be associated to spermatozoa aneuploidy, probably as a consequence of meiotic chromosome motility disturbance. Sperm nuclei from patients presenting Kartagener syndrome (1 patient) and stump tail syndrome (2 patients) were explored by 3-color fluorescence in situ hybridization (FISH). We also evaluated the outcome of ICSI in the 2 stump tail patients.

Materials and Methods

Patients and Controls

Patient 1 was a 30-year-old man who presented at our assisted reproduction center for primary infertility exploration. There was no previous medical history of sterility among his relatives. Se-

men analysis on 3 ejaculates spaced by 3 months revealed a normal sperm concentration, 100% immotile spermatozoa, a normal vitality, and many morphologically abnormal spermatozoa, 50% of them carried a short flagella (Table 1; Figure 1a and b).

Ultrastructural sperm characteristics observed by transmission electron microscopy (TEM) showed (Figure 1c and d) 43% of sperm heads with normal morphology, 100% of flagella with an abnormal structure (very long midpiece [100%] with an abnormal organization of the fibrous sheath [90%], including absence of fibrous sheath [31%], abnormal fibrous sheath without axonemal complex [17%], axonemal complex without fibrous sheath [52%]).

Urological and ultrasound examination were normal, as well as testicular hormonal plasmatic levels (follicle stimulating hormone [FSH], luteinizing hormone [LH], testosterone, 17β estradiol) and constitutional karyotype.

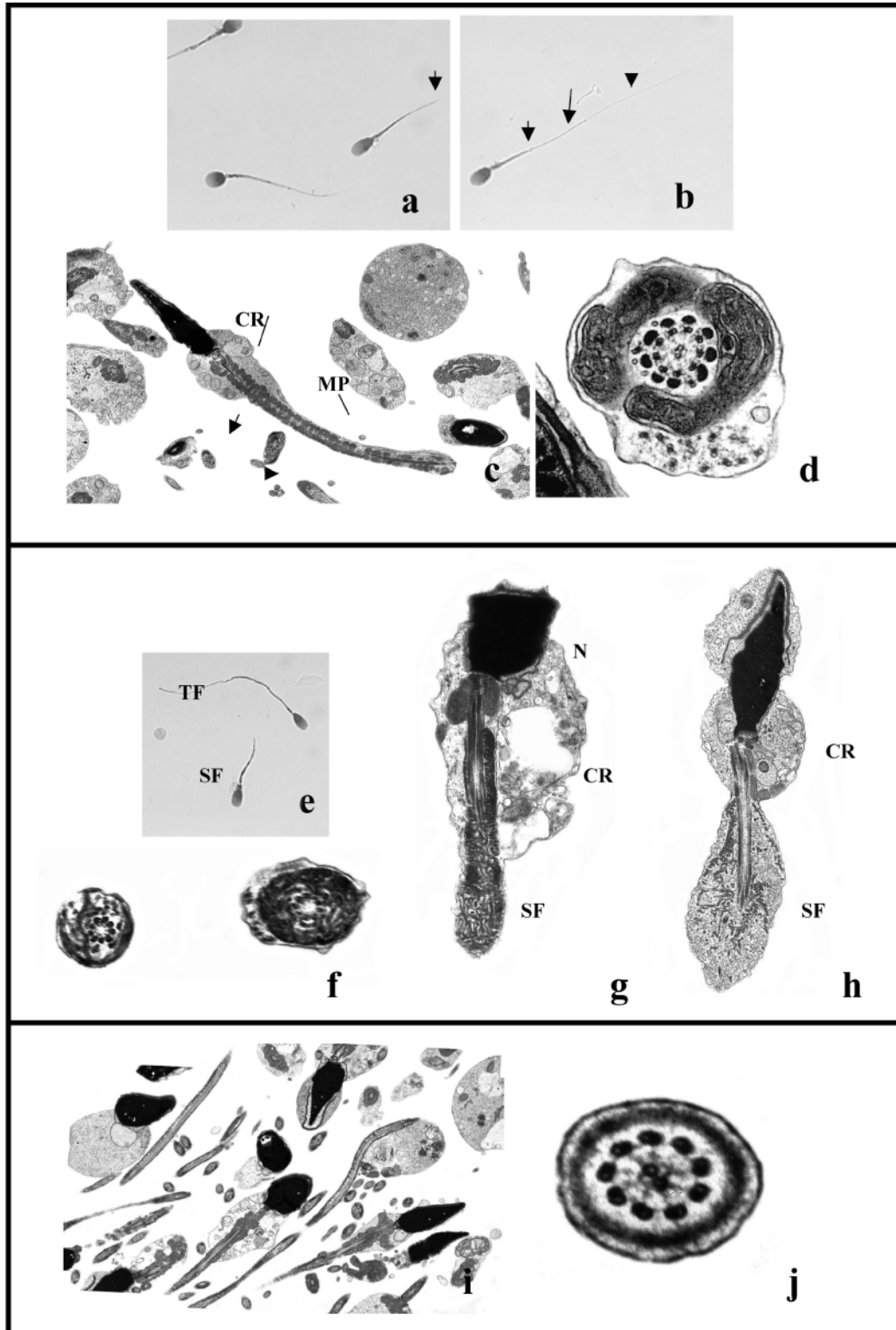
Patient 2 was a 23-year-old man who was also referred to our assisted reproduction center for exploration of a male factor infertility. Three spermograms showed a normal sperm concentration and vitality, 100% immotile spermatozoa, and a severe teratozoospermia (Table 1). Approximately 75% of spermatozoa displayed shortened, rigid, and thick flagella (Figure 1e).

