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Incidence of sperm chromosomal abnormalities in a risk population: relationship with sperm quality and ICSI outcome

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BACKGROUND: An increased incidence of chromosome abnormalities has been reported in sperm samples of many infertile men by fluorescence in-situ hybridization (FISH). METHODS: Sperm aneuploidy and diploidy rates for chromosomes 13, 18, 21, X and Y were evaluated in 63 patients with normal karyotypes using dual and triple-colour FISH techniques. Indications for sperm FISH analysis were: recurrent miscarriages of unknown aetiology (RM, $n = 40$), repeated implantation failure after intracytoplasmic sperm injection (ICSI) (IF, $n = 19$), previous Down's syndrome pregnancies ($n = 3$), and meiotic abnormalities (MA, $n = 1$). Nine healthy normozoospermic donors were also evaluated as a control group. RESULTS: A significant increase in the incidence of sex chromosome disomies was found in the RM, IF and MA groups. Oligoasthenoteratozoospermic patients ($n = 21$) showed significantly higher rates of diploidy and disomies for sex chromosomes and chromosomes 18 and 21 than normozoospermic patients ($n = 14$). Thirty-one patients with normal and seven with abnormal FISH results had undergone several ICSI treatments (108 and 23 cycles respectively). Couples with abnormal sperm FISH results showed decreased pregnancy and implantation rates and increased miscarriage rates. CONCLUSIONS: Patients with a clinical background of recurrent miscarriages of unknown aetiology or implantation failure after ICSI are at risk of showing sperm chromosomal abnormalities, the incidence of which is higher in oligoasthenoteratozoospermic patients.

Key words: aneuploidy/FISH/ICSI/miscarriage/spermatozoa

Introduction

Analysis of sperm chromosomal aneuploidies by fluorescence in-situ hybridization (FISH) is of great interest for several reasons. It has been estimated that about 60% of first trimester spontaneous abortions are caused by chromosomal abnormalities (Hassold *et al.*, 1980), the majority of which are the result of non-disjunction during gametogenesis. Although most of them are of maternal origin, molecular studies have shown that 8–12% of abortions with trisomy 13, 18 and 21 are of paternal origin (Nicolaidis and Petersen, 1998). Using sperm FISH analysis, an increased incidence of sex chromosome disomies in sperm samples from recurrent spontaneous abortion couples, when compared with controls, has been recently reported by our group in a preliminary study (Rubio *et al.*, 1999).

On the other hand, chromosome abnormalities are increasingly found in the spermatozoa of many infertile men. This makes the direct analysis of sperm aneuploidy of clinical relevance, since male infertility is now treated by intracytoplasmic sperm injection (ICSI), which has the implicit risk of transmitting chromosomal aberrations from the paternal side. In fact, a higher incidence of sex chromosomal aneuploidies and structural *de novo* chromosomal abnormalities has been found in prenatal karyotypes following ICSI compared with the general population, which could be attributed to the characteristics of the infertile men treated (In't Veld *et al.*, 1995; Liebaers *et al.*, 1995; Bonduelle *et al.*, 1998). The importance of analysing the cytogenetic constitution of ejaculated spermatozoa is emphasized by meiotic studies, showing that 17.6–26.7% of patients with severe oligozoospermia ($\leqslant 1\times10^6$ sperm/ml), have synaptic chromosome anomalies restricted to the germ cell line, which are not detectable by peripheral blood karyotype (Egozcue *et al.*, 1983, 2000a; Vendrell *et al.*, 1999). In this regard, the available literature on FISH analysis of human spermatozoa confirms higher rates of sperm aneuploidy in infertile men as compared with fertile men (Moosani *et al.*, 1995; Lahdetie *et al.*, 1997; Bernardini *et al.*, 1998; Arán *et al.*, 1999; Pang *et al.*, 1999;

Pfeffer *et al.*, 1999; Ushijima *et al.*, 2000; Vegetti *et al.*, 2000). An inverse correlation between sperm quality and sperm aneuploidy rates has been reported (Bernardini *et al.*, 2000; Nishikawa *et al.*, 2000; Ushijima *et al.*, 2000; Vegetti *et al.*, 2000), but contradictory results have been published concerning the relationship of sperm aneuploidy with specific sperm defects.

Another important issue is how sperm aneuploidy may influence ICSI outcome in infertile patients. There are only a few reports on small ICSI series performed on oligoasthenoteratozoospermic patients with increased sperm aneuploidy rates (Bernardini *et al.,* 1998, 2000; Pang *et al.*, 1999; Pfeffer *et al.*, 1999), but their results suggest that sperm aneuploidy may be associated with implantation failure and/or early fetal loss. However, larger series comparing ICSI outcome in infertile patients with and without increased sperm aneuploidy rates are necessary in order to confirm these preliminary experiences.

