Asian Journal of Andrology (2009) 11: 325–332

www.nature.com/aja

Original Article

18,X,Y aneuploidies and transmission electron microscopy studies in spermatozoa from five carriers of different reciprocal translocations

Elena Moretti[1], Nicola Antonio Pascarelli[1], Valentina Giannerini[1], Michela Geminiani[1], Cecilia Anichini[2], Giulia Collodel[1]

[1]*Department of Biomedical Sciences, Applied Biology Section, University of Siena, Siena 53100, Italy*
[2]*Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Siena 53100, Italy*

Abstract

We analysed ejaculated spermatozoa from five infertile men with different balanced reciprocal translocations to contribute to the study of meiotic segregation of chromosomes 18, X and Y and also to evaluate sperm morphology by transmission electron microscopy (TEM) analysis. Conventional lymphocyte karyotype analyses highlighted different reciprocal balanced translocations: t(12;13), t(4;9), t(X;8), t(8;10) and t(3;16). Semen analysis was performed by light and TEM. Fluorescence *in situ* hybridization was performed directly on sperm nuclei using centromeric probes for chromosomes 18, X and Y. The carriers of the balanced reciprocal translocations considered in the present study showed a very similar pattern of sperm pathologies: diffused presence of apoptosis and immaturity. All patients showed meiotic segregation derangements, highlighted by the presence of sperm diploidies and sex chromosome disomies particularly related to the failure of the first meiotic division. However, an increased incidence of chromosome 18 aneuploidy was detected in spermatozoa from t(X;8) and t(8;10) carriers. We have also reported values from sex chromosomes such as t(X;8), although the X chromosome was involved in translocation. Since patients with reciprocal translocations and spermatogenetic impairment are candidates for intracytoplasmic sperm injection cycles, the study of sperm parameters, and particularly of the level of aneuploidy rates, would provide better information for couples at risk and would contribute to the data in the literature for a better understanding of the effects of chromosomal rearrangement on the whole meiotic process and, in particular, on chromosomes not involved in translocation.

Asian Journal of Andrology (2009) 11: 325–332. doi: 10.1038/aja.2008.31; published online 6 April 2009.

Keywords: electron microscopy, fluorescence *in situ* hybridization, altered karyotype, spermatozoa, reciprocal translocation

1 Introduction

The possible correlation between human male infertility due to impaired spermatogenesis and chromosome anomalies was postulated many years ago and recently it has been extensively confirmed [1].

Reciprocal translocations are the most frequent structural alterations in humans and, among infertile males, balanced reciprocal translocation carriers do not usually exhibit any peculiar phenotypes, but generally show oligozoospermia or even azoospermia.

Patients carrying balanced reciprocal translocations are subject to meiosis nondisjunction risk. Indeed, the

Correspondence to: Dr Giulia Collodel, Department of Biomedical Sciences, Applied Biology Section, University of Siena, Policlinico S. Maria alle Scotte, Siena 53100, Italy.
Fax: +39-0577-233527 E-mail: collodel@unisi.it
Received: 6 March 2008 Revised: 5 May 2008
Accepted: 1 June 2008 Published online: 6 April 2009

mispairing of translocated chromosomes during the first meiotic division can give rise to different forms of segregation, which can result in aneuploidy of the translocated chromosomes [2]. As a general rule, reciprocal translocation carriers produce more unbalanced spermatozoa than normal or balanced spermatozoa [3]. Whether meiotic association of the translocated chromosomes could interfere with the meiotic behaviour of chromosomes not involved in the rearrangement is still under debate. This phenomenon is known as interchromosomal effect (ICE). Studies of synaptonemal complexes of human spermatocytes have revealed that the presence of a structural reorganization can give rise to ICE in pairing anomalies and/or a reduction in the number of chiasmata at pachytene and to the production of heterosynapses between the chromosomes involved in the reorganization and the sex vesicle or with other partially unpaired autosomal bivalent leading to meiotic arrest or to the production of chromosomally unbalanced spermatozoa [4].

ICE may depend on the reorganization and on the chromosome and chromosome region involved resulting in a particular meiotic behaviour. In particular, a higher frequency of nondisjunction of the sex chromosomes and chromosome 21 was reported [5].

The issue of ICE is still very controversial because this effect has been found by some authors [2, 6, 7], but not by others [5, 8]. Shi and Martin [9] and Douet-Guilbert *et al.* [10] reviewed the literature and concluded that ICE is present on some specific chromosomes in spermatozoa from men who are translocation carriers.

