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Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men

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Spermatozoa from 32 infertile patients and 13 controls with normal semen parameters were analysed using dual and triple colour fluorescence in-situ hybridization (FISH) techniques, in order to investigate the rates of aneuploidy for chromosomes 13, 18, 21, X and Y. The patients were divided into three groups according to their karyotypes or the karyotypes of their offspring: 15 were infertile men with abnormal semen parameters and normal karyotypes (group 1), 13 were infertile men with abnormal karyotypes and normal or abnormal semen (group 2) and four were infertile men with abnormal semen and normal karyotypes but whose wives conceived a child (or a fetus) with a numerical chromosomal abnormality through an intracytoplasmic sperm injection cycle (group 3). Patients with abnormal semen parameters showed a significantly higher aneuploidy rate for the investigated chromosomes in their spermatozoa compared to controls ($P < 0.005$). Our data suggest the presence of a correlation between poor semen parameters and an increase in aneuploidy rate of chromosomes 13, 18, 21, X and Y in spermatozoa ($r = –0.81071$, $P < 0.002$); therefore the risk of a chromosomal aneuploidy in spermatozoa seems to be inversely correlated to sperm concentration and total progressive motility. Patients with abnormal karyotypes showed a higher incidence of diploidy and chromosomal aneuploidies compared to controls ($P < 0.002$). This strongly suggests the presence of an interchromosomal effect of the cytogenetic rearrangement. Men who fathered a child with an abnormal karyotype through intracytoplasmic sperm injection did not present a higher aneuploidy rate for the investigated chromosomes in spermatozoa compared to patients with infertility due to a similar male factor but showed higher incidence of chromosomal aneuploidy compared to normal controls.

Key words: aneuploidy/chromosomes/FISH/male infertility/reciprocal translocation

Introduction

The development of intracytoplasmic sperm injection (ICSI) for treatment of infertility due to severe male factor has improved the chances of achieving pregnancy in many infertile couples (Van Steirteghem *et al*., 1993, 1996). Nevertheless, concerns have been raised about the possible high risk of chromosomal aneuploidies from paternal origin in the concepti, especially since natural sperm selection does not occur in the ICSI procedure (Chandley and Hargreave, 1996). This potential hazard seems to be confirmed by reports of a higher incidence of sex chromosomal aneuploidies of paternal origin and structural de-novo chromosomal abnormalities in children conceived after ICSI, compared to the general population (In't Veld *et al*., 1995; Liebaers *et al*., 1995; Bonduelle *et al*., 1998).

Since men with abnormal sperm parameters, and especially those with severe oligozoospermia, make up the majority of ICSI candidates, it is of great interest to study the chromosomal constitution in their spermatozoa. After the first attempts to study chromosomal constitution of human spermatozoa using differential staining techniques (Pearson and Brobow, 1970; Brobow *et al*., 1972; Geraedts and Pearson, 1973), in 1976 the zona-free hamster oocyte–sperm fusion technique was introduced (Yanagimachi *et al*., 1976). Two years later, this technique was applied for the first time to visualize and analyse the chromosomal constitution of human spermatozoa (Rudak *et al*., 1978). Since then, many studies have been published (reviewed by Guttenbach *et al*., 1997a): more than 20 000 sperm chromosome complements from healthy, normal donors and spermatozoa from about 50 men with constitutional chromosome aberrations have been analysed (reviewed by Guttenbach *et al*., 1997a). The majority of these latter patients were carriers of reciprocal translocations. Although this technique provides valuable information on the whole chromosomal complement of spermatozoa, it is cumbersome, time-consuming, expensive and has been applied by few laboratories. Another drawback of this technique is the possibility to analyse the chromosomal complements in only a limited number of spermatozoa able to fertilize the hamster eggs. The primed-in-situ (PRINS) labelling technique is another tool that has been applied to detect chromosomal aneuploidies in human spermatozoa by the French group in Montpellier (Pellestor *et al*., 1996a,b). In the last 10 years, non-isotopic in-situ hybridization (ISH) and fluorescent in-situ hybridization (FISH) have provided a reliable and simple tool for cytogenetic studies. Using whole chromosome painting probes or locus-specific probes it is possible to study numerical and structural chromosomal abnormalities on metaphase chromosomes (Pinkel *et al*., 1988; Schröck *et al*., 1996). Centromeric

or locus-specific probes make chromosome enumeration in interphase nuclei possible and since 1990 this technique has been applied to investigate sperm chromosomal aneuploidies. After the first reports using an isotopic ISH technique (Joseph *et al*., 1984; West *et al*., 1989), many reports using non-isotopic ISH (Guttenbach and Schmid, 1990; Guttenbach *et al*., 1994a,b) and FISH (reviewed by Downie *et al*., 1997; by Guttenbach *et al*., 1997a and by Egozcue *et al*., 1997) have been published to study numerical chromosomal abnormalities in human sperm nuclei from normal fertile men. These results have demonstrated the reliability of this technique in studying human sperm aneuploidies and are comparable, at least in normal men, with data obtained using sperm fusion with the zona-free–hamster egg technique (Martin *et al*., 1996; Estop *et al*., 1997a; Guttenbach *et al*., 1997a). Major advantages of the FISH technique versus the zona-free hamster egg–human sperm fusion technique are that it is simpler, faster and allows for the analysis of thousands of spermatozoa (except in patients with very low sperm count). However, an important disadvantage of the FISH technique applied on interphase nuclei, is that chromosomal structural abnormalities are impossible to detect (except for an attempt to study telomeric deletion and duplication with centromeric and telomeric probes: Van Hummelen *et al*., 1996). Another disadvantage of applying FISH on interphase nuclei is that so far, it has been impossible to study more than five chromosomes simultaneously (and not more than three in the smaller sperm nuclei).

Using dual and triple colour FISH techniques, some authors recently reported a higher frequency of chromosomal aneuploidies in spermatozoa of infertile men compared to normal fertile donors, at least for some chromosomes (Moosani *et al*., 1995; Pang *et al*., 1995; Colombero *et al*., 1997; Bernardini *et al*., 1997; Lahdethie *et al*., 1997; Martin *et al*., 1997; Rives *et al*., 1998a; Storeng *et al*., 1998; Pang *et al*., 1999). Conversely, in other reports no differences between infertile and fertile men were found (Miharu *et al*., 1994; Guttenbach *et al*., 1997b). Results from these (or from some of these) previous studies will be discussed later on.

In this study, using dual and triple colour FISH techniques, we analysed the rates of aneuploidy for chromosomes 13, 18, 21, X and Y in 32 infertile patients and in 13 controls with normal semen parameters. We decided to study these chromosomes because trisomies 13, 18 and 21, together with the sex chromosome aneuploidies, are the most common numerical chromosomal abnormalities in human live births (Nielsen and Wohlert, 1991). The patients were divided into three groups according to their karyotypes or the karyotypes of their offspring: 15 were infertile men with abnormal semen parameters and normal karyotypes, 13 were infertile men with abnormal karyotypes and normal or abnormal semen, and four were infertile men with abnormal semen and normal karyotypes but whose wives conceived a child (or a fetus) with a numerical chromosomal abnormality through an ICSI cycle. We compared the aneuploidy rates of the different chromosomes among the three patient groups and controls. We also evaluated the interchromosomal variation in aneuploidy rates and investigated the correlation between frequencies of aneuploidy and different abnormal semen parameters (sperm concentration, total progressive motility and normal morphology).

Materials and methods

Patients

Thirty-two patients attending the Centre for Reproductive Medicine of the Dutch-speaking Brussels Free University were recruited. Samples were provided by 15 infertile men with normal karyotype and abnormal semen (group 1), 13 infertile men with abnormal karyotype, 11 of whom had abnormal semen (group 2), four infertile men with abnormal semen who fathered a child (or a fetus) with abnormal karyotype following an ICSI procedure (group 3). We also recruited 13 healthy controls with normal peripheral blood karyotype and normal semen analysis. All the controls were selected from the semen donor programme. Characteristics of the analysed patients ($n = 32$) and controls ($n = 13$) are detailed in Table I.

Karyotype analysis

Blood (10 ml) was drawn into tubes containing heparin to prevent clotting. Metaphase spreads were made from phytohaemagglutinin-stimulated peripheral lymphocytes using standard cytogenetic techniques (Rooney and Czepulhowski, 1992).

Semen preparation and fixation

All the semen samples were first analysed to evaluate concentration, motility, according to the WHO parameters (WHO, 1992), and morphology according to strict criteria (Kruger *et al*., 1986). Semen samples with volume $\geqslant$2 ml, sperm concentration $\geqslant 20\times10^6$/ml, percentage of spermatozoa with total progressive motility $\geqslant$50% and with normal morphology $\geqslant$14% were regarded as normal. Except for 15 samples which were cryopreserved in liquid nitrogen and then thawed and fixed after a few months, all remaining samples were fixed immediately after collection according to the following procedure. The sperm samples were washed three times in phosphate-buffered saline (PBS), pH 7.2, centrifuged at 280 *g* for 10 min and the sediment was then fixed in methanol/acetic acid (3:1). The fixed specimens were stored at –20°C until further processing. The fixed spermatozoa were spread on Silane-coated slides (Silane-Prep; Sigma Diagnostics, St Louis, MO, USA) and kept at room temperature for 1–3 days. At least two slides were prepared for each patient.

Decondensation treatment

Slides were washed in 2× standard saline citrate solution (SSC) and incubated for 5 min in 1 mol/l Tris buffer, pH 9.5, containing 25 mmol/l dithiothreitol (DTT) (Martini *et al*., 1995). After decondensation, the slides were washed once in 2×SSC, once in 1×PBS and finally dehydrated through an ethanol series and air-dried. This decondensation treatment is simple and fast and allows the maintenance of sperm structure, including the tail. It also makes the differentiation between spermatozoa and other cells present in the ejaculate easier and unequivocal.

DNA probes

The commercially available kit Aneuscreen™ (Vysis, Downers Grove, IL, USA) was used. Aneuscreen™ consists of two separate probe mixtures in hybridization buffer.

Probe mixture no. 1 consists of three repetitive DNA sequence centromeric probes (chromosome enumeration probe, CEP®) for chromosome 18 (D18Z1), chromosome X (DXZ1) and chromosome Y (alpha satellite region, DYZ3), directly labelled respectively with

the Spectrum Aqua™, Spectrum Green™ and Spectrum Orange™ fluorophores.

Probe mixture no. 2 consists of unique sequence DNA locus specific identifier probes (LSI™) specific for chromosome 13 (Retinoblastoma gene, *RB-1*, located at region 13q14) and 21 (Loci D21S259, D21S341 and D21S342, located at region 21q22.13–q22.2), directly labelled with the Spectrum Green™ and the Spectrum Orange™ fluorophores respectively.