In the present study, we have retrospectively analysed our FISH results on spermatozoa in a series of patients with normal karyotypes presenting with a risk of sperm chromosomal abnormalities because of several factors independent of sperm quality, such as recurrent spontaneous miscarriages, repeated implantation failures after ICSI, previous pregnancies with chromosomal abnormalities or meiotic abnormalities in testicular biopsies. Objectives of the study were: (i) to investigate whether these indications were actually associated with an increased incidence of sperm aneuploidy and diploidy; (ii) to examine the correlation between sperm chromosomal abnormalities and the basic sperm parameters in this at risk population; and (iii) since some of the couples included in this series had undergone several ICSI treatments, to evaluate the effect of sperm chromosome abnormalities on ICSI outcome in terms of fertilization, implantation, pregnancy and miscarriage rates.

Materials and methods

Patients

From October 1998 to June 2000, FISH was performed on 63 sperm samples from a population at increased risk of having sperm chromosomal abnormalities. All patients had normal 46,XY karyotypes and mean age (± SD) was 37.5 (± 1.0) years. Indications for sperm FISH analysis were not related to the quality of the sperm sample, but were related to the following reproductive background: (i) Recurrent miscarriage (RM) of unknown aetiology after routine work-up for this pathology (n = 40). Diagnostic work-up for RM couples included endocrine screening [oestradiol, LH, FSH, thyroid stimulating hormone (TSH), prolactin, progesterone, glucose and plasmatic homocystein], ultrasound examination, screening for uterine abnormalities (hysteroscopy, hysterosalpingography), analysis of immunological factors (lupus anticoagulant, anticardiolipin antibodies, C3c and C4), analysis of infectious factors (hepatitis B and C, rubella, toxoplasma, HIV, syphilis and chlamydia), and evaluation of coagulation disorders (protein C, protein S and APCR). The mean number of previous first trimester miscarriages (weeks 6–12) was 3.0 ± 0.3. (ii) Repeated implantation failure (IF) after ICSI (three or more failed attempts), associated with poor embryo quality (slow developmental rates, high fragmentation degree, presence of vacuoles and/or multinucleated blastomeres) (n = 19). The mean number of ICSI failures in these patients was 3.7 ± 0.4 (mean ± SD). (iii) Previous Down's syndrome (DS) pregnancies (n = 3). (iv) Meiotic abnormalities (MA) (n = 1); a patient with partial meiotic arrest on testicular biopsy.

Sperm samples were evaluated according to WHO criteria (World Health Organization, 1992). To establish whether sperm chromosomal abnormalities in this series were related to sperm quality, sperm samples were classified into five groups according to the three standard sperm parameters: Normozoospermia (n = 14), isolated asthenozoospermia (<50% progressive motile spermatozoa, n = 14), isolated teratozoospermia (<30% normal forms, n = 8), asthenoteratozoospermia (AT) (<50% progressive motile spermatozoa and <30% normal forms, n = 6) and oligoasthenoteratozoospermia (OAT, $<20 \times 10^6$/ml spermatozoa, <50% progressive motile spermatozoa and <30% normal forms, n = 21). To correlate the rate of chromosomal abnormalities with the severity of oligozoospermia, OAT patients were further classified into three subgroups according to sperm count: $<5 \times 10^6$/ml (n = 9), 5–10 $\times 10^6$/ml (n = 5), and $\geqslant$10–20 $\times 10^6$/ml (n = 7).

Individual results in each patient were compared with those of a control group of nine normozoospermic donors without a history of infertility (Blanco *et al.*, 1996, 1997). Individual FISH results were considered as abnormal when a statistically significant increase in any of the analysed parameters (disomy rates for chromosomes 18, 21, X and Y and total diploidy rate) was observed when compared with the control group. The disomy rate for chromosome 13 was not evaluated in the controls, and therefore no statistical comparisons were performed for this parameter.

A total of 131 ICSI cycles was performed in 38 patients. ICSI outcome, including fertilization, embryo cleavage, pregnancy, implantation and miscarriage rates, of 108 cycles carried out in 31 patients with normal FISH results was compared with that of 23 cycles in seven patients with abnormal FISH results.

Informed consent regarding sperm FISH analysis and the ICSI procedure was obtained from all patients. The study was approved by our local ethical committee.

FISH protocol

For FISH analysis one semen sample from each patient was centrifuged with Sperm medium (Medicult, Copenhagen, Denmark) the pellet was fixed in methanol:acetic acid (3:1), sperm nuclei were decondensed by slide incubation in 5 mmol/l dithiothreitol (DTT) and 1% Triton X-100 and hybridization was performed following the manufacturer's protocol. Numerical abnormalities for chromosomes 13, 18, 21, X and Y were evaluated in different slides from the same sample, using triple-colour FISH for 18, X and Y chromosomes and dual-colour FISH for chromosomes 13 and 21. Centromeric DNA probes for chromosome 18 (locus D18Z1, CEP 18 Spectrum Aqua; Vysis Inc. Downers Grove, IL, USA), chromosome X (locus DXZ1, CEP X Spectrum Green; Vysis Inc.) and Chromosome Y (locus DYZ1, CEP Y Spectrum Orange; Vysis Inc.) were used for the triple-colour FISH analysis. Locus-specific DNA probes for chromosome 13 (locus RB, LSI 13 Spectrum Green; Vysis Inc.) and chromosome 21 (loci D21S259, D21S341, D21S342, LSI 21 Spectrum Orange; Vysis Inc.) were used for the dual-colour FISH analysis. FISH incubation and detection were performed according to the manufacturer's instructions.