Several studies have reported the disomy and diploidy rates in spermatozoa of male carriers of a translocation; in most of them, disomy of chromosome 21 and sex chromosomes have been analyzed and an increased of meiotic nondisjunction was observed. More rarely, disomy of chromosome 13 and 18 have been investigated [10].

One of the main difficulties in proving the existence ICE arises from the fact that the vast majority of patients analyzed have shown not only a translocation but also altered sperm parameters. Pellestor *et al.* [11] analyzed sperm samples from eight carriers of translocation divided into two groups: the first group having normal semen features and the second having oligoasthenozoospermia; the diploidy rate was higher in both groups compared with controls whereas the disomy rates of 13, 21, X and Y and XY were higher in the sole group with abnormal semen.

Another study [6] found a higher incidence of diploidy and aneuploidy in the spermatozoa of infertile men with abnormal karyotypes compared with normal controls.

In this kind of research, structural sperm anomalies are generally highlighted by optical microscope; however, a detailed structural analysis of spermatozoa performed by transmission electron microscope (TEM) could be useful to better define sperm alterations. For example, diffused ultrastructural anomalies have been highlighted by TEM in spermatozoa from a t(10;15) reciprocal translocation carrier, and a high frequency of sperm aneuploidies has been detected by fluorescence *in situ* hybridization (FISH) [12]. Using the same methods, severe morphological and meiotic spermatogenetic impairments were studied in seven patients with Robertsonian translocation carriers [13].

In the present paper, we contribute to the study of possible ICE in spermatozoa from five male carriers of balanced reciprocal translocations, focussing on the study of sperm morphology performed by TEM analysis. The percentages of chromosomes 18, X and Y aneuploidies were evaluated in all sperm samples.

2 Materials and methods

2.1 Patients

Five infertile patients (aged 30 to 37) carrying balanced reciprocal translocations, referred to our center for semen analysis after several years of unprotected sexual intercourse without conception, were enrolled in the study. The translocations studied were t(12;13) (q21;q12), t(4;9) (q25;p22), t(X;8) (p22.3;p23.1), t(8;10) (q13;p24) and t(3;16) (q25; p10).

Sexual development, medical history and physical examination were normal and serum hormone concentrations (follicle stimulating hormone [FSH], luteinizing hormone [LH], prolactin, androstenedione, dehydroep iandrosterone sulfate, estradiol, testosterone and free-testosterone) were within the standard range. Microbiological investigations did not reveal any genitourinary infections. None of the patients had ever received hormone therapy.

Before this study, each patient was informed of the investigation and gave his consent.

2.2 Karyotype

Conventional cytogenetic analysis of 24–48-h cultures of blood lymphocytes of the patients was per-

formed using standard techniques and evaluated by Giemsa-Trypsin-Giemsa (GTG) banding at about the 400 band level according to the 1995 International System for Human Cytogenetic Nomenclature (ISCN, 1995).

2.3 Semen analysis

2.3.1 Light and electron microscopy

Semen samples of patients were collected by masturbation after 4 days of sexual abstinence and examined after liquefaction for 30 min at 37ºC. Volume, pH, concentration and motility were evaluated according to World Health Organization (WHO) guidelines [14].

For electron microscopy, sperm samples were fixed in cold Karnovsky fixative and maintained at 4ºC for 2 h. Fixed semen was washed in 0.1 mol L^{-1} cacodylate buffer (pH 7.2) for 12 h, postfixed in 1% buffered osmium tetroxide for 1 h at 4ºC, and then dehydrated and embedded in Epon-Araldite. Ultra-thin sections were cut using a Supernova ultramicrotome (Reickert Jung, Vienna, Austria), mounted on copper grids, stained with uranyl acetate and lead citrate and observed and photographed with a Philips CM10 TEM (Philips Scientifics, Eindhoven, The Netherlands).

Three hundred spermatozoa were analyzed in ultra-thin sections for each sample. Major submicroscopic characteristics were recorded by highly trained examiners who were blind to the experiment. TEM data were evaluated using a mathematical formula [15] able to quantify electron microscopy analysis calculating the number of spermatozoa free of structural defects ("healthy") and the percentages of three main phenotypic sperm pathologies: immaturity, necrosis and apoptosis. We observed that the lowest number of spermatozoa devoid of ultrastructural defects ("healthy") assuring normal fertility is a little less than two million [16].