FISH procedure

The FISH procedure was performed according to the protocol recommended by Vysis: slides were denatured for 5 min in a 70% formamide 20×SSC solution pre-warmed at 73 ± 1°C in a waterbath. Slides were then dehydrated through an ethanol series and air-dried. Ten µl of each probe mixture was added to each slide (one slide per probe set and two slides per patient) and covered with an 18 mm square glass coverslip. The coverslips were sealed with rubber cement and hybridization took place overnight in a humidified chamber at 37°C. Post-hybridization washings were carried out as follows: coverslips were removed and the slides were immersed immediately in a 0.4×SSC/0.3% NP-40 solution at 73 ± 1°C for 2 min in a waterbath and then in a 2×SSC/0.1% NP-40 solution for 1 min at room temperature. Finally the slides were mounted in Vectashield antifade medium (Vector Laboratories Inc., Burlingame, CA, USA) containing 450 ng/ml 4′,6-diamidino-2-phenyl-indole (DAPI) counterstain.

Microscopy and scoring criteria

Slides were observed using a Zeiss-Axioscope fluorescence microscope with the appropriate filter sets (Vysis, Downers Grove, IL, USA): single band pass filter (DAPI), triple band pass filter (DAPI/Orange/Green), dual band pass filter (Aqua/Orange) and a single band pass filter (Green). Only slides with a hybridization rate ⩾98% were analysed and, with few exceptions, at least 2000 sperm nuclei per slide (4000 per patient) were scored.

For slide scoring we applied the stringent scoring criteria (Williams *et al*., 1993). Only intact spermatozoa bearing a similar degree of decondensation and clear hybridization signals were scored; disrupted or overlapping spermatozoa were excluded from analysis. Spermatozoa were regarded as abnormal if they presented two (or more) distinct hybridization signals for the same chromosome, each equal in intensity and size to the single signal found in normal monosomic nuclei. We considered only clear hybridization signals, similar in size, separated from each other by at least one signal domain and clearly positioned within the sperm head. Divided (splitted) signals were not scored erroneously as disomies. Since we performed dual and triple colour FISH procedures, we were also able to evaluate nullisomies: spermatozoa were scored as nullisomic if they did not show any signal of the investigated chromosomes and the signal(s) of the other tested chromosome(s) was present. Finally, a spermatozoon was considered diploid if it exhibited two signals for each tested chromosome and if the tail was evident, as well as the normal oval shape of the head.

Statistical analysis

Student's *t*-test was used to test the homogeneity of mean ages between patients and controls. Differences in aneuploidy rates for each chromosome among groups were analysed by Wilcoxon score (rank sums), and Spearman correlation coefficients were used to correlate total chromosomal abnormalities and sperm parameters (SAS System, SAS Institute Inc., Cary, NC, USA). The χ^2-test was used to verify that the proportion of X- and Y-bearing spermatozoa did not deviate from the expected 1:1 ratio and to test the homogeneity of disomy rates for all the different analysed chromosomes among groups (SPSS for Windows, version 6.1.2, SPSS Inc., USA).

Results

Age, karyotype, semen parameters and reproductive history of patients and controls are detailed in Table I. The mean age of the three groups of patients was not significantly different when compared to controls. Six patients in group 1, two patients in group 2 and three patients in group 3 showed oligoasthenoteratozoospermia (OAT); seven patients in group 1 and five patients in group 2 showed asthenoteratozoospermia; one patient in group 2 showed oligoteratozoospermia; two patients in group 1 and three patients in group 2 showed teratozoospermia only, one patient in group 3 showed asthenozoospermia only and two patients in group 2 showed a normal semen analysis as well as all the controls.

A total of 251 277 spermatozoa was scored: 89 828 in group 1, 51 974 in group 2, 23 906 in group 3 and 112 918 in the control group.

The total rate of chromosomally abnormal spermatozoa was 1.4% in group 1, 2.7% in group 2, 0.8% in group 3, 1.2% in group 1+3 and 0.46% in the control group. Pooling the data of the three patient groups ($n = 32$), a total of 1.7% of spermatozoa was found to be abnormal.

The number of spermatozoa scored, the nullisomy, disomy and diploidy rates for each patient and each control in the four study groups are reported in Tables II (chromosome 13 and 21) and III (chromosome 18, X and Y). In the calculation of the total means shown in the tables, the data of chromosomes involved in a constitutional translocation were excluded: chromosome 21 in patient no. 13, chromosome 18 in patient no. 19, and chromosome 13 in patient no. 31. These data were also excluded from the final statistical analysis.

Chromosomes 13 and 21

The incidence of spermatozoa with nullisomy of chromosomes 13 or 21 was significantly higher compared to controls in group 1 ($P = 0.0030$ and $P = 0.0015$ respectively) and in group 2 ($P = 0.0022$ for both chromosomes) but not in group 3 ($P = 0.1441$ for both chromosomes). The incidence of disomy for chromosome 13 was significantly higher in group 1 and in group 2 ($P = 0.0231$ and $P = 0.0022$ respectively) but not group 3 ($P = 0.4652$). Similarly, the incidence of disomy for chromosome 21 was significantly higher in group 1 and in group 2 compared to controls ($P = 0.0015$ and $P = 0.0029$ respectively).

We also analysed the pooled data of patients from groups 1 and 3, because all these patients were infertile, had abnormal semen parameters and normal karyotypes while the karyotypes of the offspring were abnormal only in patients from group 3. Moreover, statistical analysis of patients from group 3 is influenced by the low number of subjects ($n = 4$).

After pooling these data, in the 19 patients from group 1 and 3, nullisomy rates for chromosomes 13 and 21 were significantly higher compared to controls ($P = 0.0030$ and $P = 0.0015$ respectively), as was the incidence of disomy for these two chromosomes ($P = 0.0231$ and $P = 0.0015$

Table IA. Semen parameters of three groups (nos. 1–3) of patients and a control group (no. 4)

No.	Karyotype	Age (years)	Semen parameters				Result
			Volume (ml)	Concentration ($\times 10^6$/ml)	Total progressive motility (%)	Normal morphology (%)	
Group 1							
4	46,XY	37	2.9	1.8	11	0	OAT
12	46,XY	41	0.7	3.5	48	1	OAT
13	46,XY	40	5.1	1.4	24	8	OAT
16	46,XY	32	4.7	1.3	42	7	OAT
23	46,XY	34	1.2	0.7	5	1	OAT
24	46,XY	29	1.5	5.6	38	4	OAT
3	46,XY	34	2.6	76	37	1	AT
5	46,XY	40	4.0	23.6	42	4	AT
8	46,XY	40	1.4	28.5	22	5	AT
21	46,XY	31	5.7	20.2	12	10	AT
32	46,XY	35	2.8	24.8	39	3	AT
34	46,XY	40	4.5	108	43	7	AT
36	46,XY	43	2.4	87	37	7	AT
35	46,XY	29	4.1	78.5	51	9	T
37	46,XY	30	5.0	37	55	10	T
Total (mean ± SD)		35.6 ± 4.9	3.2 ± 1.6	33.2 ± 36.3	33.7	5.1	
(range)		(29–43)	(0.7–5.7)	(0.66–108.0)	(5–55)	(0–10)	
Group 2							
7	46,XY,t(2;7) (q23;p22)	33	6.5	7.8	5	0	OAT
10	45,XY,der(14;21) (q10;q10)	34	2.1	2.9	3	0	OAT
25	46,XY,t(11;12) (q24.3;q12)	28	2.5	18.5	60	11	OT
2	46,XY,t(17;22) (q11;q11)	41	2.5	21	40	12	AT
11	46,XY,t(2;5) (p25;p12)	39	2.5	98	49	5	AT
15	46,XY,t(3;18) (p27.3;q21.1)	31	7.3	39	49	5	AT
19	46,XY,t(6;7) (q21;p21)	41	1.5	36.5	40	8	AT
22	45,XY,der(13;15) (q10;q10)	38	3.9	26.5	28	3	AT
1	46,XY,t(2;10) (q23;q11.2)	30	3.7	73	52	7	T
9	46,XY,t(5;20) (p22;p13)	36	5.1	54	61	2	T
26	46,XY/47,XYY	31	1.2	30	54	5	T
6	46,XY,t(6;15) (p22;q26.3)	42	3.5	31	50	14	N
33	46,XY,t(1;2) (p36.1p11.2)	29	8.0	62	61	14	N
Total (mean ± SD)		34.8 ± 4.9	3.9 ± 2.2	38.5 ± 27.0	42.5	6.6	
(range)		(28–42)	(1.2–8)	(2.9–98.0)	(3–61)	(0–14)	
Group 3							
14	46,XY	32	4.6	11.5	38	9	OAT
18	46,XY	33	2.2	1.3	12	1	OAT
20	46,XY	36	3.0	0.11	9	1	OAT
17	46,XY	31	2.0	56	31	18	A
Total (mean ± SD)		33.0 ± 2.2	3.0 ± 1.2	17.2 ± 26.3	22.5	7.3	
(range)		(31–36)	(2.0–4.6)	(0.11–56.0)	(9–38)	(1–18)	
Group 4							
27	46,XY	34	5.0	42.5	51	14	N
28	46,XY	32	4.5	34	56	14	N
29	46,XY	33	4.2	131.5	53	21	N
30	46,XY	28	5.5	31	53	16	N
31	46,XY	28	3.0	159	60	20	N
38	46,XY	39	3.5	201	70	26	N
39	46,XY	37	4.0	45	60	32	N
40	46,XY	37	3.2	136	54	17	N
41	46,XY	39	5.0	97	70	23	N
42	46,XY	40	4.6	40	60	22	N
43	46,XY	46	4.4	100	55	28	N
44	46,XY	41	3.8	75	65	17	N
45	46,XY	39	4.7	105	50	15	N
Total (mean ± SD)		36.3 ± 5.2	4.3 ± 0.7	98.1 ± 53.9	57.1	20.3	
(range)		(28–46)	(3–5.5)	(31.0–201.0)	(43–70)	(13–32)	

Table IB. Clinical characteristics of three groups (nos. 1–3) of patients and a control group (no. 4)

