High-magnification sperm selection does not decrease the aneuploidy rate in patients who are heterozygous for reciprocal translocations

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Abstract

Problem This study sought to evaluate the value of motile sperm organelle morphology examination (MSOME) for selecting euploid spermatozoa in six patients who were heterozygous for a reciprocal translocation.

Method of study We used sperm fluorescence in situ hybridization (FISH) to screen for aneuploidy of the chromosomes involved in the translocations and a putative interchromosomal effect (ICE) for chromosomes 18, X and Y. This procedure was performed on (i) whole sperm (i.e. no selection) and on normal spermatozoa selected (ii) at a magnification typically used for intracytoplasmic sperm injection (ICSI), referred to as “ICSI-like”, and (iii) with MSOME.

Results The balanced translocation rates did not differ significantly ($p=0.81$) when comparing whole sperm (57.2 %) with spermatozoa after ICSI-like selection (56.3 %) or after MSOME (53.7 %). Similarly, the aneuploidy rates for ICEs did not differ significantly ($p=0.14$) when comparing whole

sperm (1.9 %), ICSI-selected spermatozoa (3.4 %) and MSOME-selected spermatozoa (1.0 %).

Conclusion For patients who are heterozygous for reciprocal translocations, MSOME does not improve the selection of euploid spermatozoa.

Keywords Sperm FISH · IMSI · MSOME · Reciprocal translocation · Interchromosomal effect (ICE)

Introduction

Palermo et al. first described the microscopic selection of morphologically normal spermatozoon (using magnifications of between $\times 200$ and $\times 400$) for subsequent use in intracytoplasmic sperm injection (ICSI) [25]. However, this method does not provide information on the sperm's chromosomal status and thus does not enable the exclusion of spermatozoa with chromosomal abnormalities [9]. Hence, in carriers of chromosomal abnormalities, spermatozoa with potentially damaged chromosomes can be injected into oocytes during an ICSI cycle and may produce miscarriages or infants with a congenital malformation.

Motile sperm organelle morphology examination (MSOME) is performed at high magnification ($>\times 6000$) with Nomarski contrast [3, 4]. It enables the accurate, real-time selection of motile “top spermatozoa” with a morphologically and morphometrically normal head [1, 4, 7]. When available, vacuole-free spermatozoa or spermatozoa presenting a single, small vacuole [6] that occupies less than 4 % of the total head area [10, 31] can then be used for intracytoplasmic morphologically selected sperm injection (IMSI) [7]. It has been suggested that in patients with ICSI cycle failures, implantation efficiency and pregnancy rates may be increased by the use of IMSI [1]. Use of IMSI-like

Capsule High-magnification sperm selection nor ICSI selection do not decrease the aneuploidy rate in patients who are heterozygous for reciprocal translocations.

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selection appears to be associated with better-quality spermatozoa and could improve embryonic development up to the blastocyst stage, at least [31]. Some researchers consider that there is a relationship between sperm morphology (as observed in MSOME) and chromosome content. Indeed, it has been reported that spermatozoa with large vacuoles (>13.0 % of the sperm head area) present higher aneuploidy and diploidy rates [18, 27]. However, other researchers have not observed this relationship. It has been shown that morphometrically normal spermatozoa with large sperm-head vacuoles (>25 % of the sperm head area) [8] do not have significantly higher or lower aneuploidy rates. Hence, in patients with a normal karyotype, the use of IMSI or MSOME for selecting euploid spermatozoa is still a matter of debate. Sperm aneuploidy rates are known to be low in patients with a normal karyotype. In order to evaluate the impact of MSOME on spermatozoa aneuploidy, one needs to select patients with a high malsegregation risk, i.e. those who are heterozygous for a chromosomal rearrangement (the prevalence of which ranges from 2 to 14 % in infertile men [30]). In patients who are heterozygous for a reciprocal translocation, meiotic malsegregation rates vary between 20 % and 80 % [5]. These abnormalities lead to the production of unbalanced spermatozoa that may increase the risk of chromosomal malsegregation and thus implantation failure, miscarriage or congenital malformation.