Analysis was carried out using an Olympus AX70 epifluorescence microscope equipped with a triple-band pass filter for 4′6-diamidino-2-phenylindole (DAPI)/Texas Red/fluorescein isothiocyanate (FITC) and single-band pass filters for FITC, Texas Red and Aqua Blue. Sperm nuclei scoring was done strictly according to established criteria (Blanco *et al.*, 1996) and only sperm samples with hybridiza-

Table I. Sperm disomy and diploidy frequencies according to the indications for FISH

	RM (n = 40)	IF (n = 19)	DS (n = 3)	MA (n = 1)	Control (n = 9)
No. sperm studied X/Y/18	64 730	28 345	5970	894	51 399
Sex chrom. disomies (%)	293 (0.45)[a]	151 (0.53)[b]	23 (0.38)	10 (1.12)[c]	188 (0.37)
Disomy, 18 (%)	27 (0.04)	12 (0.04)	1 (0.02)	1 (0.11)	48 (0.10)
No. sperm studied 13/21	61 474	27 199	5963	730	28 044
Disomy, 13 (%)	67 (0.11)	45 (0.17)	7 (0.12)	2 (0.27)	0.10–0.20*
Disomy, 21 (%)	97 (0.16)	43 (0.16)	11 (0.18)	5 (0.68)	91 (0.37)
Diploidy (%)	157 (0.12)	138 (0.25)	13 (0.11)	1 (0.06)	203 (0.25)

[a]$P = 0.0124$, [b]$P = 0.0003$, [c]$P = 0.0004$, significantly higher than control group.
*Estimated from the literature.
n = number of sperm samples.
RM = Recurrent miscarriage of unknown aetiology; IF = Repeated implantation failure after ICSI; DS = Previous Down's syndrome pregnancies; MA = Meiotic abnormalities.

tion efficiency over 95% were evaluated. Because of the difficulty of discriminating between nullisomic spermatozoa and hybridization failures, nullisomies were not directly scored. In order to decrease the subjectivity of the observations the incidence of nullisomy for each chromosome was estimated conservatively to be similar to the incidence of disomy for the same chromosome**.** A total of 99 939 spermatozoa were assessed for chromosomes X, Y and 18, and 95 366 spermatozoa for chromosomes 13 and 21. When possible, 2000 sperm cells per patient were scored for each hybridization. In sperm samples from severely oligozoospermic patients, however, only a small number of cells could be evaluated. This may reduce the power of statistical analysis and therefore, final conclusions have to be drawn with caution. Hybridization efficiency was >95% in all the samples evaluated.

Statistical analysis

The incidence of disomy and diploidy for the analysed chromosomes in different groups of patients according to the indications for FISH studies and to sperm quality, was compared using χ^2 test (with Yates' correction when necessary) and Fisher's exact test (Graphpad Instat v. 2.05a; Graphpad Software, San Diego, CA, USA). Individual results in each patient were also compared with the results of the control group using the same tests. For comparisons of fertilization, embryo cleavage, pregnancy, implantation and miscarriage rates after ICSI, Mann–Whitney and Fisher's exact tests were used when indicated. A $P < 0.05$ was considered as statistically significant.

Results

An increase in the incidence of sex chromosome disomies was observed in three of the four indications for FISH studies: RM ($P = 0.0124$), IF ($P = 0.003$) and MA ($P = 0.0004$), but no significant differences were found in the group of patients with a previous history of Down's syndrome pregnancies when compared with the control group (Table I).

When results were analysed according to sperm parameters, in the groups of patients with isolated asthenozoospermia, isolated teratozoospermia and AT, disomy and diploidy rates were not significantly different from those observed in normozoospermic patients (Table II). However, OAT patients showed significantly higher rates of diploidy ($P < 0.0001$), sex chromosome disomies ($P < 0.0001$), disomy for chromosome 18 ($P = 0.0368$) and disomy for chromosome 21 ($P = 0.0423$) than normozoospermic patients.

Patients with sperm counts between 10–20 $\times 10^6$/ml and between 5 and 10 $\times 10^6$/ml only showed significant increases in the diploidy rate ($P = 0.0202$ and $P = 0.0135$ respectively) when compared with patients with normozoospermia (Table III). The highest incidence of sperm chromosomal abnormalities was found in the subgroup of patients with $<5 \times 10^6$/ml, in which there were significant increases in disomy rates for sex chromosomes ($P < 0.0001$), chromosome 18 ($P = 0.0179$) and chromosome 21 ($P = 0.0002$), as well as in the diploidy rate ($P < 0.0001$), when compared with normozoospermic patients (Table III).

