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Could Sperm Aneuploidy Rate Determination Be Used as a Predictive Test Before Intracytoplasmic Sperm Injection?

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ABSTRACT: Chromosome abnormalities in embryos are a major cause of implantation and development failures. Some couples with normal karyotypes have repeated implantation failures after intracytoplasmic sperm injection (ICSI). In order to value patients at risk for genetic ICSI failures and the validity of sperm aneuploidy analysis, we have studied cytogenetic abnormalities in sperm from ICSI patients. Twenty-nine patients with normal karyotypes were included. Ten patients had at least 4 ICSI treatments without pregnancy (group A). Nine patients had a pregnancy after 1 to 3 ICSI treatments (group B). Ten fertile men with normal semen parameters were studied as controls (group C). Fluorescent in situ hybridization (FISH) was used for sperm nucleus cytogenetic analysis using chromosomes 8, 9, 13, 18, 21, X, and Y specific probes. Aneuploidy for each chromosome and diploidy rates were significantly higher in group A than in group B and in group B than in group C ($P < .05$). Considering each patient in groups A and B, aneuploidy rate for each chromosome was too variable to be considered as a significant test. We proposed analysis of the total sperm aneuploidy. Chromosomal sperm nuclei profile could be used as a predictive biological test before ICSI in order to improve genetic counseling for oligoasthenoteratozoospermia patients.

Key words: Chromosomal profile, fluorescent in situ hybridization, spermatozoa.

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During spermatogenesis, spermatogonia go through 2 meiosis divisions. Chromosomes are separated during meiosis I: each daughter cell receives 1 chromosome of each pair. During meiosis II, each chromosome is separated in 2 chromatides. At the end of spermatogenesis, the spermatozoon nucleus normally contains 23 chromosomes with 1 chromatide. In normal human spermatogenesis, 5% to 10% of the spermatozoa are aneuploid (Shi and Martin, 2000). In situations with poor semen quality, it is now well established that aneuploidy rate is increased and can affect success of assisted reproductive techniques (Storeng et al, 1998; Pang et al, 1999; Van Dyk et al, 2000).

Since 1992, intracytoplasmic sperm injection (ICSI) has been used successfully to treat male infertility (Palermo et al, 1992). However, during ICSI procedure, spermatozoon nuclear quality is never known. Fluorescent in situ hybridization (FISH) on oligoasthenoteratozoospermia (OAT) patient spermatozoa suggests that male infertility is a risk factor for chromosomal abnormalities in sperm nuclei (In't Veld et al, 1995; Bernardini et al, 1997; Aran et al, 1999; Pang et al, 1999; Acar et al, 2000; Schultz et al, 2000; Ushijima et al, 2000; Härkönen et al, 2001). In recent studies, authors have established a correlation between semen parameters (sperm concentration, motility, morphology) and sperm aneuploidy rate (Vegetti et al, 2000; Calogero et al, 2001). So ICSI is considered as a situation at genetic risk for the offspring, and recent observations suggest that high sperm aneuploidy may have a negative impact on the success of the ICSI procedure (Pang et al, 1999; Rubio et al, 2001).

In order to determine the validity of sperm aneuploidy determination as a predictive test for ICSI success, we analyzed chromosomal abnormalities in sperm from 2 subpopulations of ICSI patients: men who did not achieve an ICSI success after at least 4 attempts and men who achieved an ICSI success after 1 to 3 attempts.

Materials and Methods

Patients

Nineteen ICSI patients were included in this study. ICSI was proposed because of sperm deficiency. Patients with an obstruction on the vas deferens were excluded. They were divided into 2 groups: 10 patients (group A: patients P1 to P10) who did not attain a pregnancy (βhCG negative) after at least 4 ICSI treatments (4 to 7, mean 5.2) with embryo transfer (6 to 19 embryos transferred, mean 12.5) and 9 patients (group B: patients T1 to