No.	Karyotype	Reproductive history	Duration of infertility (months)	Offspring	Comments
Group 1					
4	46,XY	Primary infertility	60	Yes (after ICSI)	2 healthy live births
12	46,XY	Primary infertility	82	No	
13	46,XY	Primary infertility	24	Yes (after ICSI)	1 healthy live birth, 2 miscarriages
16	46,XY	Primary infertility	60	Yes (after ICSI)	1 healthy live birth
23	46,XY	Primary infertility	24	Yes (after ICSI)	1 healthy live birth
24	46,XY	Primary infertility	18	Yes (after ICSI)	1 healthy live birth
3	46,XY	Primary infertility	12	Yes (after ICSI)	1 healthy live birth
5	46,XY	Primary infertility	60	Yes	1 TOP after ICSI [fetus affected by a de-novo translocation: 46,XY,t(8;14) (q24.3;q13)
8	46,XY	Secondary infertility	36	Yes (after ICSI)	1 healthy live birth, 1 miscarriage
21	46,XY	Habitual abortions and secondary infertility	84	Yes (after ICSI)	1 healthy live birth, 4 miscarriages (1 after ICSI) 2 failed ICSI+PGD for X-linked mental retardation
32	46,XY	Habitual abortions and secondary infertility	36	Yes	1 healthy live birth, 4 miscarriages
34	46,XY	Primary infertility	84	Yes (after ICSI)	1 healthy live birth, 2 miscarriages
36	46,XY	Primary infertility	60	No	
35	46,XY	Secondary infertility	24	Yes	2 healthy live births
37	46,XY	Primary infertility	24	No	
Total (mean ± SD) (range)			45.9 ± 25.4 (12–84)		
Group 2					
7	46,XY,t(2;7) (q23;p22)	Primary infertility	36	No	1 miscarriage. Patient had growth retardation in childhood
10	45,XY,der(14;21) (q10;q10)	Primary infertility	48	No	
25	46,XY,t(11;12) (q24.3;q12)	Primary infertility	48	No (failed ART)	3 failed cycles of ICSI+PGD
2	46,XY,t(17;22) (q11;q11)	Primary infertility	60	No (failed ART)	Various failed cycles of IUI and ICSI
11	46,XY,t(2;5) (p25;p12)	Habitual abortions	14	No	3 miscarriages
15	46,XY,t(3;18) (p27.3;q21.1)	Primary infertility	24	No	1 miscarriage after ICSI
19	46,XY,t(6;7) (q21;p21)	Primary infertility	120	No	
22	45,XY,der(13;15) (q10;q10)	Primary infertility	48	No	Partner carrier of chromosomal inversion: 46,XX,inv (10) (p11.1q21.2)
1	46,XY,t(2;10) (q23;q11.2)	Primary infertility	28	Yes (after ICSI)	2 healthy live births (twin ICSI pregnancy)
9	46,XY,t(5;20) (p22;p13)	Habitual abortions and secondary infertility	36	Yes (after IVF)	1 healthy live birth, 4 miscarriages
26	46,XY/47,XYY	Primary infertility	30	Yes	46,XY/47,XYY in 200/4 interphase and 200/1 metaphase lymphocytes 1 ongoing pregnancy, 1 TOP (fetus 47,XYY)
6	46,XY,t(6;15) (p22;q26.3)	Habitual abortions and secondary infertility	36	Yes	2 healthy live births, 7 miscarriages
33	46,XY,t(1;2) (p36.1p11.2)	No attempts	–	No	Abnormal karyotype found on preconception screening for consanguinity
Total (mean ± SD) (range)			44.0 ± 27.0 (14–120)		
Group 3					
14	46,XY	Primary infertility	36	Yes (after ICSI)	1 live birth 47,XYY
18	46,XY	Primary infertility	60	No	47,XYY, miscarriage after ICSI
20	46,XY	Primary infertility	48	Yes (after ICSI)	1 healthy live birth, 1 twin TOP after ICSI (47,XY+21 and 47,XXY)
17	46,XY	Primary infertility	24	No	47,XY+16, miscarriage after ICSI. Frozen spermatozoa available only (previous chemo-radiotherapy for Hodgkin's disease)
Total (mean ± SD) (range)			42.0 ± 15.5 (24–60)		

Table IB. *Continued*

No.	Karyotype	Reproductive history	Duration of infertility (months)	Offspring	Comments
Group 4					
27	46,XY	Donor	–	Yes	Proven fertility
28	46,XY	Donor	–	Yes	Proven fertility
29	46,XY	Donor	–	Yes	Proven fertility
30	46,XY	Donor	–	Yes	Proven fertility
31	46,XY	Donor	–	Yes	Proven fertility
38	46,XY	Donor	–	Yes	Proven fertility
39	46,XY	Donor	–	Yes	Proven fertility
40	46,XY	Donor	–	Yes	Proven fertility
41	46,XY	Donor	–	Yes	Proven fertility
42	46,XY	Donor	–	Yes	Proven fertility
43	46,XY	Donor	–	Yes	Proven fertility
44	46,XY	Donor	–	Yes	Proven fertility
45	46,XY	Donor	–	Yes	Proven fertility
Total (mean ± SD) (range)					

Group 1 = normal karyotype, abnormal sperm parameters; Group 2 = abnormal karyotype, normal or abnormal sperm parameters; Group 3 = normal karyotype, abnormal sperm parameters, child with abnormal karyotype; Group 4 = fertile controls with normal karyotype (from the donor semen progamme). O = oligozoospermia; ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination; A = asthenozoospermia; IVF = in-vitro fertilization; PGD = preimplantation genetic diagnosis; T = teratozoospermia; ART = assisted reproduction techniques; TOP = termination of pregnancy; N = normozoospermia.

respectively). There was no statistically significant difference between group 1 and group 3 with respect to aneuploidy rates for chromosomes 13 and 21.

The diploidy rate was only significantly higher compared to controls in patients from group 2 ($P = 0.0019$). Patients from groups 1 and 3, analysed separately and taken together, did not show a significantly higher rate of diploidy compared to controls ($P = 0.0597$, $P = 0.4652$ and $P = 0.7150$ respectively).

Chromosome 18 and sex chromosomes

The incidence of spermatozoa with nullisomy of chromosome 18 or with nullisomy of the sex chromosomes was significantly higher compared to controls in group 1 and in group 2 (group 1: $P = 0.0015$ and $P = 0.0019$ respectively; group 2: $P = 0.0022$ and $P = 0.0015$ respectively) but not in group 3 ($P = 0.0679$ and $P = 0.0679$ respectively). The incidence of disomy for chromosome 18 was significantly higher in group 1 and in group 2 ($P = 0.0058$ and $P = 0.0022$ respectively) but not in group 3 ($P = 1.0000$). For disomy of sex chromosomes we considered the incidence of XX, YY and XY disomies as well as the total sex chromosome disomy rate. XX, YY and XY disomy rates were significantly higher compared to controls in group 1 ($P = 0.0071$, $P = 0.0030$ and $P = 0.0024$ respectively) and also in group 2 ($P = 0.0019$, $P = 0.0071$ and $P = 0.0019$ respectively). In group 3, XX, YY and XY disomy rates were not statistically different when compared to controls. The total rate of disomy of sex chromosomes was significantly higher compared to controls in group 1 and in group 2 ($P = 0.0019$ for group 1, $P = 0.0015$ for group 2) but not in group 3 ($P = 0.0679$).

Analysing the pooled data from patients of groups 1 and 3, we obtained statistically significantly higher rates of nullisomy and disomy for all chromosomes: $P = 0.0015$ and $P = 0.0058$ for chromosome 18 nullisomy and disomy rates, $P = 0.0019$ for sex chromosomes nullisomy rate, $P = 0.0071$ for XX disomy rate, $P = 0.0030$ for YY disomy rate and $P = 0.0024$ for XY disomy rate and $P = 0.0019$ for the total sex chromosomes disomy rate. Comparing groups 1 and 3, we found no statistically significant difference between these two groups with respect to the aneuploidy rates of chromosome 18 and the sex chromosomes.

The diploidy rate was significantly higher in patients from group 1 and group 2 ($P = 0.0058$ and $P = 0.0107$ respectively). Patients from groups 1 and 3, taken together, also showed a significantly higher rate of diploidy compared to controls ($P = 0.0058$).

Interchromosomal variations

We analysed the disomy rates of the different chromosomes to evaluate if they were differently prone to meiotic non-disjunction. The disomy rate for chromosomes X and Y was significantly higher compared to the disomy rate for chromosome 18 in all three groups of patients and also in the control group ($P < 0.001$ for all groups). The disomy rate for chromosome 21 was significantly higher compared to the disomy rate for chromosome 13 only in group 1 ($P = 0.025$). In groups 2, 3 and in the control group there was no statistically significant difference in the disomy rates for chromosomes 13 and 21.

X- and Y-bearing spermatozoa

Among the normal spermatozoa, the percentages of X- and Y-bearing spermatozoa was respectively 49.74% and 50.26% in group 1, 49.31% and 50.69% in group 2, 49.89% and 50.11% in group 3 and 49.47% and 50.53% in group 4. Statistical

Table II. Incidence of sperm nullisomy and disomy and diploidy for chromosomes 13 and 21 (values are expressed in %) in 35 patients and six controls

Patient no.	Karyotype	Semen Analysis	Spermatozoa counted	Chromosome 13		Chromosome 21		Diploidy
				Nullisomy	Disomy	Nullisomy	Disomy	
Group 1								
4	46,XY	OAT	2095	0.81	0.53	1.19	0.67	0.29
12	46,XY	OAT	925	0.76	0.11	0.43	0.22	0.00
13	46,XY	OAT	2039	0.78	0.34	0.64	0.39	0.25
16	46,XY	OAT	2046	1.12	0.39	0.93	0.54	0.24
23	46,XY	OAT	2017	1.14	0.50	0.74	0.45	0.25
24	46,XY	OAT	2048	0.93	0.49	0.59	0.34	0.20
3	46,XY	AT	5022	0.20	0.08	0.20	0.20	0.00
5	46,XY	AT	4047	0.42	0.10	0.44	0.27	0.00
8	46,XY	AT	4325	0.25	0.05	0.21	0.21	0.00
21	46,XY	AT	2032	0.44	0.34	0.64	0.30	0.20
32	46,XY	AT	2135	0.37	0.33	0.52	0.37	0.14
34	46,XY	AT	4022	0.12	0.07	0.20	0.12	0.02
36	46,XY	AT	4012	0.10	0.02	0.10	0.07	0.00
35	46,XY	T	4018	0.10	0.05	0.15	0.12	0.02
37	46,XY	T	4019	0.12	0.07	0.17	0.10	0.00
Total mean (range)			2986.8 (925–5022)	0.51 (0.10–1.14)	0.23 (0.02–0.53)	0.48 (0.10–1.19)	0.29 (0.07–0.67)	0.11 (0.00–0.29)
Group 2								
7	46,XY,t(2;7) (p23;p22)	OAT	1046	0.57	0.29	0.67	0.38	0.19
10	45,XY,der(14;21) (q10;q10)	OAT	2163	0.65	0.37	3.79[a]	3.14[a]	0.32
25	46,XY,t(11;12) (q24.3;q12)	OT	2085	0.29	0.19	0.19	0.19	0.10
2	46,XY,t(17;22) (q11;q11)	AT	2049	0.20	0.10	0.24	0.20	0.00
11	46,XY,t(2;5) (p25;p12)	AT	2064	0.19	0.15	0.19	0.10	0.10
15	46,XY,t(3;18) (p21.3;q21.1)	AT	2125	0.38	0.24	0.28	0.24	0.24
19	46,XY,t(6;7) (q21;p21)	AT	2065	0.34	0.19	0.39	0.29	0.29
22	45,XY,der(13;15) (q10;q10)	AT	2045	12.81[a]	7.19[a]	0.44	0.24	0.24
1	46,XY,t(2;10) (q23;q11.2)	T	2045	0.34	0.15	0.24	0.10	0.24
9	46,XY,t(5;20) (p22;p13)	T	2084	0.24	0.19	0.24	0.14	0.10
26	46,XY/47,XYY	T	2039	0.39	0.25	0.34	0.25	0.10
6	46,XY,t(6;15) (p22;q26.3)	N	2042	0.29	0.15	0.39	0.10	0.10
33	46,XY,t(1;2) (p36.1;p11.2)	N	2151	0.42	0.28	0.56	0.46	0.09
Total mean (range)			2000.2 (1046–2163)	0.36 (0.19–0.65)	0.21 (0.10–0.37)	0.35 (0.19–0.67)	0.22 (0.10–0.46)	0.16 (0.00–0.32)
Group 3								
14	46,XY	OAT	5040	0.28	0.10	0.24	0.22	0.08
18	46,XY	OAT	766	0.52	0.00	0.26	0.26	0.26
20	46,XY	OAT	412	0.24	0.24	0.49	0.24	0.00
17	46,XY	A	8610	0.12	0.12	0.10	0.10	0.01
Total mean (range)			3707.0 (412–8610)	0.29 (0.12–0.52)	0.11 (0.00–0.24)	0.27 (0.10–0.49)	0.21 (0.10–0.26)	0.09 (0.00–0.26)
Group 4								
27	46,XY	N	10 029	0.14	0.09	0.12	0.08	0.02
28	46,XY	N	10 010	0.12	0.09	0.12	0.09	0.02
29	46,XY	N	8037	0.10	0.06	0.09	0.06	0.05
30	46,XY	N	10 065	0.13	0.08	0.12	0.06	0.04
31	46,XY	N	3020	0.13	0.07	0.13	0.10	0.03
38	46,XY	N	2018	0.10	0.10	0.10	0.05	0.05
39	46,XY	N	2166	0.14	0.09	0.14	0.09	0.00
40	46,XY	N	2034	0.10	0.05	0.10	0.05	0.05
41	46,XY	N	2010	0.15	0.10	0.10	0.10	0.05
42	46,XY	N	2100	0.14	0.05	0.14	0.10	0.05
43	46,XY	N	2018	0.20	0.10	0.15	0.10	0.05
44	46,XY	N	2194	0.14	0.09	0.09	0.05	0.05
45	46,XY	N	2050	0.15	0.05	0.10	0.05	0.05
Total mean (range)			4442.4 (2010–10 065)	0.13 (0.10–0.20)	0.08 (0.05–0.13)	0.11 (0.09–0.15)	0.07 (0.05–0.10)	0.03 (0.00–0.05)