Ultrastructural examination of spermatozoa by TEM revealed (Figure 1f through h) abnormal morphology of sperm head (nuclei with irregular limits [82%], structural defects and absence of acrosome [78%]) and flagellum (absence of midpiece and mitochondria [100%], absence of axonemal complex central structures [100% central sheath, set of microtubules]).

Urological and genital ultrasound examination, as well as the constitutional karyotype, were normal. Hormonal tests revealed normal levels of FSH, LH, testosterone, and 17β estradiol.

Patient 3, a 19-year-old male, presented with a Kartagener syndrome associated bronchiectasis, chronic sinusitis, and situs

Figure 1. Light microscopy (a, b, e) and electron microscopic (c, d, f, g, h, i, j) photomicrographs of spermatozoa. Patient 1: (a) spermatozoa with a flagellum of various lengths, some of them very truncated (arrow); (b) abnormal distribution of the flagellar pieces, with an elongated midpiece (small arrow), a shorter principal piece (arrow) and an end-like piece showing an extensive length (arrowhead); (c) longitudinal section of a spermatozoon with a very long midpiece (MP) and a large cytoplasmic remnant (CR), flagellar sections of the principal piece show either a fibrous sheath disorder (arrow), or an absence of the fibrous sheath (arrowhead); (d) transverse sections through the midpiece with supernumerary axonemal components. Patient 2: (e) a spermatozoon with a flagellum showing a thicker and irregular principal piece in its proximal region (TF) and a spermatozoon with a



short flagellum (SF); **(f)** transverse sections through the principal piece of 2 spermatozoa showing an absence of the central structures of the axoneme; **(g)** longitudinal section of a spermatozoon containing a poorly elongated nucleus (N), a large cytoplasmic remnant (CR), a short flagellum characterized by an atrophic midpiece, and a short principal piece with a misassembled and thicker fibrous sheath; **(h)** longitudinal section of a spermatozoon with a large cytoplasmic remnant (CR), an absence of the mitochondrial sheath, and a short and enlarged principal piece containing a misassembled fibrous sheath in a cytoplasmic droplet. Patient 3: **(i)** longitudinal sections of spermatozoa showing various anomalies of the sperm head shaping; **(j)** transverse section of a sperm flagellum with an absence of the outer and inner dynein arms.