For TEM analysis, semen samples from twenty-five fertile men (aged 22 to 40) with normal karyotype and without anatomical problems or infections were used as controls. These fertile men had fathered one or more children during past 3 years [16].

2.3.2 FISH analysis of spermatozoa

To evaluate aneuploidy frequency, FISH was performed according to Baccetti *et al*. [12] in the sperm nuclei of the five patients. A combination of α-satellite DNA probes (CEP, Chromosome Enumeration Probes, Vysis, IL, USA) for chromosomes 18, X, and Y, directly labeled with different fluorochromes, was used.

2.3.3 Scoring criteria

The overall hybridization efficiency was > 98%. Sperm nuclei were scored if they were intact, non-overlapped and had a clearly defined border. The presence of a sperm tail was confirmed in the case of aneuploidy. A spermatozoon was considered disomic if the two fluorescent spots were of the same colour, similar in size, shape and intensity and were inside the edge of the sperm head, at least one domain apart. Diploidy was recognized by the presence of two double fluorescent spots, according to the above criteria. Observation and scoring were performed using a Leitz Aristoplan Optical Microscope (Leica, Wetzlar, Germany), equipped with a fluorescence apparatus with a triple bandpass filter for aqua, orange and green fluorochromes (Vysis) and a monochrome filter for DAPI (4',6-diamidino-2-phenylindole).

Semen samples from 25 fertile men (aged 22 to 40 years) were analyzed and used as controls [16]. Whenever possible, nearly 5 000 sperm nuclei were scored for each sample.

2.4 Statistical analysis

Statistical analysis was performed using the SAS system (version 8, SAS Institute, Cary, NC, USA).

The control group was used to build normal ranges, which were determined indicating the 25th and 75th percentiles, owing to the low number of controls [16]. A location test (signed ranked test) was performed to compare sperm characteristics from the reciprocal translocation carriers among the controls. $P < 0.05$ was considered significant.

3 Results

Lymphocyte karyotyping performed with conventional GTG banding revealed that the patients showed different reciprocal balanced translocations of t(12;13), t(4;9), t(X;8), t(8;10) and t(3;16).

Regarding semen parameters, t(4;9) and t(3;16) carriers had normal sperm concentration and the t(8;10) carrier had almost normal sperm concentration according to WHO guidelines [14], whereas t(12;13) and t(X;8) carriers were severely oligozoospermic. Total progressive motility was strongly reduced in all cases (Table 1).

Mathematically elaborated TEM analysis provided a fertility index expressed as the number of spermatozoa devoid of ultrastructural defects ("healthy") and the percentage of sperm pathologies, such as immaturity,

apoptosis and necrosis, values of which are reported in Table 2. These figures revealed severe spermatogenetic impairment in all patients and their fertility indexes appeared significantly lower than those of controls.

Sperm pathologies were also quantified by a mathematical method and, in particular, apoptosis and immaturity were significantly more frequent in spermatozoa from the analyzed patients than in spermatozoa from control subjects (Table 2). The calculation of the percentage of sperm pathologies is based on the quantification of the presence of typical ultrastructural defects in sperm organelles of each pathology. Features of immaturity were irregularly shaped acrosomes, generally elliptical or spherical nuclei with uncondensed chromatin (Figure 1) and cytoplasmic residues embedding the head or midpiece regions. Spermatids and binucleated or multinucleated spermatozoa were generally observed. The tails, often coiled into large cytoplasmic residues, had lost the normal axonemal and periaxonemal structures.

Apoptotic spermatozoa (Figure 2) were frequently present and were characterized by a misshapen acrosome and a nucleus with marginated chromatin. Swollen mitochondria, irregularly organized and embedded into large cytoplasmic residues with translucent vacuoles, were a typical ultrastructural marker for this pathology.

FISH sperm analysis was performed on the sperm nuclei of the five translocated patients.

In total, 20 684 spermatozoa were scored, evaluating the disomy and diploidy frequencies of chromosomes 18, X and Y (Table 3). Means ± SD, median, 25th and 75th percentiles of FISH values obtained from 25 fertile males, used as controls, are shown in Table 3.

In the group of translocation carriers, the diploidy rate was significantly higher compared with controls,

Table 1. Seminal parameters of five infertile reciprocal balanced translocation carriers. Normal values are also reported in WHO manual [14].