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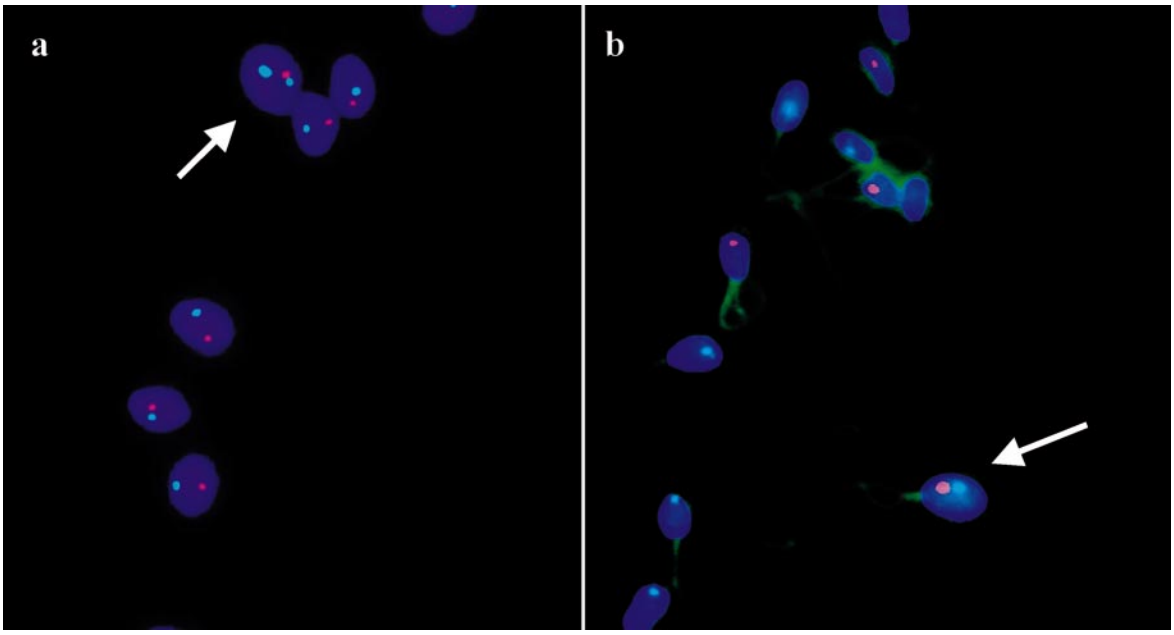

(a) FISH with chromosome 8 centromere and chromosome 9 centromere specific probes. Arrow shows a spermatozoon with 2 chromosomes 9 and 1 chromosome 8. **(b)** FISH with chromosome X centromere and chromosome Y centromere specific probes. Arrow shows a spermatozoon with 1 chromosome X and 1 chromosome Y.

T9) who obtained a pregnancy (βhCG positive) after 1 to 3 ICSI treatments (mean 1.9) with embryo transfer (2 to 11 embryos transferred 4.8). Ten fertile men with normal semen parameters (WHO, 1999) were studied as controls (group C: patients C1 to C10).

All the patients had a normal 46,XY karyotype. All the women had a normal 46,XX karyotype.

Sperm Preparation and Sperm Head Swelling

After liquefaction at 37°C for 30 minutes, each semen sample was prepared using the PureSperm (JCD, Lyon, France) migration technique if progressive sperm mobility was upper 5%, or a simple wash in Ferticult (JCD) if progressive sperm mobility ranged from 0% to 5%. Each pellet was then washed in Ferticult (JCD). After centrifugation at 500 × *g*, the pellets were resuspended in Carnoy's solution (methanol/acetic acid 3:1). Sperm preparations were dropped onto slides and air dried.

The sperm head decondensation was performed using NaOH solution (1 mol/L) for 2 minutes at room temperature (Frydman et al, 2001).

Chromosome Probes

To detect sperm aneuploidy for chromosomes 13, 18, 21, X, and Y, we used a commercial kit Aneuvysion™ (Vysis, Downers Grove, Ill) with DNA probes specific for chromosome 13 (LSI 13 SpectrumGreen™ [Vysis, Downers Grove, Ill] 13q14) and for chromosome 21 (LSI 21 SpectrumOrange™ [Vysis, Downers Grove, Ill] 21q22.13-q22.2) and DNA probes specific for chromosome 18 (CEP 18 SpectrumAqua™ [Vysis, Downers Grove, Ill] 18p11.1-q11.1), for chromosome X (CEP X SpectrumGreen™ Xp11.1-q11.1) and for chromosome Y (CEP Y SpectrumOrange™ Yp11.1-q11.1). We used DNA probes (Vysis, Downers Grove, Ill) specific for chromosome 8 (CEP 8 SpectrumOrange™ 8p11.1-q11.1) and for chromosome 9 (CEP 9 SpectrumGreen™ 9p11.1-q11.1) in 1:2 mixture allowing to obtain good hybridization signals (Figure). Hybridization frequencies were tested on 100 normal male lymphocyte metaphases per probe and were 100%.