Hence, the objective of the present study was to confirm previous finding whereby the observation of spermatozoa at high magnification in translocation carriers cannot select spermatozoa with a balanced chromosomal content [10]. To this end, we studied translocation segregation in (i) whole sperm, (ii) morphologically normal spermatozoa selected at an ICSI-like magnification ($\times 200$) and (iii) morphologically normal spermatozoa selected at high magnification (as in MSOME, $>\times 6000$).

A second chromosomal risk relates to the potential for an interchromosomal effect (ICE), since the reciprocal translocation may disturb the meiotic segregation of chromosomes not involved in the primary recombination event. Although

it was initially postulated that the parents of children with Down's syndrome had a greater incidence of translocation [21], the significance of ICEs is still subject to debate [19, 26]. Given that reciprocal translocations could cause meiotic segregation disturbance of chromosomes not involved in this rearrangement, we also studied the sperm euploid rates for chromosomes 18, X and Y.

Population

The six patients in the present study were heterozygous for reciprocal autosomes translocations. The whole sperm's characteristics were evaluated according to the WHO criteria (WHO [34]) and Kruger's sperm morphology classification [20]. Our reference value for typical forms was 15 %. Only one of the six patients had normal sperm characteristics and one had a severe oligoasthenoteratozoospermia. The mean ($\pm$ SEM) sperm count, progressive motility (a+b) and typical form rates were respectively $135.5 \pm 11.2 \times 10^6$ /ejaculate, 35 ± 4 %, and 10 ± 3 % (Table 1).

After genetic counselling, the patients gave their informed, written consent for ICSI-like selection, MSOME and meiotic segregation analysis with FISH. All protocols were approved by the local independent ethics committee and registered with the French health authorities. The work was funded by the French Biomedicine Agency.

Methods

Semen preparation and parameters for whole sperm

Each semen sample was collected by masturbation after 2 to 5 days of sexual abstinence. The liquefied, fresh semen samples were incubated for 30 min at 37 °C and then centrifuged (10 min at 600 g). After elimination of the seminal plasma, the pellet was washed twice with 1.5 ml of sterile water.

Table 1 General and sperm-related characteristics of patients who were heterozygous for reciprocal translocations

Patient	Reciprocal translocation	Age (years)	Volume (ml)	Sperm count (million per ejaculate)	Progressive motility a + b (%)	Typical form (%)	Semen profile
P1	46,XY,t(10;13)(q11;q34)	43	8.6	292.4	20	4	AT
P2	46,XY,t(5;8)(p15.1;q22.1)	36	4.8	26.9	41	2	T
P3	46,XY,t(4;11)(p15.2;q21)	28	2.8	0.2	22	10	OAT
P4	46,XY,t(5;21)(q15;q22.2)	30	2.8	267	55	29	NI
P5	46,XY,t(8;9)(q21.1;q22.2)	32	7.2	165.6	45	10	T
P6	46,XY,t(6;10)(p23;q11.2)	47	3.2	60.8	30	7	AT
	Mean $\pm$ SEM	36 $\pm$ 3	4.9 $\pm$ 1.6	135.5 $\pm$ 11.2	35 $\pm$ 4	10 $\pm$ 3	

O oligozoospermia; A asthezoospermia; T teratozoospermia; NI normozoospermia

Spermatozoa were fixed (in ethanol:acetic acid, 3:1 v/v) and stored at 4 °C. Next, spermatozoa were spread on a slide for the FISH assay. This fraction will be referred to henceforth as “whole sperm”. At least 1000 spermatozoa were counted for each sample, when available.