O = oligozoospermia; A = asthenozoospermia; T = teratozoospermia; N = normospermia

[a]Not included in the statistical evaluation: the analysed chromosomes are involved in the translocation

Table III. Incidence of sperm nullisomy and disomy and diploidy for chromosomes 18, X and Y in 35 patients and six controls. Values are expressed as percentages

	Patient no.	Karyotype	Semen analysis	Spermatozoa counted	Chromosome 18		Sex chromosomes				Diploidy	Normal X-bearing	Normal Y-bearing
					Nullisomy	Disomy	Nullisomy	XX	YY	XY			
Group 1	4	46,XY	OAT	2111	0.71	0.33	0.71	0.24	0.38	0.52	0.24	49.88	50.12
	12	46,XY	OAT	1029	0.39	0.19	0.58	0.00	0.19	0.19	0.19	49.46	50.54
	13	46,XY	OAT	1911	0.52	0.37	0.58	0.31	0.21	0.21	0.26	48.50	51.50
	16	46,XY	OAT	1916	0.73	0.42	0.99	0.37	0.31	0.47	0.26	49.24	50.76
	23	46,XY	OAT	2129	0.99	0.47	0.99	0.23	0.52	0.70	0.28	49.12	50.88
	24	46,XY	OAT	1615	0.93	0.56	1.05	0.31	0.25	0.31	0.19	48.81	51.19
	3	46,XY	AT	5034	0.26	0.12	0.16	0.06	0.06	0.02	0.02	49.97	50.03
	5	46,XY	AT	4027	0.27	0.07	0.25	0.07	0.05	0.05	0.02	50.09	49.91
	8	46,XY	AT	5034	0.28	0.04	0.28	0.06	0.08	0.14	0.00	50.36	49.64
	21	46,XY	AT	2089	0.62	0.24	0.72	0.29	0.14	0.34	0.24	48.65	51.35
	32	46,XY	AT	2025	0.44	0.30	0.49	0.35	0.25	0.25	0.15	48.99	51.01
	34	46,XY	AT	4038	0.20	0.10	0.32	0.12	0.05	0.10	0.05	n.d.	n.d.
	36	46,XY	AT	4028	0.25	0.00	0.25	0.02	0.07	0.07	0.02	n.d.	n.d.
	35	46,XY	T	4024	0.10	0.07	0.25	0.02	0.07	0.07	0.00	n.d.	n.d.
	37	46,XY	T	4016	0.10	0.07	0.15	0.05	0.02	0.00	0.00	n.d.	n.d.
Total mean (range)				3001.7 (1029–5034)	0.45 (0.10–0.99)	0.22 (0.00–0.56)	0.52 (0.15–1.05)	0.17 (0.00–0.37)	0.18 (0.02–0.52)	0.23 (0.00–0.70)	0.13 (0.00–0.28)	49.74 (48.50–50.36)	50.26 (49.64–51.50)
Group 2	7	46,XY,t(2;7) (p23;p22)	OAT	638	0.47	0.31	0.31	0.16	0.31	0.31	0.16	49.44	50.56
	10	45,XY,der(14;21) (q10;q10)	OAT	2164	0.55	0.32	0.32	0.05	0.09	0.18	0.37	49.74	50.26
	25	46,XY,t(11;12) (q24.3;q12)	OT	2129	0.38	0.14	0.42	0.14	0.19	0.19	0.09	48.47	51.53
	2	46,XY,t(17;22) (q11;q11)	AT	2118	0.33	0.19	0.38	0.09	0.09	0.14	0.00	49.43	50.57
	11	46,XY,t(2;5) (p25;p12)	AT	2059	0.34	0.19	0.24	0.05	0.05	0.10	0.00	50.22	49.78
	15	46,XY,t(3;18) (p21.3;q21.1)	AT	2283	6.83[a]	2.45[a]	0.53	0.09	0.22	0.39	0.09	49.44	50.56
	19	46,XY,t(6;7) (q21;p21)	AT	2134	0.47	0.14	0.28	0.09	0.05	0.09	0.05	48.41	51.59
	22	45,XY,der(13;15) (q10;q10)	AT	2167	0.60	0.23	0.37	0.05	0.05	0.18	0.09	48.38	51.62
	1	46,XY,t(2;10) (q23;q11.2)	T	2034	0.39	0.15	0.34	0.05	0.10	0.05	0.34	48.88	51.12
	9	46,XY,t(5;20) (p22;p13)	T	2074	0.39	0.14	0.34	0.10	0.19	0.10	0.05	49.34	50.66
	26	46,XY/47,XYY	T	2037	0.20	0.10	0.15	0.05	0.10	0.10	0.05	49.55	50.45
	6	46,XY,t(6;15) (p22;q26.3)	N	2087	0.43	0.14	0.29	0.10	0.10	0.05	0.05	49.25	50.75
	33	46,XY,t(1;2) (p36.1;p11.2)	N	2047	0.34	0.20	0.39	0.20	0.10	0.20	0.15	50.62	49.38
Total mean (range)				1997.8 (638–2283)	0.41 (0.20–0.60)	0.19 (0.10–0.32)	0.34 (0.15–0.53)	0.09 (0.05–0.20)	0.12 (0.05–0.31)	0.16 (0.05–0.39)	0.12 (0.00–0.37)	49.31 (48.38–50.62)	50.69 (49.38–51.62)
Group 3	14	46,XY	OAT	1 006	0.40	0.20	0.40	0.10	0.30	0.20	0.00	52.22	47.78
	18	46,XY	OAT	604	0.50	0.00	0.83	0.00	0.33	0.33	0.17	49.24	50.76
	20	46,XY	OAT	381	0.26	0.00	0.26	0.00	0.26	0.00	0.00	47.62	52.38
	17	46,XY	A	7087	0.17	0.07	0.24	0.06	0.10	0.07	0.03	49.74	50.26
Total mean (range)				2269 (381–7087)	0.33 (0.17–0.50)	0.07 (0.00–0.20)	0.43 (0.24–0.83)	0.04 (0.00–0.10)	0.25 (0.10–0.33)	0.15 (0.00–0.33)	0.05 (0.00–0.17)	49.89 (47.62–52.22)	50.11 (47.78–52.38)
Group 4	27	46,XY	N	10 008	0.12	0.07	0.16	0.04	0.04	0.07	0.01	49.56	50.44
	28	46,XY	N	10 033	0.09	0.06	0.10	0.04	0.05	0.06	0.03	49.90	50.10
	29	46,XY	N	8916	0.16	0.04	0.12	0.03	0.04	0.04	0.03	50.40	49.60
	30	46,XY	N	6196	0.15	0.05	0.19	0.03	0.06	0.03	0.03	49.43	50.57
	31	46,XY	N	3186	0.16	0.06	0.16	0.03	0.06	0.06	0.03	50.38	49.62
	38	46,XY	N	2134	0.09	0.05	0.14	0.05	0.05	0.00	0.05	48.94	51.06
	39	46,XY	N	2355	0.13	0.04	0.17	0.04	0.08	0.04	0.00	48.48	51.52
	40	46,XY	N	2020	0.15	0.10	0.15	0.00	0.05	0.05	0.00	47.76	52.24
	41	46,XY	N	2023	0.15	0.05	0.20	0.05	0.05	0.05	0.05	48.98	51.02
	42	46,XY	N	2008	0.10	0.05	0.10	0.00	0.05	0.05	0.05	47.50	52.50
	43	46,XY	N	2076	0.19	0.10	0.14	0.05	0.05	0.10	0.05	48.11	51.89
	44	46,XY	N	2157	0.14	0.05	0.14	0.00	0.05	0.05	0.00	49.81	50.19
	45	46,XY	N	2055	0.19	0.05	0.10	0.05	0.00	0.05	0.05	48.41	51.59
Total mean (range)				4243.6 (2008–10033)	0.13 (0.09–0.19)	0.06 (0.03–0.10)	0.14 (0.10–0.20)	0.03 (0.00–0.05)	0.05 (0.00–0.08)	0.05 (0.00–0.10)	0.03 (0.00–0.05)	49.47 (47.50–50.40)	50.53 (49.60–52.50)

n.d. = not determined; O = oligozoospermia; T = teratozoospermia; A = asthenozoospermia; N = normospermia.
[a]Not included in the statistical analysis: the analysed chromosome is involved in the translocation.

analysis demonstrated that the proportion of X- and Y-bearing spermatozoa was not significantly different from the 1:1 ratio expected in all patients and controls (individual data are reported in Table III).

Correlation between aneuploidy rates and sperm parameters

We evaluated the correlation between the total abnormality rates (sum of nullisomies and disomies of all the investigated chromosomes and of diploidies) and three sperm parameters: concentration of spermatozoa (10^6/ml), percentage of spermatozoa with progressive motility and percentage of spermatozoa with normal morphology.