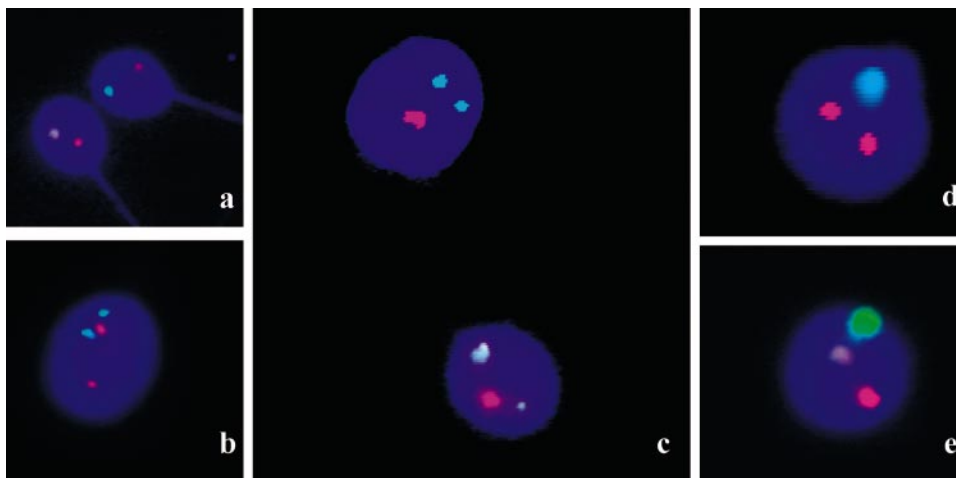


Figure 2. Fluorescence in situ hybridization on decondensed spermatozoa using centromeric probes for chromosomes X (green), Y (yellow), and 18 (red). (a) Haploid spermatozoa; (b) diploid spermatozoa (18, 18, X, X); (c) disomic X spermatozoon, disomic Y spermatozoon; (d) disomic 18 spermatozoon, hyperhaploid XY spermatozoon.

inversus. Spermatic parameters revealed normal sperm concentration, morphology, and vitality associated with a complete asthenospermia (motility $d = 100\%$). Urological examination was normal. This patient had no reproductive intention.

The ultrastructure of the sperm flagellum (Figure 1i and j) revealed an absence of inner and outer dynein arms (100%), in agreement with the ciliary axoneme anomaly, and normal structure of the axonemal complex (1 set of microtubules was absent in only 10% of spermatozoa). Examination of sperm heads showed normal structure (19%), irregular nuclear limits (52%), nonelongated or abnormal elongation of sperm nuclei (29%), defects of acrosomal structure (46%), and cytoplasmic residue (27%).

Eight probands of proved fertility, aged between 28 and 42 years, were included in the study as a control group. Their spermatic parameters were normal, and there was no previous medical history of either chronic illness, professional exposure, or environmental exposure. Their constitutional karyotype was normal 46,XY.

Semenogram analysis was performed according to the World Health Organization (WHO) guidelines (WHO, 1999) and verified in 3 different samples with an interval of 3 months. The study was carried out on a single ejaculate. All participants gave their informed consent to participate in the study.

Semen Processing

Semen samples were analyzed after liquefaction at 37°C according to standard procedures (WHO, 1999). Semen volume, sperm concentration, and total sperm count, as well as frequency of motile and morphologically normal spermatozoa, were assessed. For the FISH analysis, semen samples were processed as described by Rives et al (2000). Briefly, simultaneous decondensation and denaturation of sperm nuclei were performed by incubation for 10 minutes with 3 M NaOH at room temperature.

FISH Procedure

A multicolor FISH analysis was performed using chromosome specific α -satellite DNA probes for chromosomes X, Y, and 18

(Adgenix, Voisins Le Bretonneux, France). The probes were directly labeled with spectrum orange for chromosome X and with spectrum green for chromosome 18. A 1:1 mixture of probes for chromosome Y labeled with spectrum orange and spectrum green was used to obtain a yellow signal. Hybridization and detection were carried out as described previously (Rives et al, 2000). Slides were counterstained with a solution of 4', 6-diamidino-2-phenylindole diluted in antifade mounting medium (Adgenix, Voisins Le Bretonneux, France).