Fluorescent In Situ Hybridization

Before hybridization, sperm DNA slides were dehydrated in ethanol (70%, 90%, and 100%) and air dried. Three microliters of DNA probes mixture were applied to the sperm nucleus preparation and then covered with a coverslip and sealed with rubber cement. The denaturation was performed simultaneously for sperm nuclei and probes for 1 minute at 73°C for centromeric probes and for 2 minutes at 73°C for locus specific probes. Slides were then hybridized in a dark, moist chamber at 37°C for 15 to 18 hours. The coverslips were then removed and slides were washed for 2 minutes in 0.4 × SSC 0.3% NP40 solution at 73°C and for 30 seconds in 2.0 × SSC 0.1% NP40 solution at room temperature. Nuclei were then counterstained with 4,6-diamino-2-phenylindole dihydrochloride (DAPI) in an antifade solution (Vectashield, Vector Laboratories, Burlingame, Calif).

Dual color FISH slides were screened using an X-100 objective on an Olympus epifluorescent microscope equipped with fluoresceine isothiocyanate (FITC)/rhodamine double band-pass filter. Aqua FISH slides were screened using an X-64 objective on a Zeiss epifluorescent microscope equipped with aqua, fluoresceine isothiocyanate (FITC) and rhodamine single band-pass filters. A total of 1000 sperm nuclei were counted for each probe. Only individual and well-delineated spermatozoa were scored. We used the scoring criteria defined by Martin and Rademaker (1995). A spermatozoon was scored as disomic if it showed 2

Table 1. *Intracytoplasmic sperm injection (ICSI) attempts for patients in group A*

	Group A: 4 or More ICSI Attempts											
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Means	SD*
Number of cycle	4	4	6	5	4	7	6	6	6	4	5.2	1.1
Male partner age, y	34	33	36	40	39	38	37	30	31	34	35.2	3.4
Female partner age, y	32	35	34	30	36	37	37	25	31	33	33.0	3.7
Number of oocytes	29	31	78	28	21	45	66	46	54	30	42.8	18.6
Estradiol per oocyte, pg/mL	210	173	183	209	304	329	186	182	247	211	223	54
Oocyte/ICSI	7.3	7.8	13.0	5.6	5.3	6.4	11.0	7.7	9.0	7.5	8.0	2.4
Number of embryos	7	8	41	9	12	25	31	10	29	21	19.3	11.8
Fertilization rate, %	24.1	25.8	52.6	32.1	57.1	55.6	47.0	21.7	53.7	70.0	44.0	17.0
Grade A embryos	2	0	1	2	0	4	1	0	1	1	1.2	1.2
% grade A	28.6	0.0	2.4	22.2	0.0	16.0	3.2	0.0	3.4	4.8	8.1	10.4
Grade B embryos	4	1	13	4	0	6	8	2	13	6	5.7	4.5
% grade B	57.1	12.5	31.7	44.4	0.0	24.0	25.8	20.0	44.8	28.6	28.9	16.7
Grade C embryos	0	5	21	3	9	9	15	8	12	6	8.8	6.1
% grade C	0.0	62.5	51.2	33.3	75.0	36.0	48.4	80.0	41.4	28.6	45.6	23.6
Grade D embryos	1	2	66	0	3	3	7	0	3	8	3.3	2.8
% grade D	14.3	25.0	14.6	0.0	25.0	12.0	22.6	0.0	10.3	38.1	16.2	11.8
Number of embryos transferred	3	6	18	8	10	13	19	9	17	13	11.6	5.3
Biochemical pregnancy	0	0	0	0	0	0	0	0	0	0	. . .	. . .
Clinical pregnancy	0	0	0	0	0	0	0	0	0	0	. . .	. . .
Deliveries	0	0	0	0	0	0	0	0	0	0	. . .	. . .