When FISH results from each patient were compared individually with the control group, abnormal FISH results were observed in 14 patients (Tables IV and V). According to the indication for FISH studies (Table VI), abnormal FISH results were observed in seven out of 40 samples (17.5%) of RM patients, in six out of 19 (31.6%) couples with IF, and in none of the couples with previous Down's syndrome. The patient with MA had an increased sex chromosome disomy rate.

Tables IV, V and VI also show the distribution of abnormal FISH results according to sperm parameters: OAT patients showed the highest percentage of abnormal FISH results—nine out of 21 patients (47.6%). This was more evident in patients with sperm concentration $<5 \times 10^6$/ml, in which seven out of the nine samples analysed (77.8%) showed increased sperm chromosomal abnormalities. In the groups of patients with isolated asthenozoospermia and teratozoospermia only one out of 14 (7.1%) and one out of eight patients (12.5%) showed abnormal FISH results respectively. FISH analysis was normal in all the six patients with AT. In normozoospermic patients, three out of 14 samples (21.4%) had abnormal FISH results. Overall, in 42 patients with $> 20 \times 10^6$/ml , only five (11.9%) had FISH results significantly different from the control group. It is of interest that in all five of these patients the indication for FISH analysis was recurrent spontaneous miscarriage.

ICSI outcome of the cycles performed in these couples with normal or abnormal FISH results is detailed in Tables IV, V and VII. Fertilization rates were similar in both groups, however pregnancy rate per transfer (36.5 versus 26.3%) and implantation rate (14.9 versus 9.9%) were higher in the couples

Table II. Sperm disomy and diploidy frequencies according to sperm parameters

	Normo (n = 14)	Astheno (n = 14)	Terato (n = 8)	AT (n = 6)	OAT (n = 21)
No. sperm studied X/Y/18	26 381	25 730	10 731	6153	30 944
Sex chromosomes disomies (%)	117 (0.44)	92 (0.36)	45 (0.42)	18 (0.29)	210 (0.68) [a]
Disomy, 18 (%)	4 (0.02)	11 (0.04)	3 (0.03)	1 (0.02)	14 (0.05) [b]
No. sperm studied 13/21	26 499	24 414	9451	6266	28 736
Disomy, 13 (%)	38 (0.14)	22 (0.09)	7 (0.07)	7 (0.11)	50 (0.17)
Disomy, 21 (%)	45 (0.17)	30 (0.12)	9 (0.10)	5 (0.08)	69 (0.24) [c]
Diploidy (%)	54 (0.10)	53 (0.11)	30 (0.15)	7 (0.06)	165 (0.28) [d]

[a]$P < 0.0001$, [b]$P = 0.0368$, [c]$P = 0.0423$, [d]$P < 0.0001$, versus normozoospermic patients.
Normo = normozoospermia; Astheno = isolated asthenozoospermia; Terato = isolated teratozoospermia; AT = asthenoteratozoospermia; OAT = oligoasthenoteratozoospermia.

Table III. Sperm disomy and diploidy frequencies according to sperm concentration in oligoasthenoteratozoospermic patients

Sperm concentration	Normo (n = 14)	⩾10–20 $\times 10^6$/ml (n = 7)	5–10 $\times 10^6$/ml (n = 5)	<5 $\times 10^6$/ml (n = 9)
No. sperm studied X/Y/18	26 381	12 006	7596	11 342
Sex chromosome disomies (%)	117 (0.44) [a]	60 (0.50)	23 (0.30)	128 (1.13) [b]
Disomy 18 (%)	4 (0.02) [c]	3 (0.02)	4 (0.05)	7 (0.06) [d]
No. sperm studied 13/21	26 499	10 926	7051	10 759
Disomy 13 (%)	38 (0.14)	15 (0.14)	11 (0.16)	24 (0.22)
Disomy 21 (%)	45 (0.17) [e]	22 (0.20)	7 (0.10)	40 (0.37) [f]
Diploidy (%)	54 (0.10) [g]	37 (0.16) [h]	26 (0.18) [i]	99 (0.45) [j]

[a–b]$P < 0.0001$, [c–d]$P = 0.0179$, [e–f]$P = 0.0002$, [g–h]$P = 0.0202$, [g–i]$P = 0.0135$, [g–j]$P < 0.0001$.
Normo = normozoospermia.

with normal FISH results. There was also a higher incidence of miscarriages in the group of abnormal FISH results (80 versus 54.8%). However, none of these differences reached statistical significance.

In the group with normal FISH results, there have been eight live births from seven pregnancies; the remaining seven pregnancies are still ongoing. In all of them, prenatal karyotypes were normal. However, in the group with abnormal FISH results there was only one live birth. In this couple, the husband suffering from OAT, three previous ICSI attempts in another IVF-centre were unsuccessful in establishing a pregnancy. Sperm FISH analysis revealed only a moderate increase in the percentage of diploid sperm (0.44%). Despite this, the couple decided to undergo a further ICSI cycle, which resulted in a singleton pregnancy. At week 16 of gestation, a normal 46,XX karyotype was assessed by amniocentesis and a healthy girl (2 800 g) was born at week 35 of gestation by Caesarian section.