The total abnormality rates for chromosomes 13 and 21 and for chromosomes 18, X and Y were significantly and inversely correlated in group 1 with the concentration of spermatozoa ($r = –0.907$, $P = 0.0001$ for chromosome 13 and 21 total aneuploidies and –0.810, $P = 0.0002$ for chromosome 18, X and Y total aneuploidies) (Figures 1 and 2) and with the total progressive motility ($r = –0.524$, $P = 0.0449$ for chromosome 13 and 21 total aneuploidies and –0.556, $P = 0.0313$ for chromosome 18, X and Y total aneuploidies) (Figures 3 and 4). Such a correlation was not present in group 1 with the normal morphology and in the other groups with all the three sperm parameters considered (data not shown).

Discussion

Since 1990, many studies have been published using FISH to investigate the prevalence of aneuploidy in human spermatozoa. In the first published studies, results were hampered by the use of a single probe technique. This approach allowed for the study of only a single chromosome: disomic from diploid spermatozoa could not be discriminated, and neither could nullisomic spermatozoa be differentiated from the ones with lack of hybridization. Applying two-colour FISH with two probes labelled with two different fluorochromes or three-colour FISH with three probes labelled with three different fluorochromes, it is possible to obtain reliable data on nullisomy, disomy and diploidy rates of the analysed spermatozoa. Not all the chromosomes have been studied so far: for example the aneuploidy rates for chromosomes 19 and 22 have been reported only by one group (Rives *et al*., 1998b).

The reported frequencies of disomy in normal fertile men range from 0.23 to 0.28% for chromosome 13, 0 to 0.39% for chromosome 18 and 0.1 to 0.48% for chromosome 21. With regard to sex chromosomes, the reported frequencies range from 0.018 to 0.7% for XX disomy, 0.009 to 0.6% for YY disomy and 0.062 to 0.42% for XY disomy; the diploidy rates range from 0.06 to 0.97% (see Downie *et al*., 1997 and Egozcue *et al*., 1997 for review). These results are often not homogeneous when several studies are compared. This variability may be explained in different ways: first, the patients belonged to various geographical areas, criteria for patient selection were dissimilar and the semen samples were probably obtained after variable periods of abstinence; second, FISH protocols were not homogeneous: decondensation procedures were different and probes with various hybridization efficiencies were used; third, the same scoring criteria were not always applied.

The decondensation treatment is crucial in order to obtain a proper hybridization efficiency: this makes it possible for the probes to access the extremely highly histamine-packed DNA of the spermatozoa. The decondensation procedure is one of the most frequent variables when comparing different studies. Taking into account these variables, and especially the stringent scoring criteria (Williams *et al*., 1993) applied in our study (which can explain our relatively low percentages of disomic and diploid spermatozoa), the results in our control group are comparable with results previously reported in literature for normal fertile men.

In 1994 the first report on spermatozoa from infertile men analysed by FISH was published (Miharu *et al*., 1994). Since then, more studies on infertile men with abnormal semen parameters have been published. At least 11 out of these reports analysed chromosomes 13, 18, 21, X and Y (Table IV) (Miharu *et al*., 1994; Moosani *et al*., 1995; Pang *et al*., 1995, 1999; Bernardini *et al*., 1997; Colombero *et al*., 1997; Estop *et al*., 1997a; Francisco *et al*., 1997; Guttenbach *et al*., 1997b; Storeng *et al*., 1998; Rives *et al*., 1998a). Another study (Lähdetie *et al*., 1997) investigated chromosomes 1 and 7 in infertile patients with normal or nearly normal semen parameters and in OAT patients. The reported findings are not unequivocal. In one study (Miharu *et al*., 1994), the rate of disomy for chromosomes 1, 16, X and Y in spermatozoa from nine fertile and 21 infertile subjects using FISH with single colour chromosome-specific probes and the rate of disomy for chromosomes 17 and 18 using FISH with a dual colour technique were investigated. They analysed a total of 450 580 spermatozoa and they did not observe differences between fertile and infertile men for either diploidy or disomy for the investigated chromosomes. On the contrary, another group (Pang *et al*., 1995), found a significant increase in the frequencies of diploidy, autosomal and sex chromosomal aneuploidies in sperm samples from nine OAT infertile patients, compared to four donor controls. They investigated chromosomes 7, 11, 12, 18, X and Y using a two-probe, two-colour FISH; 1000 spermatozoa for each probe set were scored and in OAT patients higher disomy rates for chromosome 18 and for the sex chromosomes, together with a higher diploidy rate, were found. In the same year, chromosomes 1, 12, X and Y using a dual-colour FISH were investigated (Moosani *et al*., 1995), together with a complete sperm karyotyping using the zona-free hamster egg–human sperm fusion technique. This group reported an increased frequency of chromosomal abnormalities in five men with OAT and infertility. In 1997, in three studies (Bernardini *et al*., 1997; Colombero *et al*., 1997; Francisco *et al*., 1997), using multiprobe, multicolour FISH, a higher frequency of autosomal and/or sex chromosomal disomies in OAT infertile men was demonstrated. In one of these studies (Francisco *et al*., 1997) it was also demonstrated that the swim-up semen preparation did not allow for the separation of haploid from aneuploid spermatozoa. In the same year another group (Guttenbach *et al*. 1997b) did not find any difference in disomy rates for chromosomes 1, 7, 10, X and

Table IVA. Studies of sperm disomy in infertile men for chromosomes 13, 18, 21, X and Y using in-situ hybridization (ISH) and fluorescent in-situ hybridization (FISH)

Study	Technique	Patient characteristics			Sperm characteristics (mean, range)		
		No.	Mean age (years)	Diagnosis	Concentration ($\times 10^6$/ml)	Motility (%)	Morphology (%)
Miharu *et al.*, 1994	Dual colour FISH	12	n.r.	Infertility (OAT and unexplained)	n.r.	n.r.	n.r
		9	n.r.	Controls	n.r.	n.r.	n.r.
Moosani *et al.*, 1995	Dual colour FISH	5	31.6 (29–35)	Infertility (OAT)	13–50	30–49	24–51
		12	n.r.	Controls	n.r.	n.r.	n.r.
Pang *et al.*, 1995	Dual colour FISH	9	n.r.	Infertility (OAT)	8 (2.1–18.5)	34.7 (17.9–69.1)	<7 (1–6)
		4	n.r.	Controls	n.r.	n.r.	n.r.
Bernardini *et al.*, 1997	non isotopic ISH	9	37 (30–44)	Infertility (OAT)	4.5 (0.01–20)	n.r.	<20 (0–20)
		6	34 (29–44)	Infertility (Unexplained)	50 (13–122)	n.r.	>20 (45–70)
		3	36 (33–37)	Controls	77 (42–117)	n.r.	>40 (40–70)
Guttenbach *et al.*, 1997b	Single and double non-isotopic ISH	45	23–42	Infertility (OAT)	0.5–49.8	0–75	n.r.
		6	23–57	Controls	n.r.	n.r.	n.r.
Colombero *et al.*, 1997	Triple colour FISH	14	n.r.	Infertility (OAT)	n.r.	n.r.	n.r.
		4	n.r.	Controls	n.r.	n.r.	n.r.
Francisco *et al.*, 1997	Multi colour FISH	4	n.r.	Infertility (OAT)	n.r.	n.r.	n.r.
Estop *et al.*, 1997a	Dual/triple colour FISH	5		Infertility (OAT)	n.r.	n.r.	0–4
Storeng *et al.*, 1998	Dual colour FISH	9	29–60	Infertility (OAT)	n.r.	n.r.	n.r.
		10	31–46	Infertility	n.r.	n.r.	n.r.
Rives *et al.*, 1998a	Dual colour FISH	50	n.r.	OAT	n.r.	n.r.	n.r.
		10	n.r.	Controls	n.r.	n.r.	n.r.
Pang *et al.*, 1999	Dual/triple colour FISH	9	34.3 (25–39)	Infertility (OAT)	2–15	18–41	1–44
		4	31.3 (29–33)	Controls	79–160	63–80	17–30
Present study	Dual/triple colour FISH	15	35.6 ± 4.8 (29–43)	Infertility (OAT, normal karyotype)	33.2 ± 36.3 (0.66–108.0)	33.7 (5–55)	5.1 (0–10)
		13	34.8 ± 4.9 (28–42)	Infertility (abnormal karyotype)	38.5 ± 27.0 (2.9–98.0)	42.5 (3–61)	6.6 (0–14)
		4	33.0 ± 2.2 (31–36)	Infertility (OAT, abnormal child)	17.2 ± 26.3 (0.11–56.0)	22.5 (9–38)	7.3 (1–18)
		13	36.3 ± 5.2 (28–46)	Controls	98.1 ± 53.9 (31.0–201.0)	57.1 (43–70)	20.3 (13–32)

Table IVB. Studies of sperm disomy in infertile men for chromosomes 13, 18, 21, X and Y using in-situ hybridization (ISH) and fluorescent in-situ hybridization (FISH)

Study	Technique	Sperm counted per probe set	Chromosomes disomy (mean %, range)						Diploidy (mean %, range)
			13	18	21	XX	YY	XY	
Miharu *et al.*, 1994	Dual colour FISH	≅4000				0.16 (0.05–0.30)	0.11 (0.05–0.20)		0.23 (0.05–0.55)
		≅4000				0.13 (0.06–0.20)	0.08 (0.02–0.15)		0.18 (0.05–0.47)
Moosani *et al.*, 1995	Dual colour FISH	10 000				0.05–0.11	0.06–0.18	0.16–0.47	
		10 000				0.03–0.17	0.10–0.43	0.08–0.24	
Pang *et al.*, 1995	Dual colour FISH	1000		5.49 (1.5–9.5)			4.99 (2.5–14.6)[a]		2.07 (0.3–9.8)
		1000		2.07 (0.3–9.8)			0.48 (0.2–1)[a]		0.1 (0–0.1)
Bernardini *et al.*, 1997		≅4500				0.36 (0.18–0.48)	0.27 (0.13–0.43)	0.70 (0.27–1.15)	
		≅4000				0.19 (0.09–0.30)	0.14 (0.07–0.28)	0.42 (0.24–0.77)	
		≅8000				0.15 (0.07–0.22)	0.25 (0.14–0.4)	0.45 (0.4–0.52)	
Guttenbach *et al.*, 1997b	Single and double non-isotopic ISH	2000				0.14 (0–0.35)	0.10 (0.0.35)		0.1 (0–1.6)
		2000				0.11 (0.05–0.20)	0.12 (0–0.20)		0.05 (0–0.10)
Colombero *et al.*,	Triple colour	1542 (mean)		2.4[b]					
1997	FISH	1542 (mean)		0.67 [b]					
Francisco *et al.*,	Multi-colour 1000 (fresh)			0–1.7[c]			0–0.7[a]		0.1–0.3
1997	FISH	1000 (pellet)		0.1–1.1[c]			0.1–0.8[a]		0.15–2.6
		300 (swim-up)		0–2.67[c]			0–3[a]		0–0.33
Estop *et al.*, 1997a	Dual/triple colour FISH	300		0–100%[d] in abnormal spermatozoa versus 1.4%[d] (mean) in normal spermatozoa					
Storeng *et al.*,	Dual colour	≅150				0.28	0.28	0.90	
1998	FISH	≅ 300				0.03	0.24	0.47	
Rives *et al.*,	Dual colour	≅5000	0.32	0.51	0.48	0.21	0.24	0.54	
1998a	FISH	≅5000	0.21	0.24	0.22	0.22	0.17	0.22	
Pang *et al.*, 1999	Dual/triple	≅1000	1.1–3.5	0–3.1	0.6–3.4		1.6–4.9 [a]		0.4–9.6
	colour FISH	1000–2000	0.20	0.11	0.18		0.15 [a]		0.04
Present study	Dual/triple colour FISH	≅3000	0.23 (0.02–0.53)	0.22 (0.00–0.56)	0.29 (0.07–0.67)	0.17 (0.00–0.37)	0.18 (0.02–0.52)	0.23 (0.00–0.70)	0.12 (0.00–0.29)
		≅2000	0.21 (0.10–0.37)	0.19 (0.10–0.32)	0.22 (0.10–0.46)	0.09 (0.05–0.20)	0.12 (0.05–0.31)	0.16 (0.05–0.39)	0.14 (0.00–0.37)
		≅3000	0.11 (0.00–0.24)	0.07 (0.00–0.20)	0.21 (0.10–0.26)	0.04 (0.00–0.10)	0.25 (0.10–0.33)	0.15 (0.00–0.33)	0.07 (0.00–0.26)
		≅7000	0.08 (0.05–0.13)	0.06 (0.03–0.10)	0.07 (0.05–0.10)	0.03 (0.00–0.05)	0.05 (0.00–0.08)	0.05 (0.00–0.10)	0.03 (0.00–0.05)