Sperm Scoring and Statistical Analysis

The slides were examined at 100 \times magnification with an epifluorescence microscope (DMRD, Leica, Germany). Preparations were scored with a triple band-pass filter (FITC/Rhodamine/DAPI). A minimum of 10 000 sperm nuclei were evaluated per semen specimen, using strict scoring criteria (Rives et al, 2000) for the determination of sperm aneuploidy.

All statistical analyses were performed using Statview for Windows 95 (Abacus Concepts, Inc, Berkeley, Calif). The scores of the 3 patients were compared with those observed in the control group using χ^2 tests. A value of $P < .05$ was considered to be significant. In the control groups, the values are noted as mean plus or minus standard error (SE).

Results

FISH Analysis on Spermatozoa

A total of 121 301 spermatozoa were scored; from patient 1 there were 10 172, from patient 2 there were 10 376, from patient 3 there were 20 315, and from the control group there were 80 438 (Figure 2). The disomy and diploidy rates for each patients and controls are reported in Table 2.

The frequencies of X-bearing and Y-bearing sperm were not significantly different from the expected 50% in the 3 patients. Furthermore, the rate of disomic 18 and

Table 2. *Sperm chromosome aneuploidy frequencies for chromosomes X, Y, and 18 estimated by 3-color fluorescence in situ hybridization*

Presumed Karyotypes	% of Sperm			
	Patient 1	Patient 2	Patient 3	Control Group
23, X	48.70	49.06	50.12	50.06 ± 0.28
23, Y	49.78	50.13	48.53	49.36 ± 0.24
24, XY	0.13	0.13	0.13	0.09 ± 0.02
24, XX	0.40 (<.0001)*	0.18 (<.007)	0.22 (<.0001)	0.09 ± 0.03
24, YY	0.51 (<.0001)	0.16 (<.009)	0.19 (<.0001)	0.08 ± 0.04
Diploidy	0.16 (=0.23)	0.17 (=0.006)	0.46 (<.0001)	0.09 ± 0.02
24, X/Y, +18	0.06	0.01	0.08	0.08 ± 0.02

* Data in parentheses are *P* values.

hyperhaploid XY sperm cells did not differ significantly from the control group. However, the frequency of disomic XX and YY spermatozoa, as well as the rate of diploidy, were significantly greater in the 3 patients when compared with controls.

ICSI Attempts

Microinjection of the oocytes was carried out for patients 1 and 2 with ejaculated spermatozoa (Table 3). On the day of ICSI, ejaculated spermatozoa were initially completely immotile for the 2 patients and after migration on 2 layers of gradient density (Pureperm, JCD, Lyon, France). Some motile spermatozoa were obtained after treatment of the suspension with Pentoxifylline (Torental, Aventis, France) at a final concentration of 3.5 mM. Only motile spermatozoa with the optimal morphological appearance were used for ICSI.

Patient 1

Three ICSI procedures were performed in the couple. The first, second, and third attempts led to fertilization rates of 69.23%, 83.4%, and 81.25%, respectively. Among the 35 injected oocytes during the 3 attempts, 7 fertilized oocytes were triploid (20%). No pregnancy was obtained

after fresh (first and second attempts) and frozen-thawed embryos (first and third attempts) transfer.

Patient 2

Three ICSI attempts were carried out. The fertilization rate was 100% for the first procedure, but no cleavage was obtained. The second and third attempts had a fertilization rate of 66.7% and 50%, respectively; however, they did not result in pregnancy. Only 1 fertilized oocyte (5%) was triploid.