* SD indicates standard deviation.

hybridization signals of the same color, size, and intensity. Two spots separated by less than the diameter of 1 hybridization domain were scored as a single signal. The absence of hybridization signal for a single chromosome was scored as nullisomy for this chromosome only when the other probed chromosome gave a signal. The aneuploidy rate was determined by the sum of nullisomy and disomy rates.

Statistical Analysis

Aneuploidy and diploidy rates were compared among groups A, B, and C. Unpaired *t* test or Kruskal-Wallis test were used as appropriate. Differences were considered to be statistically significant when the probability value was less than .05.

Results

Women's characteristics and ICSI attempts are indicated in Tables 1 and 2. In order to exclude ovarian disorders responsible for sterility, women's age and hormonal determinations (day 3 FSH, day 3 LH, day 3 estradiol) were compared between groups A and B. No difference was observed considering age and hormonal determination were normal in the 2 groups (data not shown). Considering the results of ICSI attempts, the fertilization rate was significantly higher in group B than in group A (respectively, 67% vs 44%, $P = .0251$). The embryo quality (number and regularity of blastomeres, presence or absence of fragments) was not different between group A and group B. In group A, no pregnancy was achieved. In group B, 1 biochemical pregnancy (βhCG positive but no cardiac activity), 1 first trimester spontaneous abortion, and 7 births resulted.

Men's characteristics and determination of aneuploidy rates are indicated in Tables 3 through 6. There was no statistical difference for semen parameters and for age between men in groups A and B. In group A, the aneuploidy rate varied from 0.83% for chromosome 9 to 2.22% for chromosome 13. The diploidy rate was 0.52%. In group B, the aneuploidy rate varied from 0.50% for chromosome 8 to 0.81% for chromosomes 13 and 21. The diploidy rate was 0.29%. In group C, the aneuploidy rate varied from 0.13% for chromosomes X/Y to 0.22% for chromosome 8. The diploidy rate was 0.12%. Aneuploidy (disomy plus nullisomy) rates for studied chromosomes and diploidy rate were significantly increased in group A and B compared with the control population ($P < .05$). The sum of aneuploidy rates for chromosomes 8, 9, 13, 18, 21, X, and Y (total aneuploidy rate) were compared in the 3 groups. It was significantly higher in ICSI patients (groups A and B) than in controls (group C). Total aneuploidy rate was significantly increased in patients with ICSI failures (group A) compared with patients who fathered after fewer than 4 ICSI treatments (group B).

Discussion

In our study, we first compared sperm aneuploidy rates for chromosomes 8, 9, 13, 21, and X/Y in 3 populations: men without ICSI success after at least 4 attempts (group

Table 2. *Intracytoplasmic sperm injection (ICSI) attempts for patients in group B*

	Group B: 1 to 3 ICSI Attempts										
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Means	SD*
Number of cycle	1	2	1	2	3	2	1	2	1	1.7	0.7
Male partner age, y	30	35	29	44	35	51	27	30	35	35.1	7.8
Female partner age, y	23	32	27	34	37	31	26	29	29	29.8	4.3
Number of oocytes	13	16	8	11	15	34	5	20	8	14.4	8.7
Estradiol per oocyte, pg/mL	124	142	135	235	244	173	254	149	422	209	94
Oocyte/ICSI	13.0	8.0	8.0	5.5	5.0	17.0	5.0	10.0	8.0	8.8	4.0
Number of embryos	11	12	8	7	5	22	4	11	4	9.3	5.7
Fertilization rate, %	84.6	75.0	100.0	63.6	33.3	64.7	80.0	55.0	50.0	67.4	20.1
Grade A embryos	0	0	3	0	0	2	0	3	1	1.0	1.3
% grade A	0.0	0.0	37.5	0.0	0.0	9.1	0.0	27.3	25.0	11.0	14.9
Grade B embryos	5	1	5	0	2	9	1	3	3	3.2	2.8
% grade B	45.5	8.3	62.5	0.0	40.0	40.9	25.0	27.3	75.0	36.1	24.0
Grade C embryos	3	7	0	0	3	9	2	5	0	3.2	3.2
% grade C	27.3	58.3	0.0	0.0	60.0	40.9	50.0	45.5	0.0	31.3	25.4
Grade D embryos	3	4	0	7	0	2	1	0	0	1.9	2.4
% grade D	27.3	33.4	0.0	100.0	0.0	9.1	25.0	0.0	0.0	21.6	32.3
Number of embryos transferred	2	6	2	5	8	5	3	4	2	4.1	2.1
Biochemical pregnancy	0	1	0	0	0	0	0	0	0	. . .	. . .
Clinical pregnancy	1	0	1	1	1	1	1	1	1	. . .	. . .
Deliveries	1	0	1	1	1	1	0	1	1	. . .	. . .