It is of interest to point out that although in most couples ICSI was performed with the patient's own oocytes, there were two patients with donated oocytes and abnormal FISH results (Tables IV and V). In this subgroup, four pregnancies were achieved after eleven embryo transfers, and all of them miscarried.

Discussion

Sperm FISH analysis to study baseline frequencies of aneuploidy has been carried out for a variety of conditions, including both fertile and infertile men with normal or abnormal blood karyotypes and patients whose female partners conceived a child or a fetus with chromosomal abnormalities (Egozcue *et al.*, 1997; Blanco *et al.*, 1998; Vegetti *et al.*, 2000). However, formal indications for sperm FISH analysis in clinical practice are still not clearly established.

We considered RM of unknown aetiology as a risk group for sperm chromosome abnormalities because an increased incidence of meiotic abnormalities (Vendrell *et al.*, 1999; Egozcue *et al.*, 2000a,b) and sperm aneuploidy (Giorlandino *et al.*, 1998; Bernardini *et al.*, 2000) had been previously reported in these patients. In a preliminary report by our group, an increased incidence of sex chromosome disomies was found in RM patients, and an increase in the diploidy rate was also found in a subset of patients with RM after ovum donation (Rubio *et al.*, 1999). Results of the present study confirm an increased incidence in sex chromosome disomies in RM patients in comparison with controls.

We also found increased incidences of sex chromosome disomies in couples with IF after ICSI. The poor embryo quality associated with the repeated IF observed in these couples could be related to defective sperm quality (Pellestor, 1991). Impaired sperm parameters have been described to adversely affect the chromosome constitution of embryos, not only because an oocyte can be fertilized by a chromosomally abnormal spermatozoon, leading to IF (In't Veld *et al.*, 1997; Pang *et al.*, 1999) but also because paternal factors derived from the centrosome can contribute to numerical chromosome abnormalities in the embryo (Obasaju *et al.*, 1999).

Table IV. Individual sperm parameters, sperm aneuploidy rates and ICSI outcome for patients with recurrent miscarriages (RM)

Patient. Number	Volume (ml)	Count ($\times 10^6$/ml)	a+b (%)	Normal (%)	Scored XY18	Sex chr. Disomies	Disomy 18	Scored 13/21	Disomy 13	Disomy 21	Total Diploidy	No. of ICSI cycle	No. of Pregnancy	No.of Abortion.	No. of Livebirth
RM1	2.3	11	46	24	1941	1.55*	0.00	2000	0.20	0.70*	0.33	-	-	-	-
RM2	2.1	70	59	41	1964	0.81*	0.10	2009	0.15	0.20	0.08	-	-	-	-
RM3	4.1	2	28	0	1709	2.17*	0.06	1846	0.22	0.76*	0.39	3	0	-	-
RM4	4.9	43	17	31	2944	0.75*	0.27*	1556	0.06	0.19	0.09	-	-	-	-
RM5	2.4	52	46	46	1771	0.40	0.00	1994	0.15	0.10	0.03	-	-	-	-
RM6	2.0	41	38	44	2296	0.09	0.00	1997	0.00	0.05	0.14	-	-	-	-
RM7	2.3	28	34	35	1967	0.31	0.05	1888	0.16	0.11	0.08	7[a]	5	4	-
RM8	2.2	25	43	45	2002	0.15	0.10	2019	0.10	0.20	0.22	-	-	-	-
RM9	3.4	95	51	46	1983	0.10	0.00	2112	0.28	0.33	0.07	5+3[a]	2	1	1[a]
RM10	2.7	45	48	36	2519	0.40	0.00	2098	0.05	0.19	0.06	-	-	-	-
RM11	3.4	37	48	46	1957	0.56	0.00	2028	0.10	0.10	0.05	3[a]	2	2	0
RM12	3.1	25	54	42	1951	0.72*	0.05	2028	0.25	0.20	0.13	-	-	-	-
RM13	2.5	65	67	31	2066	0.39	0.00	2036	0.20	0.29	0.12	1[a]	1	1	0
RM14	3.5	44	32	33	2068	0.29	0.19	2022	0.00	0.15	0.20	4[a]	2	0	2
RM15	4.2	66	64	36	2147	0.33	0.00	2071	0.10	0.14	0.05	7[a]	2	1	1
RM16	1.5	70	49	42	1740	1.21*	0.00	1934	0.31	0.31	0.11	8[a]	2	2	0
RM17	2.7	36	36	34	2040	0.20	0.05	2033	0.05	0.05	0.12	-	-	-	-
RM18	2.6	12	28	27	2051	0.24	0.05	1007	0.00	0.00	0.10	-	-	-	-
RM19	3.7	15	7	10	1949	0.26	0.10	1921	0.00	0.05	0.18	5	0	-	-
RM20	3.2	20	68	38	1990	0.20	0.00	1978	0.00	0.00	0.05	-	-	-	-
RM21	2.9	56	60	30	1020	0.00	0.00	1001	0.00	0.00	0.05	2[a]	1	0	1
RM22	2.4	13	41	20	1014	0.30	0.00	1008	0.00	0.10	0.15	1	0	-	-
RM23	2.5	200	50	40	1036	0.29	0.00	1061	0.09	0.00	0.43	-	-	-	-
RM24	3.2	32	22	33	1558	0.45	0.06	2089	0.19	0.05	0.03	-	-	-	-
RM25	2.7	48	35	32	1029	0.29	0.00	1033	0.00	0.19	0.19	4	1	0	–
RM26	3.0	58	19	31	1074	0.09	0.00	1017	0.10	0.10	0.05	8[a]	2	2	0
RM27	2.1	10	65	5	1091	0.18	0.00	1028	0.10	0.00	0.00	1[a]	0	–	–
RM28	2.5	60	24	35	1016	0.20	0.00	1020	0.00	0.00	0.10	–	–	–	–
RM29	2.5	95	54	16	1909	0.52	0.00	1034	0.19	0.10	0.00	–	–	–	–
RM30	2.8	28	21	17	934	0.11	0.00	1004	0.20	0.10	0.05	1[a]	1	0	–
RM31	7.0	57	52	16	1029	0.39	0.10	1006	0.10	0.10	0.44	–	–	–	–
RM32	2.8	28	38	11	1040	0.10	0.00	1009	0.30	0.00	0.05	1[a]	0	–	–
RM33	3.2	24	48	11	1112	0.18	0.09	1044	0.00	0.19	0.05	4[a]	0	–	–
RM34	3.0	29	62	10	1478	0.74*	0.07	1022	0.10	0.00	0.28	1+3[a]	2	2	–
RM35	2.5	106	51	18	1006	0.50	0.00	1147	0.09	0.17	0.09	2	0	–	–
RM36	3.2	83	39	14	1070	0.19	0.00	1064	0.00	0.09	0.00	1[a]	0	–	–
RM37	5.3	62	69	11	1152	0.26	0.00	1103	0.09	0.18	0.00	–	–	–	–
RM38	3.4	68	34	14	1062	0.28	0.00	1112	0.09	0.00	0.09	8[a]	2	2	0
RM39	3.2	28	73	18	1004	0.30	0.00	1039	0.00	0.00	0.15	–	–	–	–
RM40	6.0	27	49	14	2041	0.34	0.00	2056	0.06	0.05	0.20	2[a]	1	1	0

