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GENETICS

Balanced chromosomal translocations in men: relationships among semen parameters, chromatin integrity, sperm meiotic segregation and aneuploidy

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Abstract
Purpose To analyse relationships between semen parameters, sperm chromatin integrity and frequencies of chromosomally unbalanced, disomic and diploid sperm in 13 Robertsonian and 37 reciprocal translocation carriers and to compare the results with data from 10 control donors.
Methods Conventional semen analysis, Sperm Chromatin Structure Assay and FISH with probes for chromosomes involved in the individual translocations and for chromosomes X, Y, 7, 8, 13, 18 and 21.
Results Normal semen parameters were found in 30.8 % of Robertsonian and 59.5 % of reciprocal translocation carriers. The rates of unbalanced sperm were 12.0 % in Robertsonian and 55.1 % in reciprocal translocation carriers with no difference between normospermic patients and those showing altered semen parameters. Significantly increased frequencies of spermatozoa showing defects in chromatin integrity and condensation, aneuploidy for chromosomes not involved in a translocation and diploidy were detected in translocation carriers with abnormal semen parameters. Normospermic reciprocal translocation carriers showed an increase in chromosome 13 disomy compared to the control group. There was no relationship between gametic and somatic aneuploidy in 12 translocation carriers studied by FISH on sperm and lymphocytes. The frequency of motile sperm was negatively correlated with the frequency of sperm showing disomy, diploidy and defective chromatin condensation.
Conclusions Abnormal semen parameters can serve as indicators of an additional risk of forming spermatozoa with defective chromatin and aneuploidy in translocation carriers.

Keywords Spermatozoa · Chromosomal translocation · Chromatin integrity · Meiotic segregation · Aneuploidy

Capsule Translocation carriers showing abnormal semen parameters are at increased risk of forming gametes with additional numerical chromosomal aberrations and defective chromatin condensation.

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Introduction

Balanced chromosomal translocations are often found as a cause of infertility [25]. They are particularly frequent among couples experiencing recurrent miscarriages and among men showing altered semen quality [9, 11, 26, 45, 48]. Robertsonian translocations are created by a fusion of two acrocentric chromosomes after their breakage and loss of p-arms. Their incidence in the population is 1.23/1000 newborns and the most common combination is the fusion of chromosomes 13 and 14 [31]. The normal and translocated chromosomes form a trivalent by pairing in the first meiotic division (MI) and segregate by

alternate (normal/balanced) or unbalanced mode. The frequencies of unbalanced spermatozoa ranging between 3.4 % and 40 % were detected in male carriers of Robertsonian translocations [38].

Reciprocal translocations are produced by breakage and exchange of distal segments between non-homologous chromosomes. The incidence in the population is 1/712 newborn children [31]. During meiotic pairing, the normal and translocated chromosomes form a quadrivalent. Unbalanced spermatozoa were detected in male carriers of balanced reciprocal translocations in frequency of 18.6 %–80.7 % [3]. They arise by adjacent I, adjacent II, 3:1 or 4:0 segregation mode in anaphase I and their relative proportions and viability of resulting embryos depend on the chromosomes involved, position of breaks and recombination sites [3, 7, 15].

The existence of a possible interchromosomal effect of translocations (ICE), i. e. formation of gametes with another chromosomal abnormality due to meiotic disturbances caused by interactions of the translocated chromosomes with other non-homologous chromosomes, has not been fully established yet [1, 12, 21]. Significantly higher aneuploidy frequencies were reported in sperm and embryos of some balanced translocation carriers, but not in others [1, 19, 23, 33, 35, 47]. A correlation between germinal and somatic aneuploidy was described in both normospermic men and men showing abnormal semen parameters [2, 18, 39] and the underlying role of genomic instability and mitotic checkpoint in development of germinal and somatic aneuploidy was discussed in this connection.

In genetic counselling for family planning purposes, the prenatal testing, and alternatively, preimplantation genetic diagnosis (PGD) are recommended to balanced translocation carriers because these patients are at increased risk of conceiving chromosomally abnormal embryos, resulting in implantation failure, miscarriage or delivery of affected offspring [19, 28, 36]. Attempts were made to find a relationship between the frequency of unbalanced gametes, semen parameters and an outcome of PGD [8, 13, 54], but more comprehensive studies are needed. In addition to conventional semen analysis and sperm FISH, the Sperm Chromatin Structure Assay (SCSA) providing information on the frequency of spermatozoa showing DNA fragmentation (DFI–percentage of mature spermatozoa with increased chromatin damage) and high DNA stainability (HDS, immature cells) can be used as a predictor of reproduction outcome based on the comparison with threshold values for normal fertility, i. e. 30 % DFI and 15 % of HDS cells [6, 14, 20, 22, 44, 49].