OAT = oligoasthenoteratozoospermia; n.r. = not reported.
[a]Total sex chromosomal disomies.
[b]Total abnormality rates for chromosomes 18, X and Y.
[c]Total abnormality rates for chromosomes 15, 18 and 22.
[d]Total disomy rates for chromosomes 13, 18, 21, X and Y.

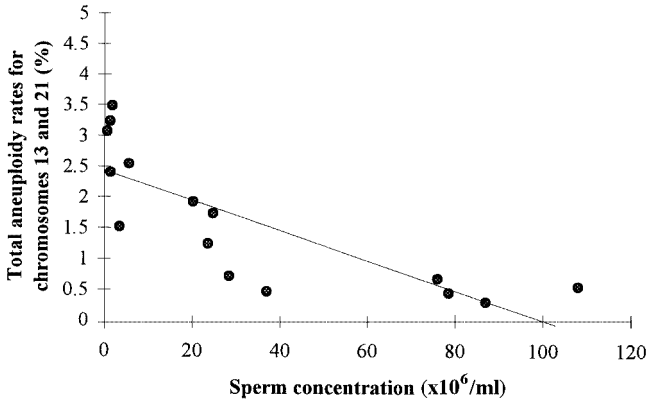

Figure 1. Scatterplot of the sperm concentration versus the total number of abnormalities (chromosomes 13 and 21) in group 1 (15 patients) (Spearman correlation coefficient = –0.907, P = 0.0001).

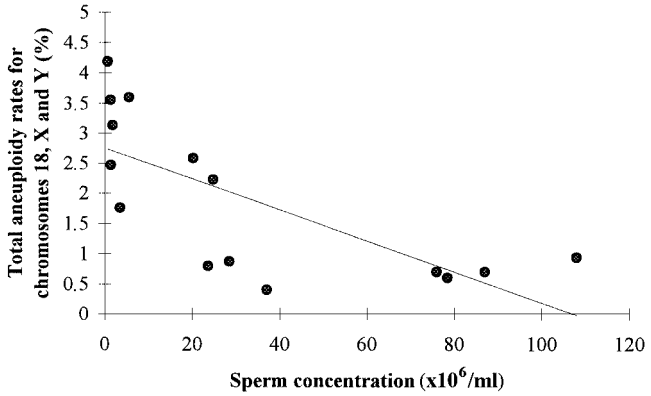

Figure 2. Scatterplot of the sperm concentration versus the total number of abnormalities (chromosomes 18, X and Y) in group 1 (15 patients) (Spearman correlation coefficient = –0.810, P = 0.0002).

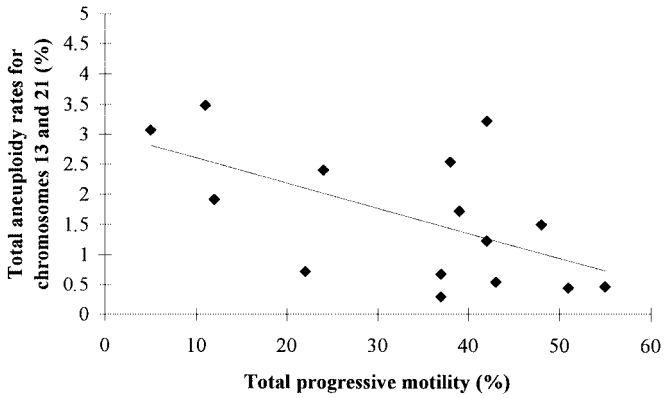

Figure 3. Scatterplot of the total progressive motility of spermatozoa versus the total number of abnormalities (chromosomes 13 and 21) in group 1 (15 patients) (Spearman correlation coefficient = –0.52415, P = 0.04).

Y between 45 infertile patients with varying degrees of semen abnormalities and six healthy controls of proven fertility.

Comparing semen samples from patients undergoing IVF and ICSI, a significantly higher incidence of sex chromosomal aneuploidies in the latter patients was found (Storeng *et al.*, 1998). A French group (Rives *et al.*, 1998a) investigated semen samples from 50 infertile males with semen abnormalities of

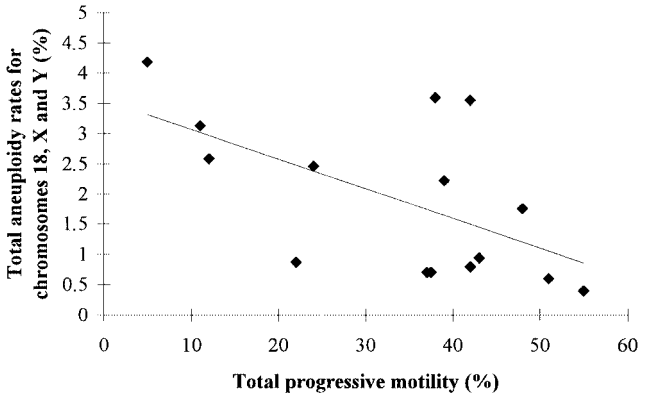

Figure 4. Scatterplot of the total progressive motility of spermatozoa versus the total number of abnormalities (chromosomes 18, X and Y) in group 1 (15 patients) (Spearman correlation coefficient = –0.55635, P = 0.0313).

various degrees and from 10 controls. Using whole chromosome paint probes for chromosomes 1, 13, 14, 18, 21, 22, X and Y they found an increased incidence of YY- and XY-bearing spermatozoa and a higher incidence of total autosomal aneuploidy in sperm nuclei from infertile OAT patients compared to control subjects with proven fertility. This study also showed a significant difference in the disomy frequencies between the different autosomes, with the highest frequency for chromosome 18 (0.51%) and the lowest for chromosome 1 (0.35%). More recently nine infertile patients undergoing ICSI were analysed for aneuploidies of chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y (Pang *et al.*, 1999). The authors found significantly elevated frequencies of diploidy, aneuploidies of autosomes and of sex chromosomes and total aneuploidy in OAT patients. The frequencies per chromosome for disomy for autosomes and sex chromosomes in OAT patients were 0–5.4% versus 0.05–0.2% in controls. Interestingly six series of ICSI in five of these patients failed to gave a viable pregnancy and the authors therefore suggested that the elevated aneuploidy in spermatozoa of these patients can contribute to their infertility.

These different results, as reported for studies in fertile men, are most probably due to the different criteria for patient recruitment, the different decondensation treatment used, the different probes and FISH protocols applied and also the different scoring criteria adopted.

Nevertheless, our results are in agreement with the majority of the reported data. According to our findings, there seems to be a higher incidence of chromosomes 13, 18, 21, X and Y aneuploidies in infertile patients with abnormal semen and normal karyotype (group 1) and in pooled patients of group 1 and group 3, compared to normal controls. Moreover, in these patients an inverse correlation between aneuploidy rates and sperm parameters (concentration and total progressive motility) was found. Such a correlation was present for all the investigated chromosomes. On the contrary we did not find a significant correlation between chromosomal aneuploidies and abnormal morphology of spermatozoa, although this finding was reported by other authors (Yurov *et al.*, 1996; Colombero *et al.*, 1997; Estop *et al.*, 1997a; In't Veld *et al.*, 1997).

Infertile patients with abnormal karyotype and normal or

abnormal semen were also investigated (group 2). Data from the literature regarding aneuploidy rates evaluated by FISH in spermatozoa from patients with abnormal karyotype are less numerous and semen samples from only a few patients have been analysed. These data are also difficult to compare among published studies because it is probable that the degree of semen parameter abnormalities might be conditioned by the different chromosomes involved in a chromosomal rearrangement and by the different breakpoints (Van Assche *et al*., 1996). To substain this hypothesis, it is interesting to note that two out of 10 infertile patients carrying a reciprocal translocation in our study presented normal semen analysis (patients no. 6 and 33).

Some other authors studied patients with reciprocal or Robertsonian translocation or inversion using FISH (Lu *et al*., 1994; Rosseaux *et al*., 1995a,b; Colls *et al*., 1997; Martini *et al*., 1998; Mercier *et al*., 1998).

However, these previously reported results are too scanty to draw any conclusion. In this study, we analysed by FISH semen samples from a group of 10 patients carrying a reciprocal translocation, two patients carrying a Robertsonian translocation and one patient with a 46,XY/47,XYY mosaic in order to evaluate the aneuploidy rates for chromosomes 13, 18, 21, X and Y. From our findings there appears to be a higher frequency of diploid spermatozoa and of aneuploidies for chromosomes 13, 18, 21, X and Y in patients carrying a translocation compared to controls. Therefore the hypothesis of an interchromosomal effect of a chromosomal rearrangement on meiotic pairing, as reported by other authors (Spriggs and Martin, 1994; Rousseaux *et al*., 1995a,b; Mercier *et al*., 1998), seems acceptable.