* SD indicates standard deviation.

A), men with ICSI success after 1 to 3 attempts (group B), and fertile men with normal semen parameters (group C). All patients have a normal karyotype.

Normal karyotype determination on blood lymphocyte cells does not exclude cytogenetic abnormalities in sperm (Calogero et al, 2003). In our series, sperm aneuploidy for each chromosome was higher in OAT patients than in normal semen men. Over the past decade, several studies have been conducted about cytogenetic analysis using fluorescent in situ hybridization (FISH) on spermatozoa from infertile men. Martin (1996) suggested that ICSI with poor quality sperm is a situation at risk of transmitting chromosomal abnormalities to offspring. For Rubio et al (2001), men with implantation failure (IF) after ICSI are at risk of showing sperm chromosomal abnormalities. In their study, IF patients had variable semen parameters (normozoospermia, asthenozoospermia, teratozoospermia), and there was no information about women investigations. Aneuploidy rates from IF patients were not compared with aneuploidy rates from patients who obtained a pregnancy.

In our study, male and female characteristics were an-

Table 3. *Sperm characteristics and fluorescent in situ hybridization analysis for patients in group A*

	Group A: 4 or More ICSI Attempts*									
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Age	34	33	36	40	39	38	37	30	31	34
Volume, mL	4.2	2.5	5.5	7.0	5.0	4.0	3.0	3.0	3.5	4.0
Density, 10^6/mL	60.0	1.0	5.0	1.8	15.0	24.0	18.0	2.0	0.5	44.0
Total motility, %	10	1	35	10	20	20	30	1	25	30
Progressive motility, %	5	0	25	0	0	10	0	0	10	20
Normal forms, %	0	0	47	20	5	12	27	0	5	41
Aneuploidy for chromosome, %										
8	0.1	1.5	1.1	2.9	3.0	0.4	1.2	ND*	0.4	0.2
9	0.1	0.4	0.8	1.1	2.4	0.3	0.4	ND	1.4	0.6
13	3.0	2.4	1.4	1.8	3.6	2.8	1.2	2.0	2.8	1.2
18	0.1	2.9	0.3	1.7	3.7	0.1	0.5	1.2	1.2	0.6
21	7.2	1.3	2.0	3.2	2.8	0.5	0.8	1.9	1.8	0.6
X/Y	0.0	1.1	1.8	2.3	2.7	1.0	0.5	1.5	1.8	0.2
Diploidy	0.4	1.5	0.6	0.3	1.2	0.4	0.2	0.4	0.1	0.1
Total aneuploidy	10.5	9.6	7.4	13.0	18.2	5.1	4.8	ND	9.4	3.4

* ICSI indicates intracytoplasmic sperm injection; ND, not determined.

Table 4. *Sperm characteristics and fluorescent in situ hybridization analysis for patients in group B*

	Group B: 1 to 3 ICSI Attempts*								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
Age	30	35	29	44	35	51	27	30	35
Volume, mL	3.5	3.6	2.0	8.0	2.0	5.0	9.0	3.5	3.0
Density, 10^6/mL	16.0	4.8	8.0	80.0	14.0	14.0	1.1	0.6	38.0
Total motility, %	30	5	25	20	35	5	10	20	15
Progressive motility, %	20	1	15	10	25	1	0	10	0
Normal forms, %	23	3	7	24	12	22	24	5	32
Aneuploidy for chromosome, %									
8	0.6	0.4	0.5	0.3	0.3	0.7	1.0	0.4	0.3
9	0.1	0.7	0.7	0.3	0.4	1.0	1.3	0.3	0.4
13	1.2	0.3	ND*	0.8	0.2	2.0	0.9	0.9	0.2
18	0.9	1.2	0.2	0.3	0.7	0.2	1.8	0.7	0.5
21	0.7	0.9	ND	1.6	0.2	0.8	0.6	1.5	0.2
X/Y	0.2	1.0	0.3	0.7	1.4	0.3	1.2	0.6	0.3
Diploidy	0.2	0.1	0.1	0.1	0.1	0.4	0.8	0.3	0.5
Total aneuploidy	3.7	4.5	ND	4.0	3.2	5.0	6.8	4.4	2.4