In this study, conventional semen analysis, SCSA and sperm FISH analysis of meiotic segregation of chromosomes involved in translocations and of aneuploidy for chromosomes X, Y, 7, 8, 13, 18 and 21 were performed in 50 translocation carriers and were compared with the results from 10 control donors. Relationships among the studied parameters were analysed by a correlation analysis. Finally, an association between sperm and somatic cell aneuploidy was tested on a subset of 12 translocation carriers.

Materials and methods

Patients and semen samples

Semen samples were obtained by masturbation from 13 carriers of Robertsonian and 37 carriers of reciprocal translocations and the control group of 10 men. For information on individual participants, see Tables 1 and 2. Samples were allowed to liquefy at room temperature, analyzed using standard techniques for semen analysis [56, 57], and stored frozen in liquid nitrogen without any cryopreservation until FISH and SCSA analysis. Samples were collected in a 6 year period (2006–2011). For the purpose of this study, the historical results of the semen analysis were compared with current reference values [57] to unify the classification of patients into groups (normal vs. abnormal). All participants gave their informed consent with the study. The study was approved by the Ethics Committee of the University Hospital Brno, Czech Republic.

Sperm chromatin structure assay (SCSA)

The integrity of sperm chromatin was measured in 11 Robertsonian and 33 reciprocal translocation carriers and 10 control donors using SCSA method as described in Rybar et al. [42]. The DNA fragmentation index (DFI) and percentage of high density staining (HDS) cells were assessed using a flow cytometer (FACSCalibur flow cytometer; Becton Dickinson, Mountain View, CA, USA) operated by the CELLQuest software. Analysis of the data was performed using SCSA-Soft software (SCSA DIAGNOSTICS, INC, Multiplex Research & Technology Center, Brookings, USA).

Sperm FISH

For the sperm FISH assay, semen samples were thawed at room temperature, smeared onto microscopic slides, and fixed by 3:1 solution of methanol:acetic acid. The sperm DNA was decondensed by incubation in DTT as described by Robbins et al. [37] and denatured in 50 % formamide at 72 °C for 4 min. The probe sets for FISH on sperm were selected for each individual translocation according to the position of breakpoints (Table 3). The multicolour FISH was performed according to the instructions of producers of probes (Oncor, Illkirch Cedex, France; Qbiogene, Illkirch Cedex, France; Vysis-Abbott, Abbott Park, IL, USA; Cytocell, Cambridge, UK; Kreatech, Amsterdam, The Netherlands). Biotinylated

Table 1 Characteristics of the study participants. Robertsonian translocation carriers and control donors

Patient	Translocation	Age	Semen parameters				Result	SCSA		Reproductive history
			Volume (ml)	Concentration ($\times 10^6$/ml)	Motility (%)	Normal morphology (%)		DFI (%)	HDS (%)	
Group A										
Rob1[a]	t(13;14)	30	1.8	155	50	30	N	18.2	10.4	1 SA, 1 ectopic pregnancy–SA
Rob2	t(13;14)	29	1.5	28	42	n.a.	N	12.1	22.9	primary infertility
Rob3	t(13;14)	34	2.3	57	60	23	N	10.7	12.9	primary infertility 24 months, 3 IVF-ET sine eff.
Rob4[a]	t(15;22)	28	3.2	44	50	5	N	41.8	12.3	primary infertility 5 months, 1 IVF-ET sine eff.
Mean ± SD		30.3±2.6	2.2±0.7	71.0±57.2	50.5±11.7	19.3±12.9		20.7±14.4	14.6±5.6	
Group B										
Rob5	t(13;14)	31	7.4	8	60	9	O	36.5	36.4	1 SA, 1 ITP for malformations
Rob6	t(13;14)	39	4.6	0.3	40	n.a.	O	35.5	28.8	primary infertility 48 months
Rob7	t(13;14)	40	2.0	9	14	11	OA	31.6	20.4	primary infertility 12 months
Rob8	t(13;14)	31	2.8	0.3	0	n.a.	OA	n.a.	n.a.	primary infertility
Rob9[a]	t(13;14)	27	4.3	6.7	12	6	OA	23.7	29.1	primary infertility 18 months, 2 IVF-ET sine eff.
Rob10	t(13;14)	35	4.2	36	40	4	T	27.0	20.5	primary infertility 48 months
Rob11	t(13;14)	36	2.0	5	45	30	O	n.a.	n.a.	primary infertility 18 months, 1 IVF-ET sine eff.
Rob12	t(13;14)	28	1.8	22	6	11	A	17.8	51.4	primary infertility 48 months
Rob13	t(14;21)	28	4.0	4	25	1	OAT	33.6	24.4	primary infertility
Mean ± SD		32.8±4.9	3.7±1.8	10.1±11.6	26.9±20.4	10.3±9.4		29.4±6.9	30.1±10.9	
Control donors[a]										
C1		26	7.2	134	70	42	N	8.2	5.9	donor
C2		29	2.3	44	60	26	N	6.8	5.8	donor
C3		28	4.3	72	50	30	N	9.4	11.0	donor
C4		27	3.5	120	70	33	N	6.8	6.5	donor
C5		27	2.4	64	60	27	N	8.9	8.9	donor
C6		28	2.1	71	60	24	N	12.7	12.8	donor
C7		27	3.8	36	70	33	N	17.3	12.7	donor
C8		29	3.2	84	60	27	N	15.6	12.5	donor
C9		27	5.3	49	60	24	N	16.7	7.2	donor
C10		24	4.6	53	55	17	N	10.7	14.0	donor
Mean ± SD		27.2±1.5	3.9±1.6	72.7±32.2	61.5±6.7	28.3±6.7		11.3±4.0	9.7±3.2	