*Significantly higher ($P < 0.05$) than controls.
[a]Cycles with donated oocytes.

Table V. Individual sperm parameters, sperm aneuploidy rates and ICSI outcome for patients with implantation failure (IF), Down syndrome pregnancies (DS) and meiotic abnormalities (MA)

Patient. Number	Volume (ml)	Count ($\times 10^6$/ml)	a+b (%)	Normal (%)	Scored XY18	Sex chr. Disomies	Disomy 18	Scored 13/21	Disomy 13	Disomy 21	Total Diploidy	No. of ICSI cycle	No. of Pregnancy	No.of Abortion.	No. of Livebirth
IF1	1.5	16	62	27	1949	0.51	0.00	2027	0.15	0.25	0.23	1	0	–	–
IF2	5.0	2.5	2	24	1955	0.72*	0.00	1949	0.15	0.21	0.03	–	–	–	–
IF3	3.6	54	46	44	2121	0.47	0.05	2009	0.10	0.15	0.05	–	–	–	–
IF4	3.7	54	63	38	2009	0.45	0.05	2103	0.05	0.10	0.17	2+1[a]	0	–	–
IF5	3.3	15	29	24	2011	0.25	0.00	1935	0.36	0.05	0.13	6[a]	1	1	0
IF6	2.8	0.1	0	0	2540	0.51	0.04	1964	0.15	0.20	1.02*	4	0	–	–
IF7	4.4	9	60	10	1772	0.51	0.00	2026	0.10	0.05	0.05	3	0	–	–
IF8	3.2	54	36	48	1873	0.53	0.00	1814	0.11	0.17	0.05	1	1	0	1
IF9	2.8	9	2	11	1031	0.10	0.10	898	0.33	0.22	0.16	2	1	0	2
IF10	2.4	5	11	13	2041	0.00	0.05	2011	0.15	0.15	0.44*	1	1	0	1
IF11	7.1	0.1	0	0	114	27.19*	2.63*	225	3.11	1.78*	6.78*	–	–	–	–
IF12	1.5	26	26	12	997	0.30	0.10	1069	0.00	0.00	0.15	5	2	1	–
IF13	5.2	9	4	9	1053	0.19	0.00	1015	0.20	0.10	0.00	4+1[a]	1	0	–
IF14	3.0	1.1	17	25	1058	0.28	0.09	1023	0.10	0.10	0.05	3	0	–	–
IF15	6.0	2	17	11	1290	0.85*	0.00	981	0.31	0.10	0.26	1	0	–	–
IF16	3.2	4	31	0	979	0.92*	0.00	1010	0.10	0.69*	0.25	2	0	–	–
IF17	3.1	33	35	13	1050	0.10	0.00	1008	0.10	0.10	0.00	1	0	–	–
IF18	4.0	6	55	1	1699	0.59	0.12	1101	0.09	0.00	0.11	1+1[a]	0	–	–
IF19	2.0	2	17	5	803	0.00	0.00	1031	0.00	0.00	0.11	4[a]	2	1	–
DS1	0.5	20	32	36	1971	0.56	0.05	1923	0.00	0.21	0.13	–	–	–	–
DS2	4.0	48	66	41	1973	0.35	0.00	2017	0.15	0.20	0.10	–	–	–	–
DS3	2.7	65	68	35	2026	0.25	0.00	2023	0.20	0.15	0.10	2	1	0	–
MA	4.1	0.2	14	21	894	1.12*	0.11	730	0.27	0.68	0.06	–	–	–	–