Discussion

Studies of chromosome aneuploidy in spermatozoa have largely focused on infertile males with multiple sperm alterations (oligoasthenozoospermia). In our 3 patients, FISH was performed to assess sperm chromosome aneuploidy due to systematic sperm abnormalities. The normal sperm count ruled out the negative effect of this parameter on spermatozoa chromosome constitution in our 3 patients. Moreover, it is now well established that spermatozoa aneuploidy is negatively correlated to the sperm count (Rives et al, 1999; Pang et al, 1999; for review see

Table 3. *Outcome of 3 intracytoplasmic sperm injection cycles in patients 1 and 2*

	Patient 1			Patient 2		
	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3
Retrieved oocytes	16	7	19	7	11	11
Injected oocytes	13	6	16	3	6	10
Fertilized oocytes	9	5	13	3	4	5
Fertilization rate, %	69.23	83.4	81.25	100	66.7	50
Obtained embryos*						
Grade 1 embryos	1	2	2		1	
Grade 2 embryos	7	2	11		2	3
Grade 3 embryos	1					
Transferred embryos	2	3	3	0	2	2
Frozen embryos	6	0	3	0	0	0

* Grade of embryos evaluated on day 2. Grade 1, blastomeres of equal size, no cytoplasmic fragments; grade 2, blastomeres of equal size, less than 20% cytoplasmic fragments; grade 3, blastomeres of unequal size, from 21% to 50% cytoplasmic fragments; grade 4, blastomeres of distinctly unequal size, more than 51% cytoplasmic fragments.

Calogero et al, 2003) and reduced sperm count might introduce biases in aneuploidy evaluation.

The 3 patients had the same sperm alteration: complete asthenozoospermia induced by a structural specific abnormality of flagella. Patients 1 and 2 displayed shortened flagella, particularly thick and irregular for patient 2. Patient 1 had an elongated midpiece coupled with disorder or absence of the fibrous sheath in the principle piece. These morphological sperm abnormalities are described as dysplasia of the fibrous sheath (DFS) (Chemes et al, 1987, 1998; for review see Chemes and Rawes, 2003). The lack of mitochondria in the sperm midpiece, another rare sperm pathology, was observed in patient 2. This defect occurs in 2 main varieties: in the first, the spermatozoa may possess a midpiece that obviously lacks the mitochondria; in the second, the spermatozoa may lack the midpiece altogether and the fibrous sheath implants directly onto the neck. This second variety, as observed in the patient 2, is part of the DFS phenotype (Zamboni, 1991; for review see Chemes and Rawe, 2003). Patient 3 had morphologically normal and stiff flagella on light microscopy and expressed also clinical signs of Kartagener syndrome (chronic sinusitis, bronchiectasis, situs inversus). Ultrastructural investigations of sperm flagella and ciliated cells revealed that the outer microtubule doublets of the axoneme lacked inner and outer dynein arms, confirming the diagnosis of immotile cilia syndrome (Afzelius and Eliason, 1979; Rossman et al, 1981).

In our 3 patients, the sex ratio in spermatozoa was not different from the expected 50%. The rate of XX- and YY-bearing sperm was significantly higher in these patients. However, the frequency of hyperhaploid XY spermatozoa was not increased compared with controls. This result suggests that nondisjunction at the second meiotic division is far greater than at the first meiotic division in our patients presenting specific flagellar anomaly. The frequency of disomic XX spermatozoa was not different from the frequency of YY spermatozoa. The X chromosome did not seem to be more prone to nondisjunction than chromosome Y at the second meiotic division. The frequency of 18 disomic sperm nuclei was closed to the controls. Several studies have explored spermatozoa chromosome constitution in infertile patients and reported that spermatozoa from low-quality semen have an increased rate of aneuploidy (Moosani et al, 1995; Lähdetie et al, 1997; Finkelstein et al, 1998; Pang et al, 1999; Rives et al, 1999; Vegetti et al, 2000), except the data from Miharu et al (1994) and Guttenbach et al (1997). In infertile males with polymorphic sperm alterations, nondisjunctions of sex chromosomes preferentially occurred at the first meiotic division (Finkelstein et al, 1998; Pang et al, 1999; Rives et al, 2000; Gole et al, 2001; Templado et al, 2002). To our knowledge, few studies have investigated the frequency of chromosome abnormalities in relation to sper-