Patients	Karyotype	Spermiogram data		
		Volume (mL)	No. sperm mL^{-1} ($\times 10^6$)	Motility (a + b) (%)
1	46,XY t(12;13)	3.5	6	10
2	46,XY t(4;9)	1.8	21.25	14
3	46,XY t(X;8)	1.5	3.9	0
4	46,XY t(8;10)	3.5	19	21
5	46,XY t(3;16)	2	34	20
WHO [14]	/	2–6	≥ 20	≥ 50

Table 2. Transmission electron microscopy (TEM) spermatological characteristics of five infertile reciprocal translocation carriers. Values from men of proven fertility used as controls were compared with those from patients of the study.

Patients	Semen profile	No. healthy sperm	Sperm pathologies (%)		
			Apoptosis	Immaturity	Necrosis
1	OAT	11**	18.09**	85.22**	38.78
2	AT	888**	21.72**	87.39**	28.35
3	OAT	312**	22.28**	83.57**	32.67
4	OAT	62 556*	21.88**	70.56*	40.23
5	AT	1 234**	14.67**	75.89	25.89
Controls					
Mean ± SD		7 386 080 ± 10 464 288	4.056 ± 2.048	48.83 ± 13.93	32.13 ± 10.58
25th percentile		2 057 544	3.59	38.59	24.74
Median		3 807 391	4.06	47.29	34.63
75th percentile		8 308 132	4.67	58.22	40.10

Abbreviations: O, oligozoospermia; A, asthenozoospermia; T, teratozoospermia.
*$P < 0.05$; **$P < 0.01$, compared with controls.
Mean percentages (± SD), 25th percentile, median and 75th percentile of values of number of healthy spermatozoa and percentage of pathologies in spermatozoa from 25 healthy men of proven fertility [16].

particularly in the cases of t(12;13), t(X;8) and t(8;10).

A significantly increased rate of disomy of chromosome 18 was observed in cases of t(X;8) and t(8;10). Almost all values referring to sex chromosome disomy were out of the normal range, in particular XY disomy was significantly higher in translocation carriers compared with controls. In patient 3, a t(X;8) carrier in which chromosome X was involved in translocation, values of XX and XY disomies were very high and ICE was only related to chromosome 18.

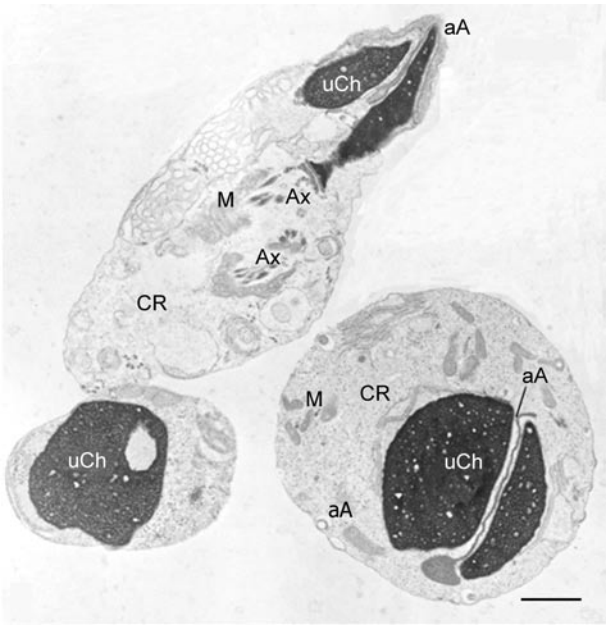

Figure 1. Transmission electron micrograph (TEM) of longitudinal and cross sections of immature spermatozoa characterized by irregular nuclei with uncondensed chromatin (uCh). Large cytoplasmic residues (CR) embed the coiled axonemes (Ax) and disorganized mitochondria (M). aA, Altered acrosome. Bar = 1 μm.