Group A–Robertsonian translocation, normospermic; Group B–Robertsonian translocation, abnormal semen

[a] [53]

n.a. not analysed; *N* normospermic; *O* oligospermic; *A* asthenospermic; *T* teratospermic

SA spontaneous abortion; *ITP* induced termination of pregnancy; *IVF-ET* in vitro fertilization cycle with embryotransfer

Table 2 Characteristics of the study participants. Reciprocal translocation carriers

Patient	Translocation	Age	Semen parameters				Result	SCSA		Reproductive history
			Volume (ml)	Concentration ($\times 10^6$/ml)	Motility (%)	Normal morphology (%)		DFI (%)	HDS (%)	
Group C										
Rec1	t(1;2)	30	2.0	18	55	20	N	10.2	14.2	primary infertility, 1 IVF-ET→SA
Rec2	t(1;6)	46	2.0	125	61	12	N	n.a.	n.a.	1 missed abort, 2 IUI sine eff., 1 IVF/PGD-ET→a girl 46,XX
Rec3	t(2;7)	30	4.2	36	70	12	N	12.3	8.8	1 IVF/PGD-ET→ twins 46,XX
Rec4	t(3;4)	30	3.8	51	78	10	N	30.7	1.2	primary infertility 18 months
Rec5	t(3;5)	30	3.2	36	68	19	N	15.4	9.6	a boy 46,XY,$t(3;5)_{pat}$
Rec6	t(4;7)	36	4.1	37	45	28	N	19.3	11.8	primary infertility 36 months
Rec7[a]	t(4;7)	38	5.8	69.5	63	19.5	N	6.9	6.8	
Rec8	t(4;8)	36	3.1	81	70	28	N	9.6	10.9	a girl 46,XX, 1 ITP for 46,XX,$der(4)_{pat}$
Rec9	t(4;11)	23	1.8	120	50	20	N	69.0	54.9	1 SA, secondary infertility 18 months, spontaneous gravidity
Rec10	t(4;16)	33	2.5	76.7	51	15	N	32.7	12.8	primary infertility 12 months
Rec11	t(5;9)	33	3.6	196	70	35	N	8.3	8.6	primary infertility
Rec12	t(5;19)	33	1.7	85	70	23	N	7.1	7.2	a boy 46,XY,$t(5;19)_{pat}$
Rec13[a]	t(6;7)	34	1.6	156	65	10.5	N	21.0	13.3	a boy 46,XY,$der(7)_{pat}$, 1 ITP for 46,XY, $der(7)_{pat}$
Rec14	t(6;14)	30	4.2	116	55	7	N	9.4	8.4	1 ITP, 1 missed abort
Rec15	t(7;9)	27	3.0	60	60	50	N	9.2	13.6	primary infertility
Rec16	t(7;10)	31	2.1	76	45	11	N	30.0	12.2	primary infertility
Rec17[ab]	t(7;10)	28	4.0	63	65	12	N	14.6	11.9	2 ITP for 46,XX, $der(7)_{pat}$; 2 IVF-ET→ a healthy girl
Rec18	t(7;12)	28	2.0	303	75	16	N	6.9	12.7	3 IVF/PGD-ET sine eff.
Rec19[c]	t(10;15)	30	n.a.	29	75	21	N	56.8	29.5	1 SA, 1 IVF-ET sine eff., 2 IVF/PGD-ET sine eff.
Rec20[ad]	t(11;18)	27	3.8	60	60	34	N	8.1	11.0	1 SA, secondary infertility
Rec21[ad]	t(11;18)	53	2.5	84	50	18	N	56.5	4.2	1 SA, a son 46,XY, a son 46,XY, $t(11;18)_{pat}$
Rec22[a]	t(13;15)	38	4.5	145	60	39	N	8.2	3.9	3 SA, 3 IVF-ET sine eff.
Mean ± SD		32.9±6.6	3.1±1.1	92.0±65.2	61.9±9.8	20.9±10.9		21.1±18.6	12.7±11.1	
Group D										
Rec23	t(1;2)	30	4.0	9	20	16	OA	1.8	11.6	2 IVF/PGD-ET sine eff.
Rec24	t(1;7)	28	2.2	1	5	20	OA	n.a.	n.a.	primary infertility 18 months, 1 IVF-ET sine eff.
Rec25	t(1;13)	33	3.0	18	33	12	A	21.9	16.3	1 IVF/PGD-ET sine eff.
Rec26	t(1;14)	29	3.0	19	10	10	A	54.2	19.9	3 IVF/PGD-ET→a boy 46,XY