In group 2 a male carrier of a low grade 46,XY/47,XYY mosaicism was included. The mosaicism was found in one out of 200 metaphase nuclei and in four out of 200 interphase nuclei of peripheral lymphocytes by FISH. This patient (no. 26) presented a similar diploidy rate and disomy rates for chromosomes 13, 18, 21, X and Y compared to normal controls and an X- and Y-bearing spermatozoa ratio not different from 1:1. There are few data in the literature on FISH analysis on spermatozoa from patients carrying an extra sex chromosome. The majority of these studies suggest a higher incidence of aneuploid spermatozoa in 47,XYY males (Martini *et al*., 1996; Mercier *et al*., 1996; Blanco *et al*., 1997) and in a Klinefelter male (Estop *et al*., 1998). Two analysed Klinefelter mosaics (Chevret *et al*., 1996; Martini *et al*., 1996) showed increased levels of aneuploidies. All these patients were found able to produce mature normal haploid spermatozoa, with an X:Y ratio not significantly different from 1:1 (Martini *et al*., 1996; Blanco *et al*., 1997) or with an excess of Y-bearing spermatozoa (Mercier *et al*., 1996; Chevret *et al*., 1997) or an excess of X-bearing spermatozoa (Chevret *et al*., 1996).

The four infertile patients from group 3 were analysed separately because they fathered a child with an abnormal karyotype through an ICSI cycle: in patients no. 14 and 18, the paternal origin of the supernumerary Y chromosome is obvious, whereas the paternal origin of the supernumerary 21 and Y chromosomes in patient no. 20 was proven by molecular analysis. With regard to patient no. 17, no DNA was available from the miscarriage for molecular analysis, thus the origin of the supernumerary 16 chromosome could not be proven. The paternal origin of the majority of prenatally detected chromosome aberrations was also demonstrated in another ICSI series (six out of nine cases) (Van Opstal *et al*., 1997).

The mean age of these four patients was 33 years (range 31–36) and the mean age of their wives was 30.2 years (range 28–33). This is the first report on an aneuploidy study of spermatozoa from infertile men who fathered offspring with abnormal karyotype through an ICSI procedure. Probably due to the small number of these subjects ($n = 4$), in this group we did not observe a significantly higher frequency of aneuploidy rate for the analysed chromosomes compared to controls. These patients also did not show any significant difference in aneuploidy rates compared to patients of group 1 and therefore seem to have the same risk for aneuploidy in offspring as patients of group 1. According to our data, the frequency of abnormal spermatozoa seems to be influenced by semen parameters more than by the history of a conceptus carrying a chromosomal aneuploidy: the incidence of aneuploidy in spermatozoa of our patients is significantly correlated with the poor semen quality, distinctively with a low sperm concentration and a low total progressive motility and it is not higher in group 3 compared to group 1. Patients with the highest risk for aneuploidies in spermatozoa and in offspring therefore seem to be men with severe oligoasthenozoospermia. Our findings in severe OAT patients are in agreement with other reports which show that the frequency of non-disjunction of autosomes and gonosomes increases when the sperm count decreases (Rives *et al*., 1998a; Storeng *et al*., 1998). These data are also confirmed by the results of a follow-up study (Bonduelle *et al*., 1998) on children conceived after ICSI: patients at a higher risk of conceiving a child with an abnormal karyotype are males with severe OAT.

On the other hand, about 90% of trisomies are maternal in origin (Koehler *et al*., 1996) and studies on oocytes of similar age groups (30–39 years) have showed an aneuploidy rate above 20% (Dailey *et al*., 1996; Martini *et al*., 1997). These data suggest that the increase in aneuploidy rate in oligoasthenozoospermic men is of limited clinical relevance, when compared to the maternal contribution to chromosomal abnormalities of the embryos. It has also been demonstrated that there is no difference in chromosome abnormalities between embryos obtained after ICSI and IVF (Gianaroli *et al*., 1997; Munné *et al*., 1998). However, in our opinion, such an increase in aneuploidy rate in spermatozoa, inversely correlated with sperm concentration and motility, should not be underestimated. If each chromosome shows this slight increase in aneuploidy rate, it is possible to speculate a total rate of aneuploidy of ~33–74% in spermatozoa from OAT men (Pang *et al*., 1999). It would also be of great interest to analyse the chromosome complements of epididymal and testicular spermatozoa, since many ICSI procedures are now performed in patients who do not have spermatozoa in the ejaculate.

As a consequence we believe that prenatal diagnosis after an ICSI pregnancy should be offered to all couples, especially to those with a severe male factor and certainly when a

structural chromosomal rearrangement in the paternal karyotype is present.

Another point of interest is to study the segregation of rearranged chromosomes in carriers of a reciprocal translocation. These patients show a different risk of conceiving a child with an unbalanced karyotype and also different semen parameters, ranging from a normal semen analysis to severe OAT, possibly depending on the chromosomes involved in the rearrangement and on the breakpoints. So far these segregation studies have been performed in <5000 spermatozoa from 36 carriers using the zona-free hamster egg–sperm fusion technique (reviewed by Guttenbach *et al*., 1997a) and in few patients using the FISH technique (Lu *et al*., 1994; Spriggs and Martin, 1994; Rousseaux *et al*., 1995a,b; Colls *et al*., 1997; Estop *et al*., 1997b; Martini *et al*., 1998; Mercier *et al*., 1998), mainly due to the difficulties in obtaining probes to study the chromosomes involved in the translocation. A segregation study on our patients carrying a reciprocal translocation (10 patients in group 2) is in progress and data from one of these patients (patient no. 25) have been published elsewhere (Van Assche *et al*., 1999).

In conclusion, our data suggest a correlation between poor semen parameters and an increase in chromosomal aneuploidy rate of spermatozoa. Nevertheless, the majority of spermatozoa in these patients seems to be normal for chromosomes 13, 18, 21, X and Y. The risk of a chromosomal aneuploidy in spermatozoa seems to be inversely correlated with sperm concentration and total progressive motility. Patients with abnormal karyotype showed a higher incidence of diploidy and chromosomal aneuploidies compared to controls, strongly suggesting the presence of an interchromosomal effect of the cytogenetic rearrangement. These observations suggest the necessity for proper counselling before ICSI in couples with a severe male factor or with a male partner carrying a chromosomal rearrangement.

Finally, men who fathered a child with an abnormal karyotype through an ICSI procedure did not present a higher aneuploidy rate for the investigated chromosomes in spermatozoa compared to patients with infertility due to similar male factor. These data, as well as other publications, support the hypothesis that the increase in sex chromosomal anomalies after ICSI is possibly the consequence of sperm aneuploidies rather than a consequence of micromanipulation.

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References

Bernardini, L., Martini, E., Geraedts, J.P.M. *et al*. (1997) Comparison of gonosomal aneuploidy in spermatozoa of normal fertile men and those with severe male factor detected by *in situ* hybridization. *Mol. Hum. Reprod*., **3**, 431–438.

Blanco, J., Rubio, C., Simón, C. *et al*. (1997) Increased incidence of disomic sperm nuclei in a 47,XYY male assessed by fluorescent *in situ* hybridization (FISH). *Hum. Genet*., **99**, 413–416.

Bonduelle, M., Aytoz, A., Van Assche, E. *et al*. (1998) Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. [Editorial] *Hum. Reprod*., **13**, 781–782.

Brobow, M., Madan, K. and Pearson, P.L. (1972) Staining of some specific regions of human chromosomes, particularly the secondary constriction of number 9. *Nature New Biol*., **238**, 122–124.

Chandley, A.C. and Hargreave, T.B. (1996) Genetic anomaly and ICSI. *Hum. Reprod*., **11**, 930–932.

Chevret, E., Rosseaux, S., Monteil, M. *et al*. (1996) Increased incidence of hyperhaploid 24,XY spermatozoa detected by three-colour FISH in a 46,XY/47,XXY male. *Hum. Genet*., **97**, 171–175.

Chevret, E., Rosseaux, S., Monteil, M. *et al*. (1997) Meiotic behaviour of sex chromosomes investigated by three-colour FISH on 35 142 sperm nuclei from two 47,XYY males. *Hum. Genet*., **99**, 407–412.

Colls, P., Blanco, J., Martínez-Pasarell, O. *et al*. (1997) Chromosome segregation in a man heterozygous for a pericentric inversion, inv(9)(p11q13), analyzed by using sperm karyotyping and two-color fluorescence *in situ* hybridization on sperm nuclei. *Hum. Genet*., **99**, 761–765.

Colombero, L.T., Hariprashad, J., Moy, F. *et al*. (1997) Impact of abnormal semen parameters on the genetic status of human spermatozoa. *J. Androl*., **18** (Suppl.), Abstr. 11, p. P-27.

Dailey, T., Dale, B., Cohen, J. and Munné, S. (1996) Association between nondisjunction and maternal age in meiosis-II human oocytes. *Am. J. Hum. Genet*., **59**, 176–184.

Downie, S.E., Flaherty, S.P. and Matthews, C.D. (1997) Detection of chromosomes and estimation of aneuploidy in human spermatozoa using fluorescence *in-situ* hybridization. *Mol. Hum. Reprod*., **3**, 585–598.

Egozcue, J., Blanco, J. and Vidal, F. (1997) Chromosome studies in human sperm nuclei using fluorescence *in-situ* hybridization (FISH). *Hum. Reprod. Update*, **3**, 441–452.

Estop, A.M., Vandermark, K.K., Munné, S. *et al*. (1997a) Sperm morphology and chromosome aneuploidy in men with infertility as determined by fluorescence *in situ* hybridization (FISH). *Fertil. Steril*. (Suppl.), Abstr. P-242, p. S208.

Estop, A.M., Cieply, K.M. and Aston, C.E. (1997b) The meiotic segregation pattern of a reciprocal translocation t(10;12)(q26.1;p13.3) by fluorescence *in situ* hybridization sperm analysis. *Eur. J. Hum. Genet*., **5**, 78–82.

Estop, A.M., Munné, S., Cieply, K.M. *et al*. (1998) Meiotic products of a Klinefelter 47,XXY male as determined by sperm fluorescence *in situ* hybridization analysis. *Hum. Reprod*., **13**, 124–127.

Francisco, R.G., Newberg, M.T., Pang, M.G. *et al*. (1997) Determining aneuploidy and Y chromosome deletions in oligoasthenoteratozoospermic patients undergoing ICSI. *J. Assist. Reprod. Genet*., **14**, (Suppl.), Abstr. OC-07–058, p. 56S.

Geraedts, J. and Pearson, P. (1973) Specific staining of the human number 1 chromosome in spermatozoa. *Humangenetik*, **20**, 171–173.

Gianaroli, L., Magli, M.C., Munné, S. *et al*. (1997) Preimplantation genetic diagnosis of aneuploidy and male infertility. *Int. J. Androl*., **20** (Suppl. 3), 31–34.

Guttenbach, M. and Schmid, M. (1990) Determination of Y chromosome aneuploidy in human sperm nuclei by nonradioactive *in situ* hybridisation. *Am. J. Hum. Genet*., **46**, 553–558.

Guttenbach, M., Schakowski, R. and Schmid, M. (1994a) Incidence of chromosome 3, 7, 10, 11, 17 and X disomy in mature human sperm nuclei as determined by nonradioactive *in situ* hybridisation. *Hum. Genet*., **93**, 7–12.

Guttenbach, M., Schakowski, R. and Schmid, M. (1994b) Incidence of chromosome 18 disomy in human sperm nuclei as detected by nonisotopic *in situ* hybridisation. *Hum. Genet*., **93**, 421–423.