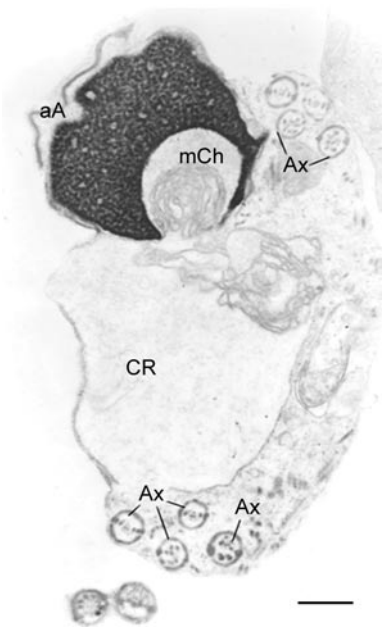

Figure 2. Transmission electron micrograph (TEM) of a longitudinal section of apoptotic spermatozoa characterized by altered nuclei with marginated chromatin (mCh), coiled axoneme (Ax) irregularly organized into large cytoplasmic residues (CR). aA, Altered acrosome. Bar = 1.5 μm.

Table 3. Incidence of sperm disomy and diploidy of chromosomes 18, X and Y in five male carriers of reciprocal translocations. Values from patients were compared with fertile men used as controls. A total number of 128 352 sperm nuclei was scored [16].

Patients	No. cells scored	Diploidy (%)	18 (%)	XX (%)	YY (%)	XY (%)
1	3 498	1.197**	0.142	0.047	0.143*	0.527**
2	5 643	0.668*	0.078	0.118*	0.078	0.220**
3§	2 549	2.197***	0.302**	0.305**	0.180*	4.100***
4§§	5 325	1.894***	0.270**	0.146*	0.073	0.219**
5	3 669	0.545*	0.108	0.081	0.081	0.218**
Controls°						
Mean ± SD		0.277 ± 0.073	0.103 ± 0.052	0.060 ± 0.057	0.048 ± 0.032	0.137 ± 0.071
25th percentile		0.243	0.066	0.02	0.034	0.076
Median		0.280	0.094	0.04	0.045	0.110
75th percentile		0.310	0.120	0.067	0.051	0.170

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared with controls.

§XX disomy was reported, but X is a chromosome involved in translocation t (X;8).

§§Multiple fluorescent signals (> 4) were detected in 0.243% of spermatozoa.

°Mean percentages (± SD), 25th percentile, median and 75th percentile of values of disomy and diploidy for the examined chromosomes in spermatozoa from 25 healthy men of proven fertility.

4 Discussion

The aim of the present study was to evaluate the spermatogenetic process in reciprocal translocation carriers with infertility problems. Spermatozoa were examined by light and electron microscopy to determine semen parameters and details of fine morphology. FISH analysis was performed to investigate the meiotic behavior of chromosomes 18, X and Y. In the t(X;8) carrier, only chromosome 18 was considered to be related to ICE, although data concerning nondisjunction of sex chromosomes were also reported.

Two out of five reciprocal translocation carriers were severely oligozoospermic, and an explanation of this phenomenon was given by Anton *et al.* [17] who, in analyzing spermatozoa from seven male carriers of Robertsonian translocation t(13;14) (q10;q10), hypothesized that any factor that delays anaphase, such as erratic separation of chromosomes, may arrest division leading to cell apoptosis.

Ultrastructural sperm examination in this group of patients showed a severely deranged spermatogenetic process. The quantification of TEM data highlighted a higher percentage of immaturity and apoptosis in reciprocal translocation carriers than in controls, whereas necrosis was not particularly diffused. Each ejaculate showed many binucleate spermatozoa, indicating alterations of germ cell division during spermiogenesis. Similar features have already been described by TEM in a carrier of reciprocal translocation (10;15) [12]. In Robertsonian translocation carriers, TEM also highlighted features of general immaturity, particularly evident in a t(14;22) carrier, together with the consistent presence of necrosis [13].

Regarding sperm apoptosis, Brugnon *et al.* [18] found phosphatidylserine externalization, a typical marker of apoptosis, and significantly higher DNA fragmentation rates in ejaculated spermatozoa from translocation carriers than in spermatozoa from donors.

Recently our research group published a paper [16] in which apoptosis and necrosis were evaluated by TEM and annexin V-propidium iodide assay. This last method enables the detection of phosphotidylserine externa-lisation in the plasma membrane (a critical stage of apoptosis) and cell membrane breakage (a precocious sign of necrosis). In this paper we found a good correlation between the two methods, TEM and the one with fluorochromes.

In the cases analyzed in the present study, the number of "healthy" spermatozoa, determined by TEM, was very low and the mathematical formula indicated that intracytoplasmic sperm injection (ICSI) is the only possible treatment for obtaining fertilization.

Because translocation carriers are candidates for ICSI, FISH analysis performed directly on sperm nuclei provides a better understanding of the meiotic process and it could improve the risk assessment of chromosomally abnormal embryos [19].