*Significantly higher ($P < 0.05$) than controls.
[a]Cycles with donated oocytes.

Table VI. Individual sperm FISH results in each patient. Distribution according to the indication for FISH analysis and to the sperm parameters

	No. of patients studied	No. of patients with abnormal FISH results (%)*
Indication for FISH		
Recurrent miscarriage	40	7 (17.5)
Implantation failure	19	6 (31.6)
Down's pregnancy	3	0
Meiotic abnormalities	1	1 (100)
Sperm parameters		
Normozoospermia	14	3 (21.4)
Asthenozoospermia	14	1 (7.1)
Teratozoospermia	8	1 (12.5)
AT	6	0
OAT	21	9 (47.6)

*When compared with the control group (see text).
AT = asthenoteratozoospermia; OAT = oligoasthenoteratozoospermia.

Table VII. ICSI outcome in patients with normal and abnormal sperm FISH results

	Normal FISH results	Abnormal FISH results
No. of cycles	108	23
No. of patients	31	7
No. of MII injected oocytes	1137	256
No. of 2-pronuclear oocytes (%)	768 (71.5)	176 (74.5)
Mean no. embryos transferred ± SD	2.6 ± 1.5	2.8 ± 1.8
No. of embryo transfers	85[a]	19[b]
No. of pregnancies (%)[c]	31 (36.5)	5 (26.3)
Implantation rate (%)	14.9	9.9
No. of miscarriages (%)[d]	17 (54.8)	4 (80.0)
No. of ongoing pregnancies	7	0
No. of live births	8[e]	1

[a]60 embryo transfers on day 3 and 25 on day 6.
[b]12 embryo transfers on day 3 and seven on day 6.
[c]Clinical pregnancy rate per transfer.
[d]Miscarriage rate (% of clinical pregnancies).
[e]Six singleton and one twin pregnancies.

Our third indication for sperm FISH analysis was previous pregnancies affected by Down's syndrome. None of the three patients included in this group presented an increase in the incidence of chromosomal abnormalities. These results agree in part with the report by Blanco *et al.* (1998), in which the overall incidence of chromosome 21 disomy in the fathers of affected children was not significantly different from the control population (Blanco *et al.*, 1998). In the same study, however, analysis of individual data demonstrates significant increases of disomy 21 in spermatozoa of the two fathers in whom the paternal origin of the extra chromosome 21 was established. In our series, this correlation could not be checked since the origin of the extra chromosome 21 was not tested.

Analysis of spermatozoa in the patient with abnormal meiosis showed an extremely significant increase in sex chromosome disomies. These results confirm that some abnormal cell lines can progress through meiosis producing chromosomally abnormal spermatozoa despite severe meiotic arrest or synaptic anomalies (Vendrell *et al.*, 1999; Bernardini, 2000).

Focusing on sperm quality, OAT was associated with significant increases in sex chromosome disomies, disomy for chromosomes 18 and 21, and in the percentage of diploid sperm, particularly in those samples with markedly reduced sperm concentration ($<5 \times 10^6$/ml spermatozoa). Our results are in accordance with previous studies that have shown increased chromosome abnormalities in OAT patients and a more frequent involvement of sex chromosomes (Arán *et al.*, 1999; Pang *et al.*, 1999; Pffefer *et al.*, 1999; Bernardini, 2000; Nishikawa *et al.*, 2000; Ushijima *et al.*, 2000; Vegetti *et al.*, 2000). In our series the mean incidence of sex chromosome disomies in the OAT patients was 0.68%, and in the subset of OAT patients with sperm counts $<5\times10^6$/ml, it was raised to 1.13%. In general, most investigators agree with the concept of a linear relationship between the severity of meiotic process disturbance and the alteration of seminal parameters (Arán *et al.*, 1999; Pfeffer *et al.*, 1999; Vegetti *et al.*, 2000). Nishikawa *et al.* (2000) also found an increase of sex chromosome disomies in OAT patients, more remarkable in sperm samples with $<10 \times 10^6$/ml (Nishikawa *et al.*, 2000). In our study, among the autosomes, chromosome 21 showed a higher incidence of disomy (0.24%), in agreement with other studies (Arán *et al.*, 1999; Pfeffer *et al.*, 1999, Ushijima *et al.*, 2000; Vegetti *et al.*, 2000). These results reflect that chromosome 21 and sex chromosomes are more prone to meiotic errors due to non-disjunction (Spriggs *et al.*, 1995; Blanco *et al.*, 1996). Diploidy is another sperm chromosomal abnormality usually increased in OAT and infertile patients. The mean diploidy rate in our general OAT series was 0.28%, reaching 0.45% in the subgroup of patients with $<5\times10^6$/ml, both being significantly higher than that observed in the group of normozoospermic patients (0.10%).