Guttenbach, M., Engel, W. and Schmid, M. (1997a) Analysis of structural and numerical chromosome abnormalities in sperm of normal men and carriers of constitutional chromosome aberrations. A review. *Hum. Genet*., **100**, 1–21.

Guttenbach, M., Martinez-Expósito, M.-J., Michelmann, H.W. *et al*. (1997b) Incidence of diploid and disomic sperm nuclei in 45 infertile men. *Hum. Reprod*., **12**, 468–473.

In't Veld, P.A., Brandenburg, H., Verhoeff, A. *et al*. (1995) Sex chromosomal abnormalities and intracytoplasmic sperm injection. *Lancet*, **346**, 773.

In't Veld, P.A., Broekmans, J.M., de France, H.F. *et al*. (1997) Intracytoplasmatic sperm injection (ICSI) and chromosomally abnormal spermatozoa. *Hum. Reprod*., **12**, 752–754.

Joseph, A.M., Gosden, J.R. and Chandley, A.C. (1984) Estimation of aneuploidy levels in human spermatozoa using chromosome specific probes and *in situ* hybridisation. *Hum. Genet*., **66**, 234–238.

Koehler, K.E., Hawley, R.S., Sherman, S. and Hassold, T. (1996) Recombination and nondisjuntion in humans and flies. *Hum. Mol. Genet*., **5**, 1495–1504.

Kruger, T.F., Menkeveld, R., Stander, F.S.H. *et al*. (1986) Sperm morphologic features as a prognostic factor in *in vitro* fertilization. *Fertil. Steril*., **46**, 1118–1123.

Lähdetie, J., Saari, N., Ajosenpää-Saari, M. and Mykkänen, J. (1997) Incidence of aneuploidy spermatozoa among infertile men studied by multicolor fluorescence *in situ* hybridization. *Am. J. Med. Genet*., **71**, 115–121.

Liebaers, I., Bonduelle, M., Van Assche, E. *et al*. (1995) Sex chromosome abnormalities after intracytoplasmic sperm injection. [Letter] *Lancet*, **346**, 1095.

Lu, P.Y., Hammitt, D.G., Zinsmeister, A.R. and Dewald, G.W. (1994) Dual color fluorescence *in situ* hybridization to investigate aneuploidy in sperm from 33 normal males and a man with a t(2;4;8) (q23;q27;p21). *Fertil. Steril*., **62**, 394–399.

Martin, R.H., Spriggs, E. and Rademaker, A.W. (1996) Multicolor fluorescence *in situ* hybridization analysis of aneuploidy and diploidy frequencies in 225 846 sperm from normal men. *Biol. Reprod*., **54**, 394–398.

Martin, R.H., Moosani, N., Rademaker, A.W. *et al*. (1997) Sperm chromosomal abnormalities in infertile men. *J. Assist. Reprod. Genet*., **14** (Suppl.), Abstr. PS-16–2, p. 25S.

Martini, E., Speel, E.J.M., Geraedts, J.P.M. *et al*. (1995) Application of different in-situ hybridization detection methods for human sperm analysis. *Hum. Reprod*., **10**, 855–861.

Martini, E., Geraedts, J.P.M., Liebaers, I. *et al*. (1996) Constitution of semen samples from XYY and XXY males as analysed by in-situ hybridization. *Hum. Reprod*., **11**, 1638–1643.

Martini, E., Flaherty, S.P., Swann, N.J. *et al*. (1997) Analysis of unfertilized oocytes subjected to intracytoplasmic sperm injection using two rounds of fluorescence in-situ hybridization and probes to five chromosomes. *Hum. Reprod*., **12**, 2011–2018.

Martini, E., von Bergh, A.R.M., Coonen, E. *et al*. (1998) Detection of structural abnormalities in spermatozoa of a translocation carrier t(3;11) (q27.3;q24.3) by triple FISH. *Hum. Genet*., **102**, 157–165.

Mercier, S., Morel, F., Roux, C. *et al*. (1996) Analysis of the sex chromosomal equipment in spermatozoa of a 47,XYY male using two-colour fluorescence in-situ hybridization. *Mol. Hum. Reprod*., **2**, 485–488.

Mercier, S., Morel, F., Fellman, F. *et al*. (1998) Molecular analysis of the chromosomal equipment in spermatozoa of a 46,XY,t(7;8) (q11.21;cen) carrier by using fluorescence *in situ* hybridization. *Hum. Genet*., **102**, 446–451.

Miharu, N., Best, R.G. and Young, S.R. (1994) Numerical chromosome abnormalities in spermatozoa of fertile and infertile men detected by fluorescence *in situ* hybridization. *Hum. Genet*., **93**, 502–506.

Moosani, N., Pattinson, H.A., Carter, M.D. *et al*. (1995) Chromosomal analysis of sperm from men with idiopathic infertility using sperm karyotyping and fluorescence *in situ* hybridization. *Fertil. Steril*., **64**, 811–817.

Munné, S., Marquez, C., Reing, A. *et al*. (1998) Chromosome abnormalities in embryos obtained after conventional *in vitro* fertilization and intracytoplasmic sperm injection. *Fertil. Steril*., **69**, 904–908.

Nielsen, J. and Wohlert, M. (1991) Chromosome abnormalities found among 34 910 newborn children: results from a 13-year incidence study in Århus, Denmark. *Hum. Genet*., **87**, 81–83.

Pang, M.G., Zackowski, J.L., Hoegerman, S.F. *et al*. (1995) Detection by fluorescence *in situ* hybridization of chromosomes 7, 11, 12, 18, X and Y abnormalities in sperm from oligoasthenoteratozoospermic patients of an *in vitro* fertilization program. *J. Assist. Reprod. Genet*., **12** (Suppl.), Abstr. OC-105, p.53S.

Pang, M.G., Hoegerman, S.F., Cuticchia, A.J. *et al*. (1999) Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence *in situ* hybridization in spermatozoa from nine patients with oligoasthenoteratozoospermia undergoing intracytoplasmic sperm injection. *Hum. Reprod*., **14**, 1266–1273.

Pearson, P.L. and Brobow, M. (1970) Fluorescent staining of the Y chromosome in meiotic stages of the human male. *J. Reprod. Fertil*., **22**, 177–179.

Pellestor, F., Quenesson, I., Coignet, L. *et al*. (1996a) Direct detection of disomy in human sperm by the PRINS technique. *Hum. Genet*., **97**, 21–25.

Pellestor, F., Girardet, A., Coignet, L. *et al*. (1996b) Assessment of aneuploidy for chromosome 8, 9, 13, 16, and 21 in human sperm by using primed *in situ* labeling technique. *Am. J. Hum. Genet*., **58**, 797–802.

Pinkel, D., Landegent, J., Collins, C. *et al*. (1988) Fluorescence *in situ* hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc. Natl. Acad. Sci. USA*, **85**, 9664–9668.

Rives, N., Mazurier, S., Sibert, L. *et al*. (1998a) Incidence of aneuploidy in sperm nuclei of infertile men. *Hum. Reprod*. **13** (Suppl.), Abstr. O-245, pp. 126–127.

Rives, N., Mazurier, S., Bellet, D. *et al*. (1998b) Assessment of autosome and gonosome disomy in human sperm nuclei by chromosome painting. *Hum. Genet*., **102**, 616–623.

Rooney, D. and Czepulkowski, B. (1992) *Human Cytogenetics. A Practical Approach*, Vol. 1. Oxford University Press, Oxford.

Rosseaux, S., Chevret, E., Monteil, M. *et al*. (1995a) Meiotic segregation in males heterozygote for reciprocal translocations: analysis of sperm nuclei by two and three colour fluorescence *in situ* hybridization. *Cytogenet. Cell Genet*., **71**, 240–246.

Rosseaux, S., Chevret, E., Monteil, M. *et al*. (1995b) Sperm nuclei analysis of a Robertsonian t(14q21q) carrier, by FISH, using three plasmids and two YAC probes. *Hum. Genet*., **96**, 655–660.

Rudak, E., Jacobs, P.A. and Yanagimachi, R. (1978) Direct analysis of the chromosome constitution of human spermatozoa. *Nature*, **274**, 911–913.

Schröck, E., du Manoir, S., Veldman, T. *et al*. (1996) Multicolor spectral karyotyping of human chromosomes. *Science*, **273**, 494–497.

Spriggs, E.L. and Martin, R.H. (1994) Analysis of segregation in a human male reciprocal translocation carrier, t(1;11) (p36.3;q13.1), by two-colour fluorescence *in situ* hybridization. *Mol. Reprod. Dev*., **38**, 247–250.

Storeng, R.T., Plachot, M., Theophile, D. *et al*. (1998) Incidence of sex chromosome abnormalities in spermatozoa from patients entering an IVF or ICSI protocol. *Acta Obstet. Gynecol. Scand*., **77**, 191–197.

Van Assche, E., Bonduelle, M., Tournaye, H. *et al*. (1996) Cytogenetics of infertile men. *Hum. Reprod*., **11** (Suppl. 4), 1–24.

Van Assche, E., Staessen, C., Vegetti, W. *et al*. (1999) Preimplantation genetic diagnosis and sperm analysis by fluorescence in-situ hybridization for the most common reciprocal translocation t(11;22). *Mol. Hum. Reprod*., **5**, 682–690.

Van Hummelen, P., Lowe, X.R. and Wyrobek, A.J. (1996) Simultaneous detection of structural and numerical chromosome abnormalities in sperm of healthy men by multicolor fluorescence *in situ* hybridization. *Hum. Genet*., **98**, 608–615.

Van Opstal, D., Los, F.J., Ramlakhan, S. *et al*. (1997) Determination of the parent of origin in nine cases of prenatally detected chromosome aberrations found after intracytoplasmic sperm injection. *Hum. Reprod*., **12**, 682–686.

Van Steirteghem, A.C., Liu, J., Joris, H. *et al*. (1993) Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum. Reprod*., **8**, 1055–1060.

Van Steirteghem, A.C., Nagy, P., Liu, J. *et al*. (1996) The development of intracytoplasmic sperm injection. *Hum. Reprod*., **11** (Suppl. 1), 59–72.

West, J.D., West, K.M. and Aitken, R.J. (1989) Detection of Y-bearing spermatozoa by DNA–DNA *in situ* hybridisation. *Mol. Reprod. Dev*., **1**, 201–207.

Williams, B.J., Ballenger, C.A., Malter, H.E. *et al*. (1993) Nondisjunction in human sperm: results of fluorescence *in situ* hybridization studies using two and three probes. *Hum. Mol. Genet*., **2**, 1929–1936.

World Health Organization (1992) *Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 3rd edn. Cambridge University Press, Cambridge.

Yanagimachi, R., Yanagimachi, H. and Rogers, B.J. (1976) The use of zona-free animal ova as a test system for the assessment of the fertilizing capacity of human spermatozoa. *Biol. Reprod*., **15**, 471–476.

Yurov, Y.B., Saias, M.J., Vorsanova, S.G. *et al*. (1996) Rapid chromosomal analysis of germ-line cells by FISH: an investigation of an infertile male with large-headed spermatozoa. *Mol. Hum. Reprod*., **2**, 665–668.

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