Several studies, recently reviewed by Douet-Guilbert *et al.* [10], have reported the disomy and diploidy rates in the spermatozoa of male carriers of a translocation. ICE of some specific chromosomes was detected in male carriers of translocation, although the origin of the increased aneuploidy rates could be related to the oligoasthenoteratozoospermia usually present in most patients enrolled in these studies. Pellestor *et al.* [11] clearly highlighted no evidence for the occurrence of ICE in spermatozoa of fertile rearrangement carriers, but significant variations were observed in the groups of translocation carriers with altered semen parameters, suggesting a direct correlation between poor quality spermatozoa and increased aneuploidy rate. These results are consistent with the study of Vegetti *et al*. [6] and the present study, in which a higher incidence of aneuploidies in spermatozoa from infertile men with abnormal karyotype was detected.

Moreover, this phenomenon showed inter-individual variations that cannot be predicted [10]. For this reason, a deep analysis of spermatozoa from translocation carriers is needed before attempting assisted reproduction techniques. In the present study, the aneuploidy of choromosome 18 was evident in spermatozoa from a t(X;8) carrier, in which all FISH values were altered, and in spermatozoa from a t(8;10) carrier.

Higher values of sex chromosomes were obviously detected in spermatozoa from a t(X;8) carrier, considering the involvement of the X chromosome in the translocation; however, an increase in nondisjunction, particularly evident in meiotic division I, was observed in all analyzed samples.

It was interesting that all diploidy frequencies were higher in carriers of translocations versus controls. Diploidy of sperm cells may be generated by a binucleate sperm head or by diploid nuclei: the first case was demonstrated by the membranous sperm septum between the two nuclei, and the second was deduced from

the presence of double sets of chromosomes 18, X, Y.

In particular, the highest frequency of aneuploidies was shown in the t(X;8) carrier. Azoospermia is the most common finding in male carriers of an X-autosomal translocation [20], as also reported recently in a reciprocal translocation t (X;11) [21]. However a few cases with severe oligozoospermia have been reported [20], including the case reported in the present study where the patient showed a severe oligoasthenoteratozoospermia and a high frequency of unbalanced chromosome complement. Translocations involving a portion of the X chromosome have a deep impact on spermatogenesis that can lead to the formation of elongated spermatids, but the process is remarkably inefficient, as indicated by the presence of a small number of spermatozoa. In these cases ICSI techniques should be performed with the risk that X-autosomal translocation can be transmitted to offspring as demonstrated by Ma *et al*. [20] in a t(X;20) carrier.

The deleterious effect on spermatogenesis of translocations involving the X chromosome was also observed in boars, in which DNA fragmentation was detected exclusively among early spermatocytes and degenerating germ cells. These findings and the absence of stages beyond the pachytene suggest that the meiocytes are arrested at pachytene and eliminated through the apoptotic process, in spite of the complete synapsis displayed by the chromosomes involved in this translocation [22].

In conclusion, we observed an increment in aneuploidies in spermatozoa from the five infertile patients examined and this phenomenon could be linked to ICE; however, since none of the carriers were classified as normozoospermic, an effect of the poor testis environment on the meiotic process should not be excluded.

Since the analyzed infertile patients showed severe morphological and meiotic spermatogenetic impairment, and inter-individual variations cannot be predicted [10], a detailed ultrastructural and chromosomal analysis of spermatozoa from every reciprocal translocation carrier is advisable before undertaking ICSI cycles, and it is important to discuss with couple the option of Preimplantation Genetic Diagnosis [23].

Acknowledgment

This research was supported by a Piano di Ateneo per la Ricerca (PAR) (University Plan for Research) grant in 2005 from the University of Siena, Italy.