matozoa morphological characteristics (Martin and Rademaker, 1988; Rosenbusch et al, 1992; Rives et al, 1999; Morel et al, 2001; Templado et al, 2002). Most of the studies, except the study of Templado et al (2002), led to the conclusion that sperm morphology deformity cannot be used as an indicator of spermatozoa chromosomal damage. However, in these different data, teratozoospermia was not related to a primary sperm abnormality. (Martin and Rademaker, 1988; Rosenbusch et al, 1992; Rives et al, 1999; Morel et al, 2001). Systematic sperm morphological abnormalities may have an increased risk of sperm numerical chromosomal disturbances, rather than heterogeneous teratozoospermia. More recently, some groups reported an association between predominant morphological sperm abnormalities and aneuploidy rates. Sperm head deformities appear to be related to defects of their chromosome contents: large sperm heads (Yurov et al, 1996; Vicari et al, 2003), large sperm heads and multiple tails (In't Veld et al, 1997; Viville et al, 2000; Benzacken et al, 2001; Devillard et al, 2002; Lewis-Jones et al, 2003), and round headed sperms or globozoospermia with acrosomal aplasia (Carrell et al, 1999, 2001; Martin et al, 2003). In the cases of large sperm heads, polyploidy is generally observed in spermatozoa. However, in acrosome aplasia, aneuploidy evaluation, based on case reports, revealed an elevated frequency of XY hyperhaploid spermatozoa (Carrell et al, 1999; Martin et al, 2003), an increased rate of XX and YY disomic sperm nuclei (Carrel et al, 2001), and no variation in the sex chromosome aneuploidy rate (Carrell et al, 1999; Viville et al, 2000; Vicari et al, 2003). Lee et al (1996) observed a significantly higher incidence of structural chromosome anomalies in spermatozoa with amorphous, round, and elongated heads, whereas the incidence of aneuploidy was not significantly different compared with normal sperm heads. Only 1 study, to our knowledge, explored aneuploidy in spermatozoa of a patient with shortened flagella (Viville et al 2000). Three-color FISH analysis using chromosome X, Y, and 1 specific probes showed aneuploidy and diploidy rates comparable with those observed in normal controls. These results are not in agreement with those observed in our patients A and B, the rates of disomic XX, YY, and diploid spermatozoa were significantly increased compared with controls. The variability of sperm aneuploidy frequency is a common feature of abnormal spermatogenesis (Rives et al, 1999) but may also depend on 1) the methodology used, 2) the scoring criteria, and 3) the chromosomes explored.

In our 3 patients, nondisjunctions occur preferentially at the second meiotic division for sex chromosomes. While we cannot exclude that nondisjunctions may also occur in ICS and DFS patients during the first meiotic division, this was not observed for sex chromosomes in our 3 patients. The segregation of homologous chromo-

somes at meiosis I depends on the loss of sister chromatid cohesion along chromosome arms, as well as on the presence of chiasmata (for review see Hassold and Hunt, 2001). The overall frequency of crossovers is reduced in bivalents that underwent spontaneous meiosis I missegregation (Page and Hawley, 2003), and nondisjunction at meiosis II is assumed to result in most cases from failure of the sister chromatids to separate (for review see Hassold and Hunt, 2001). Furthermore, persistence of centromeric cohesion during meiosis I is essential for chromatid segregation during meiosis II. Rec8p (in yeast), SCC1 (in mammals and vertebrates), and other cohesins subunits are found along the longitudinal axis of chromosomes during pachytene and are cleaved by separase at anaphase I along chromosome arms and at metaphase II in the centromeric region (Waizenegger et al, 2000; Nasmyth, 2002; Terret et al, 2003). Even if cohesins and separase play a major role in chromosome segregation and separation, kinetochores, microtubules, and their motors are also essential for spindle assembly and function as well as for chromosome movements during mitosis and meiosis (Wu and Palazzo, 1999; Endow, 1999). However, these factors do not interfere with meiotic recombination events. Flagella and centrosome components are of the same origin and composed by microtubules and their motors, and we can speculate that alteration of microtubules (probably in patients 1 [perturbation of axonemal complex] and 2 [absence of axonemal complex central structure] and their motors [dynein in patient 3]) may disturb the spindle assembly during the first and second meiotic division leading to meiotic nondisjunctions and spermatozoa aneuploidy. Furthermore, disturbances of these components probably occur at variable levels in immotile cilia syndrome and dysplasia of fibrous sheath, resulting in an increased but not excessive rate of aneuploidy for sex chromosomes.