Selection of spermatozoa at a conventional magnification (ICSI-like) and at high magnification (MSOME)

Liquefied, fresh semen samples were evaluated according to the WHO guidelines [34] and prepared on a two-layer, discontinuous concentration gradient (PureSperm 100, Nidacon, Sweden), as described previously [15]. In a glass-bottomed dish (the WillCo-dish from WillCo Wells BV, The Netherlands), 5–10 µl of the sperm fraction obtained after density gradient selection were diluted into a 4 µl microdroplet of 10 % polyvinylpyrrolidone in flushing medium (FertiPro N.V., Belgium). Spermatozoa were then selected at an ICSI-like magnification ($\times 200$) or an MSOME-like magnification with a Nikon inverted microscope equipped with an $\times 100$ objective and Nomarski contrast, as previously described [11]. We selected normal spermatozoa according to the WHO criteria (WHO [34]). At an MSOME magnification, the head was always symmetrical, oval and smooth. The head size in both axes was normal. All spermatozoa had either no vacuoles or just one single small vacuole occupying less than 4 % of the head area [31]. For each selection, up to 100 spermatozoa were selected within 4 h.

Sperm fluorescence in situ hybridization analysis

Sperm slides were washed with 2 $\times$ saline-sodium citrate (SSC) buffer at 37 °C (30 min), fixed in methanol (5 min), decondensed with 1 N NaOH (80 s) and washed with 2 $\times$ SSC (5 min) and phosphate-buffered saline (PBS) (5 min). The slide was dehydrated with 70 %, 85 % and 100 % ethanol for 90 s each. For the meiotic segregation analysis of reciprocal translocations, 3 µl of a solution of the corresponding probes (Table 2) (Abbott Laboratories, Chicago, IL, USA) were placed on separate slides. After codenaturation at 73 °C (4 min) and hybridization at 37 °C (overnight), the slides were washed with 0.4 $\times$ SSC/0.1 % IGEPAL[®] at 73 °C (105 s) and then with 2 $\times$ SSC/0.3 % IGEPAL[®] at room temperature (15 s).

After addition of 4',6-diaminido-2-phenyl-indol (DAPI), the slides were observed under a fluorescence microscope (BX60, Olympus, Tokyo, Japan) equipped with PathVysion Smart Capture software (version 1.4, Digital Scientific, Cambridge, United Kingdom).

Lastly, the slides were washed in PBS (2 min) to remove the cover slip prior to ICE analysis. They were dehydrated with alcohol, as described above. The same procedure was performed with 3 µl of mixed centromeric probes for chromosomes 18, X and Y.

Statistical analysis

Since most sperm-related measurements are not normally distributed [12], we used a non-parametric Kruskal Wallis

Table 2 Reciprocal translocation segregation analysis

Patient	Reciprocal translocation	Probes used	Whole sperm		ICSI Selection		IMSI selection	
			Sperm number	balanced translocation rate	Sperm number	balanced translocation rate	Sperm number	balanced translocation rate
P1	46,XY,t(10;13)(q11;q34)	CEP10 (SA), RB1 (SG), Subtel10q (SO)	1001	46.4 %	87	67.8 %	18	55.6 %
P2	46,XY,t(5;8)(p15.1;q22.1)	CEP 8 (SA), CDC (SG), NSD1 (SO)	213	61.0 %	153	53.5 %	96	47.9 %
P3	46,XY,t(4;11)(p15.2;q21)	CEP 4 (SO), WHS (SG), CEP 11 (SA)	513	58.7 %	140	47.8 %	113	55.8 %
P4	46,XY,t(5;21)(q15;q22.2)	CDC (SG), NSD1 (SO), DSCR1 (SO and SG)	2272	51.5 %	148	53.0 %	73	47.9 %
P5	46, XY,t(8;9)(q21.1;q22.2)	CEP8 (SA), CEP9 (SG), Subtel8q (SO)	1007	67.4 %	154	68.2 %	132	72.0 %
P6	46,XY,t(6;10)(p23;q11.2)	CEP6 (SA), CEP10 (SG), Subtel10q (SO)	1036	58.0 %	74	47.3 %	60	43.3 %
		Mean $\pm$ SEM	1007 $\pm$ 703	57.2 $\pm$ 7.4 % ^a	126 $\pm$ 36	56.3 $\pm$ 9.4 % ^a	82 $\pm$ 41	53.7 $\pm$ 10.2 % ^a
		Median and [interquartile range (IQR)]		58.25 % [7.3]		53.25 % [15.1]		51.8 % [7.8]