The correlation of sperm motility and morphology with chromosomal abnormalities is more controversial. In our series, patients with isolated impaired sperm motility or morphology did not show significant increases in disomy or diploidy rates in comparison with normozoospermic patients. While some authors believe that sperm aneuploidy correlates more with asthenozoospermic rates (Vegetti *et al.*, 2000), others found a stronger association with abnormal sperm morphology (Yurov *et al.*, 1996; Estop *et al.*, 1997; In't Veld *et al.*, 1997). Several factors may be involved in these contradictory results in the literature. Firstly, different morphology assessment criteria have been used in these studies (WHO versus Kruger's). Secondly, there are difficulties in analysing astheno and/or teratozoospermia as isolated factors, since there are few patients with single sperm defects. In fact, in many studies, astheno and/or teratozoospermic patients were also oligozoospermic. Thirdly, perhaps sperm aneuploidy is more related to specific types of abnormal morphology (Estop *et al.*, 1997) such as macrocephalia or the presence of multiple tails (In't Veld *et al.*, 1997). This is the reason why some investigators, rather than investigating specific correlations with semen parameters, look at the overall quality of the seminal sample as expressed by the total normal motility count (TNMC) and apply in-situ hybridization (ISH) rather than FISH, in order to control the normality of sperm morphology during the analysis of signal-products (Bernardini *et al.*, 1998).

On the other hand, it is important to know the influence of

sperm chromosome abnormalities detected by FISH on ICSI outcome. Pfeiffer *et al.* (1999) assessed significantly higher diploidy and total aneuploidy rates in ten OAT patients undergoing 11 ICSI cycles (Pfeiffer *et al.*, 1999). Overall fertilization rate was 70%, but only two successful pregnancies were achieved. Pang *et al.* (1999) reported a total aneuploidy rate of 33–74% in a series of nine severe OAT patients (Pang *et al.*, 1999). ICSI was performed in five of them, and no ongoing pregnancies were established, similar data have been reported by another group (Bernardini *et al.*, 2000).

In our study, ICSI outcome was assessed retrospectively in 131 cycles carried out on patients in whom sperm chromosomal constitution was later evaluated by FISH. The poor general results in this series are related to the patient selection criteria chosen in our study. To date, this is the largest series of cycles in which ICSI results are compared in patients with normal and abnormal FISH results. Fertilization rates were similar in both groups, but apparently higher pregnancy rates (36.5 versus 26.3%) and lower miscarriage rates (54.8 versus 80%) were observed in the group of patients with normal FISH results when compared with couples with abnormal FISH results, although these differences were not significantly different, probably because the number of pregnancies in the group of patients with abnormal FISH results was low. A power calculation showed that a total of 1128 ICSI cycles (205 with abnormal and 923 with normal FISH results) should be analysed to achieve significant differences in pregnancy rates. In addition, the high miscarriage rate in the group of patients with normal FISH results is related with the selection criteria used in the study, because one of such criteria was just unexplained recurrent abortion. Nonetheless, the trend observed in our series suggests that sperm aneuploidy may be associated with IF and fetal losses. Although one patient with a moderate increase in the percentage of diploid sperm was able to father a healthy child, four ICSI cycles were required to achieve a successful pregnancy. Further studies are needed to confirm this tendency.

Moreover, some patients in our series were included in our ovum donation programme. Analysis of these cases is of interest, because the sperm contribution to ICSI outcome can be analysed as an isolated factor. In this subgroup, pregnancy rates were similar in patients with normal and abnormal FISH results. Four pregnancies were achieved in patients with abnormal sperm FISH results. All four were miscarried, further suggesting a correlation between increased incidence of sperm chromosomal abnormalities and abortion.

In conclusion, our study confirms that a normal karyotype does not exclude the presence of chromosomal abnormalities in spermatozoa of patients included in an ICSI programme. Further studies are required to understand more about the risk of higher rates of sperm aneuploidy in male partners of couples with recurrent pregnancy losses and normal seminal parameters. In fact, the low semen quality represents an independent risk factor and it is often associated. It is unlikely that the small increase found in the rates of sex chromosome aneuploidy in spermatozoa of RM and IF patients might be reflected in significant changes of clinical results (pregnancy, implantation, abortion rates) but future studies on additional chromosomes in spermatozoa and embryos from these patients may help to clarify this better. Our study provides a demonstration that OAT is associated with higher sperm aneuploidy and diploidy rates, particularly in the presence of oligozoospermia. Such conditions might, in part, explain the low implantation and high abortion rates observed during ICSI cycles.

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