Diploid spermatozoa were also more numerous in our 3 patients than in the control group. It has been suggested that diploidy is the most constant and frequent chromosome abnormality detected in spermatozoa from infertile males with meiotic disorders or low sperm count (Egozcue et al, 2002). Our data permit us to extend the hypothesis that diploidy is the most common chromosome abnormality found in spermatozoa from males with systematic morphological sperm abnormalities. Alterations of microtubules or their motors can also interfere with the normal attachment and orientation of the bivalents on the metaphase plate. The chromosomes may be unable to migrate to the poles at anaphase and, as suggested by Egozcue et al (2000, 2002), any severe meiotic disorder can affect the anaphase I checkpoint, giving rise to the production of diploid spermatozoa. Diploid spermatozoa play a role in the genesis of diantric triploidy. Of the total number of triploid embryos, 65.9% are diantric generated

by diploid spermatozoa (Egozcue et al, 2002). Patient 1 had a higher rate of diploid sperm nuclei and a higher rate of triploid zygotes (20%). This result confirms the hypothesis of Egozcue et al (2002); however, we did not evaluate the origin of triploid zygotes in this patient.

Similar arrays of microtubules to those found in mitosis and meiosis have been used during fertilization to facilitate the fusion of male and female pronuclei. Furthermore, the mitotic potential of the human zygote originated from the male gamete centrosome (Palermo et al, 1994). A defective sperm centrosome will probably lead to fertilization arrest and may be a cause of male infertility (Navara et al, 1996; Hewitson et al, 1997). In our patient 2, with lack of midpiece and axonemal complex central structures, we may speculate that the absence of cleavage in the first ICSI attempt may be explained by failure of microtubules to assemble around the paternal centriole and by a defect in sperm aster formation. Centrosomal dysfunction, with insufficient sperm aster formation, lack of syngamy and cleavage, as well as defective embryos, has also been previously reported in 2 patients presenting a specific morphological anomaly: a globozoospermic patient with short tails (Nakamura et al, 2002) and a patient with head-tail junction and attachment alterations (Rawe et al, 2002). The quality of the embryos obtained after ICSI in our 2 patients were satisfying (grades 1 and 2: 96.15% for patient 1 and 100% for patient 2). Nevertheless, no pregnancy was obtained after transfer. The embryos obtained may not have a good developmental potential with reduced or abnormal blastomere mitotic division ability, excess of aneuploid embryos, or instability of blastomere mitotic divisions leading to aneuploid blastomeres. Exploration of the chromosome constitution of nontransferred or arrested embryos from ICS or DFS patients should be performed to confirm this hypothesis. Therefore, analysis of a larger group of patients with ICS or DFS will be of great interest to ensure not only the hypothesis of increased aneuploidy in spermatozoa but also to clearly evaluate their chance of pregnancy and live birth after ICSI.

Our data demonstrate that systematic sperm abnormalities concerning human flagella detected by light microscopy or by electron microscopy may be associated with an increased rate of sex chromosome aneuploidy, as well as an elevated rate of diploidy. The use of spermatozoa from patients with a primary flagellar anomaly may pose 2 different but related problems: 1) genetic risk including chromosomal and genic risk for the progeny and 2) fertilization problems with uncertain rate of pregnancy. Therefore, couples should be counseled regarding these 2 different risks prior to the procedure.

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