^a No statistical difference, using Kruskal Wallis test, between whole sperm/ICSI selection/IMSI selection: $p=0.81$

Table 3 Chromosome X, Y and 18 segregation analysis

	Patient	Reciprocal translocation	Whole sperm		ICSI Selection		IMSI selection	
			Sperm number	Euploidy rate	Sperm number	Euploidy rate	Sperm number	Euploidy rate
Reciprocal translocations	P1	46,XY,t(10;13)(q11;q34)	1038	96.24 %	90	97.78 %	13	100.00 %
	P2	46,XY,t(5;8)(p15.1;q22.1)	514	96.33 %	90	92.22 %	147	97.96 %
	P3	46,XY,t(4;11)(p15.2;q21)	1002	98.20 %	122	95.90 %	109	99.08 %
	P4	46,XY,t(5;21)(q15;q22.2)	1018	99.51 %	134	97.76 %	86	97.67 %
	P5	46, XY,t(8;9)(q21.1;q22.2)	1026	99.12 %	169	98.82 %	123	99.19 %
	P6	46,XY,t(6;10)(p23;q11.2)	1017	99.31 %	73	97.26 %	62	100.00 %
	Mean $\pm$ SEM		936 $\pm$ 207	98.1 $\pm$ 1.5 % ^a	113 $\pm$ 36	96.6 $\pm$ 2.4 % ^a	90 $\pm$ 48	99.0 $\pm$ 1.0 % ^a
	Median [interquartile range (IQR)]		99.1 % [2.2]		97.6 % [1.3]		99.1 % [1.2]	

^a No statistical difference, using Kruskal Wallis test, between whole sperm / ICSI selection / IMSI selection: $p=0.13$

test to compare the three groups of spermatozoa. The median and interquartile range (IQR) have been added to Tables 2 and 3. Statistical analyses were performed with SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA).

The threshold for statistical significance was set to $p<0.05$.

Results

Number of sperm analysed

The mean $\pm$ SD (range) number of spermatozoa analysed per patient were 1007 $\pm$ 703 (213–2272) for whole sperm, 126 $\pm$ 36 (74–154) after ICSI-like selection and 82 $\pm$ 41 (18–132) after MSOME selection.

Reciprocal translocation segregation analysis (Table 2)

Considering patients individually, spermatozoa selected on ICSI-like or IMSI-like magnification were more or less balanced than those on whole sperm. Given that the type of selection (ICSI or IMSI) did not influence balanced translocation rates for individual patients, we decided to pool the patients' data and thus avoid any effects of the translocation type and variations in the number of spermatozoa analyzed.

Meiotic segregation analysis in patients who were heterozygous for reciprocal translocations revealed that the balanced translocation rates (i.e. balanced spermatozoa) did not differ significantly ($p=0.81$) when comparing whole sperm (57.2 $\pm$ 7.4 %), spermatozoa selected by conventional ICSI-like examination (56.3 $\pm$ 9.4 %) and spermatozoa selected by MSOME (53.7 $\pm$ 10.2 %).

Analysis of the interchromosomal effect on chromosomes 18, X and Y (Table 3)

As with the reciprocal translocation segregation analysis, the data on individual patients' aneuploidy rates for chromosomes 18, X and Y were pooled. The overall euploidy rates did not differ significantly ($p=0.13$) when comparing whole sperm (98.1 $\pm$ 1.5 %), spermatozoa selected by conventional ICSI-like examination (96.6 $\pm$ 2.4 %) and spermatozoa selected by MSOME (99.0 $\pm$ 0.9 %).