* ICSI indicates intracytoplasmic sperm injection; ND, not determined.

alyzed. There was no difference between groups A and B for male and female characteristics (Table 6). Considering first steps of in vitro embryo development, we did not observe any relevant difference for number or morphology of blastomeres between group A and group B (Tables 1 and 2). Neither the sperm parameters or embryo morphology can predict ICSI success for OAT patients. Only fertilization rate was decreased in group A in comparison with group B. Aneuploidy rates observed in groups A and B were compared with the presence or absence of biochemical pregnancy in order to determine the impact of sperm aneuploidy on ICSI success.

Considering groups, aneuploidy rates were statistically higher in group A than in group B. No correlation was established between sperm aneuploidy and numeration, motility or morphology. Considering each patient individually, results of aneuploidy determination were variable between each studied chromosome (Table 6). We conclude that analysis of 1 chromosome in sperm is not sufficient to predict ICSI success because it cannot reflect genetic risk for the whole chromosome. We propose the analysis of 7 chromosomes together in order to have a global vision of the aneuploidy risk.

We compared the total sperm aneuploidy (sum of the aneuploidy rates measured for chromosomes 8, 9, 13, 18, 21, X, and Y) in groups A and B. A rate above 5% seems to be negative for ICSI (7 of 9 in group A, 1 of 8 in group B). Our results agreed with results of Burrello et al (2003), who have shown that an aneuploidy rate for chromosomes 8, 12, 18, X, and Y above 1.55% has a

Table 5. *Sperm characteristics and fluorescent in situ hybridization analysis for patients in group C*

	Group C: Fertile Donors									
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Age	39	40	39	33	30	35	33	35	32	38
Volume, mL	2.5	1.0	4.0	3.0	6.0	4.3	3.6	3.0	3.0	3.0
Density, 10^6/mL	142.0	116.0	35.0	150.0	34.0	151.0	117.0	158.0	175.0	61.0
Total motility, %	50	60	30	50	25	40	50	50	50	45
Progressive motility, %	40	40	20	40	20	30	40	40	40	35
Normal forms, %	44	54	44	64	71	46	67	67	40	33
Aneuploidy for chromosome, %										
8	0.2	0.2	0.2	0.0	0.5	0.3	0.2	0.3	0.0	0.3
9	0.4	0.0	0.1	0.1	0.4	0.1	0.4	0.3	0.1	0.1
13	0.1	0.3	0.0	0.3	0.2	0.1	0.2	0.1	0.1	0.2
18	0.2	0.4	0.2	0.1	0.2	0.3	0.1	0.0	0.0	0.0
21	0.0	0.2	0.3	0.3	0.1	0.2	0.1	0.1	0.2	0.1
X/Y	0.0	0.0	0.1	0.0	0.4	0.2	0.3	0.2	0.0	0.1
Diploidy	0.0	0.1	0.0	0.1	0.4	0.2	0.1	0.0	0.2	0.1
Total aneuploidy	0.9	1.1	0.9	0.8	1.5	1.2	1.3	1.0	0.4	0.8

Table 6. *Comparison of groups A, B, and C for intracytoplasmic sperm injection (ICSI) attempts, sperm parameters, and chromosome aneuploidies*