References

1 Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A, *et al*. Human male infertility: Chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. Hum Reprod Update 2000; 6: 93–105.
2 Machev N, Gosset P, Warter S, Treger M, Schillinger M, *et al*. Fluorescence in situ hybridization sperm analysis of six translocation carriers provides evidence of an interchromosomal effect. Fertil Steril 2005; 84: 365–73.
3 Benet J, Oliver-Bonet M, Cifuentes P, Templado C, Navarro J. Segregation of chromosomes in sperm of reciprocal translocation carriers: a review. Cytogenet Genome Res 2005; 111: 281–90.
4 Blanco J, Egozcue J, Vidal F. Interchromosomal effects for chromosome 21 in carriers of structural chromosome reorganizations determined by fluorescence in situ hybridization on sperm nuclei. Hum Genet 2000; 106: 500–5.
5 Estop AM, Cieply K, Munne S, Surti U, Wakim A, *et al*. Is there an interchromosomal effect in reciprocal translocation carriers? Sperm FISH studies. Hum Genet 2000; 106: 517–24.
6 Vegetti W, Van Assche E, Frias A, Verheyen G, Bianchi MM, *et al*. Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men. Hum Reprod 2000; 15: 351–65.
7 Palermo GD, Colombero LT, Hariprashad JJ, Schlegel PN, Rosenwaks Z. Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod 2002; 17: 570–5.
8 Oliver-Bonet M, Navarro J, Codina-Pascual M, Abad C, Guitart M, *et al*. From spermatocytes to sperm: meiotic behaviour of human male reciprocal translocations. Hum Reprod 2004; 19: 2515–22.
9 Shi Q, Martin RH. Aneuploidy in human spermatozoa: FISH analysis in men with constitutional chromosomal abnormalities, and in infertile men. Reproduction 2001; 121: 655–66.
10 Douet-Guilbert N, Bris MJ, Amice V, Marchetti C, Delobel B, *et al*. Interchromosomal effect in sperm of males with translocations: report of 6 cases and review of the literature. Int J Androl 2005; 28: 372–9.
11 Pellestor F, Imbert I, Andreo B, Lefort G. Study of the occurrence of interchromosomal effect in spermatozoa of chromosomal rearrangement carriers by fluorescence in-situ hybridization and primed *in situ* labelling techniques. Hum Reprod 2001; 16: 1155–64.
12 Baccetti B, Bruni E, Collodel G, Gambera L, Moretti E, *et al*. 10, 15 reciprocal translocation in an infertile man: ultrastructural and fluorescence *in-situ* hybridization sperm study: case report. Hum Reprod 2003; 18: 2302–8.
13 Baccetti B, Collodel G, Marzella R, Moretti E, Piomboni P, *et al*. Ultrastructural studies of spermatozoa from infertile males with Robertsonian translocations and 18, X,Y aneuploidies. Hum Reprod 2005; 20: 2295–2300.
14 World Health Organization. WHO Laboratory Manual for

the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge: Cambridge University Press, 1999.
15 Baccetti B, Bernieri G, Burrini AG, Collodel G, Crisà N, *et al*. Notulae seminologicae. 5. Mathematical evaluation of interdependent submicroscopic sperm alterations. J Androl 1995; 16: 365–71.
16 Collodel G, Moretti E. Morphology and meiotic segregation in spermatozoa from men of proven fertility. J Androl 2008; 29: 106–14.
17 Anton E, Blanco J, Egozcue J, Vidal F. Sperm FISH studies in seven male carriers of Robertsonian translocation t(13;14)(q10;q10). Hum Reprod 2004; 19: 1345–51.
18 Brugnon F, Van Assche E, Verheyen G, Sion B, Boucher D, *et al*. Study of two markers of apoptosis and meiotic segregation in ejaculated sperm of chromosomal translocation carrier patients. Hum Reprod 2006; 21: 685–93.
19 Yakut T, Ercelen N, Acar H, Kimya Y, Egeli U. Meiotic segregation analysis of reciprocal translocations both in sperms and blastomeres. Am J Med Genet 2006; 15: 1074–82.
20 Ma S, Yuen BH, Penaherrera M, Koehn D, Ness L, *et al*. ICSI and the transmission of X-autosomal translocation: a three-generation evaluation of X;20 translocation: case report. Hum Reprod 2003; 18: 1377–82.
21 Ishikawa T, Kondo Y, Yamaguchi K, Oba T, Sakamoto Y, *et al*. An unusual reciprocal X-autosome translocation in an infertile azoospermic man. Fertil Steril 2007; 88: 705.e15-7.
22 Koykul W, Baguma-Nibasheka M, King WA, Basrur PK. Meiosis and apoptosis in germ cells of X-autosome translocation carrier boars. Mol Reprod Dev 2000; 56: 448–57.
23 Georgiou I, Syrrou M, Pardalidis N, Karakitsios K, Mantzavinos T, *et al*. Genetic and epigenetic risks of intracytoplasmic sperm injection method. Asian J Androl 2006; 8: 643–73.