Discussion

The objective of the present study was to determine whether or not MSOME can be used to select balanced, normal spermatozoa (according to FISH) in patients with reciprocal translocations. This analysis was performed for three groups of spermatozoa (whole sperm, normal spermatozoa selected at an ICSI-like magnification and normal spermatozoa selected by MSOME-like magnification) by determining the proportion of balanced sperm (i.e. those resulting from 2:2 alternate modes of translocation meiotic segregation). Furthermore, we also focused on the aneuploidy rate of chromosomes not involved in these translocations. Lastly, we sought to determine whether or not MSOME was able to specifically identify balanced, euploid sperm. To the best of our knowledge, the only study to have addressed this topic found that MSOME was ineffective [10]. In contrast to previous reports, we considered only class I spermatozoa and compared the MSOME results with those obtained by ICSI-like selection. Furthermore, the mean number of spermatozoa analyzed in our series was 126 $\pm$ 36 for the ICSI selected sample and 82 $\pm$ 41 for MSOME; both numbers were much higher than that previously reported (9 $\pm$ 7) [10].

Even though the effect on spermatogenesis varies from one type of translocation to another [29], there are also significant inter-individual variations for a given translocation [2, 14, 22]. In infertile populations with implantation failure, the use of IMSI reportedly increases the pregnancy rate. Some researchers consider that this increase may be related to the technician's ability to avoid microinjection of spermatozoa with aneuploid chromosome contents or chromatin damage (e.g. DNA breaks, non-condensed chromatin, etc.) [8, 17, 27, 33]. Given that the significance of sperm head vacuole characteristics remains unclear [17], we wondered whether MSOME could be used to select euploid spermatozoa. Hence, we sought to determine the value of MSOME in patients at a high risk of chromosomally unbalanced spermatozoa (such as those with reciprocal translocations).

By studying the segregation of reciprocal translocation, we found similar balanced translocation rates for ICSI-like-selected, MSOME-selected and whole sperm ($p > 0.05$). Likewise, the three types of spermatozoa did not differ significantly in terms of the euploidy rate for chromosomes not involved in the translocation. All the balanced translocation rates were in accordance with literature values (i.e. a mean aneuploidy rate close to 50 % for translocation segregation and around 2 % for the ICE [13, 23, 28]). Due to the small sample size for ICSI and IMSI selected spermatozoa, no statistical analyses were performed.

Our data confirmed that MSOME was not able to select euploid spermatozoa or those with balanced translocations [10] and confirmed the results of previous studies in which the observed abnormalities (vacuoles) were linked to DNA damage (chromatin condensation or DNA fragmentation, for example), rather than aneuploidy [8, 17]. Even though Perdrix et al. [27] observed some aneuploidy rate differences between spermatozoa with large vacuoles and whole sperm from patients with a normal karyotype, other authors failed to observe this association [8, 32], for a review, see [24]. Similar results were also found for infertile patients with macrocephalic head syndrome, in whom no euploid spermatozoa had been retrieved after ICSI-like selection or MSOME [11]. Only diploid spermatozoa with an enlarged head could be selected by MSOME.

Lastly, the higher pregnancy rates observed after IMSI are probably not related to an increase in the euploidy rate. Since vacuoles appear to be associated with chromatin condensation failure [8, 17, 27] and, in some cases, sperm DNA fragmentation [16, 33], we suggest that the higher pregnancy rates and lower miscarriage rates observed with IMSI were related to DNA damage rather than aneuploidy.

On the basis of our results for six patients, we conclude that MSOME is inefficient (and no better than ICSI-like examination) for detecting chromosomally abnormal spermatozoa with a normal-sized head.

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