	Mean			SD*			Median			Min			Max			*P*	
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A/B/C	A/B
Female partner age, y	33.0	29.8	. . .	3.7	4.3	. . .	33.5	29.0	. . .	25.0	23.0	. . .	37.0	37.0	. . .	. . .	NS*
Number of oocytes	42.8	14.4	. . .	18.6	8.7	. . .	38.0	13.0	. . .	21.0	5.0	. . .	78.0	34.0	. . .	. . .	.0006
Estradiol per oocyte, pg/mL	223	209	. . .	53.8	94	. . .	210	173	. . .	173	124	. . .	329	422	. . .	. . .	NS
Oocyte/ICSI	8.1	8.8	. . .	2.4	4.0	. . .	7.6	8.0	. . .	5.3	5.0	. . .	13.0	17.0	. . .	. . .	NS
Number of embryos	19.3	9.3	. . .	11.8	5.7	. . .	16.5	8.0	. . .	7.0	4.0	. . .	41.0	22.0	. . .	. . .	.0187
Fertilization rate, %	44.0	67.4	. . .	16.8	20.1	. . .	49.8	65.0	. . .	21.7	33.0	. . .	70.0	100.0	. . .	. . .	.0253
Male partner age, y	35.2	35.1	35.4	3.4	7.8	3.4	35.0	35.0	35.0	30.0	27.0	30.0	40.0	51.0	40.0	. . .	NS
Volume, mL	4.2	4.5	3.3	1.4	2.7	1.5	4.0	3.6	3.0	2.5	2.0	1.0	7.0	9.0	6.0	. . .	NS
Density, 10^6/mL	21.1	28.3	113.9	21.1	27.3	52.2	16.5	15.0	129.5	1.0	8.0	34.0	60.0	80.0	175.0	. . .	NS
Total motility, %	18.2	18.3	45.0	12.2	10.6	10.5	20.0	20.0	50.0	1.0	5.0	25.0	35.0	35.0	60.0	. . .	NS
Progressive motility, %	7.0	9.1	34.5	9.2	9.4	8.3	2.5	10.0	40.0	0.0	0.0	20.0	25.0	25.0	40.0	. . .	NS
Normal forms, %	15.7	16.9	53.0	17.5	10.3	13.4	8.5	22.0	50.0	0.0	3.0	33.0	47.0	32.0	71.0	. . .	NS
Aneuploidy for chromosome																	
8	1.20	0.50	0.22	1.10	0.23	0.15	1.10	0.40	0.20	0.10	0.30	0.00	3.00	1.00	0.50	.0017	. . .
9	0.83	0.58	0.20	0.72	0.38	0.16	0.60	0.40	0.10	0.10	0.10	0.00	2.40	1.30	0.40	.0040	. . .
13	2.22	0.81	0.16	0.83	0.61	0.10	2.20	0.85	0.15	1.20	0.20	0.00	3.60	2.00	0.30	$<$.0001	. . .
18	1.23	0.72	0.15	1.22	0.52	0.14	0.90	0.70	0.15	0.10	0.20	0.00	3.70	1.80	0.40	.0014	. . .
21	2.21	0.81	0.16	1.97	0.52	0.10	1.85	0.75	0.15	0.50	0.20	0.00	7.20	1.60	0.30	.0002	. . .
X/Y	1.29	0.67	0.13	0.89	0.44	0.14	1.30	0.60	0.10	0.00	0.20	0.00	2.70	1.40	0.40	.0002	. . .
Diploidy	0.52	0.29	0.12	0.47	0.24	0.12	0.40	0.20	0.10	0.10	0.10	0.00	1.50	0.80	0.40	.0211	. . .
Total aneuploidy	9.02	3.91	1.02	4.64	1.57	0.37	9.40	4.00	0.95	3.40	1.70	0.40	18.20	6.80	1.80	$<$.0001	. . .

* NS indicates not significant; SD, standard deviation.

negative impact on ICSI outcome. This limit was fixed by analyzing 14 normozoospermic healthy men.

We propose that sperm aneuploidy analysis for at least 7 chromosomes could be used as an additional predictive test before ICSI for 46,XY men. This test could be used in 2 therapeutic schemas. First, aneuploidy determination in sperm could be proposed to each couple before the first ICSI as predictive test. Second, this test could be proposed in second intention as a diagnostic test after 3 ICSI failures. Thus, an aneuploidy rate less than or equal to 5% could lead to an ICSI attempt, whereas aneuploidy rate more than 5% could convince the couple to have a genetic counseling and to discuss preimplantation genetic diagnosis for aneuploidies (Voullaire et al, 2002; Munné, 2003).

In conclusion, these results suggest that chromosomal sperm nuclei profile could be used as a predictive test before ICSI in order to improve genetic counseling for OAT patients. Further studies are needed to determine the chromosomes to analyze and the threshold to be used.

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