Table 2 (continued)

Patient	Translocation	Age	Semen parameters				Result	SCSA		Reproductive history
			Volume (ml)	Concentration ($\times 10^6$/ml)	Motility (%)	Normal morphology (%)		DFI (%)	HDS (%)	
Rec27	t(2;7)	30	2.0	12	8	n.a.	OA	40.7	42.0	primary infertility 30 months, 3 IVF/ PGD-2 ET sine eff.
Rec28	t(2;7)	33	4.5	29	31	16	A	37.9	19.0	1 IVF/PGD-ET sine eff.
Rec29	t(4;6)	30	3.0	34	26	6	A	13.4	53.8	2 ITP for malformations, 2 IVF/PGD-ET→a boy 46,XY
Rec30[a]	t(4;10)	30	6.5	37	35	13	A	18.3	22.5	primary infertility 18 months, 2 IVF-ET sine eff.
Rec31	t(5;15)	34	2.0	72	47	4	T	11.8	20.2	a son 46,XY,der(15)$_{pat}$, 2 IVF-ET→ITP unbalanced karyotype
Rec32[ab]	t(7;10)	25	5.7	76	50	3	T	n.a.	n.a.	
Rec33[a]	t(7;13)	29	7.5	76	55	3	T	34.3	14.9	primary infertility 6 years
Rec34	t(8;13)	29	4.0	7	30	21	OA	41.8	10.5	primary infertility 48 months, 1 IVF-ET sine eff.
Rec35	t(11;22)	28	6.2	10	34	8	OA	31.6	21.0	primary infertility 24 months, 2 IVF/ PGD-ET sine eff.
Rec36	t(13;15)	35	4.6	21	20	2	AT	29.5	20.2	
Rec37	t(16;17)	38	4.5	17	45	3	T	n.a.	n.a.	primary infertility 12 months
Mean ± SD		30.7±3.3	4.2±1.7	29.2±25.5	29.9±15.4	9.8±6.6		28.1±15.0	22.7±12.6	

Group C–reciprocal translocation, normospermic; Group D–reciprocal translocation, abnormal semen

[a] [53]; [b] [52]; [c] [51]; [d] [50]

n.a. not analysed; *N* normospermic; *O* oligospermic; *A* asthenospermic; *T* teratospermic

IVF in vitro fertilization cycle; *PGD* preimplantation genetic diagnosis; *ET* embryotransfer; *SA* spontaneous abortion; *IUI* intrauterine insemination; *ITP* induced termination of pregnancy

probes used in some patients were detected by incubation with a 1:1 mixture of avidin-FITC (Vector Laboratories, Burlingame, CA) and avidin-Cy3 (Amersham, Arlington Heights, IL, USA). In the interchromosomal effect study, α-satellite probes for chromosomes X, Y, 7, 8, and 18 (Vysis-Abbott) and locus specific probes for chromosomes 13 (Kreatech) and 21 (Vysis-Abbott) were used in combinations of two and three probes on three separate slides in each sample. The preparations were mounted using the antifade solution (Vector Laboratories, Burlingame, CA, USA) containing 0.01 μg/ml DAPI (Sigma Chemical Co., St. Louis, MO, USA). The slides were examined using an Olympus BX60 fluorescence microscope equipped with necessary fluorescent filters and phase-contrast optics. Strict scoring criteria were used [40]. Briefly, only morphologically well defined, nonoverlapping spermatozoa were scored. The sperm was considered disomic for a chromosome when two fluorescence signals of the same colour, size, and intensity separated by a distance of at least one fluorescence domain diameter were observed inside the nucleus.

Somatic aneuploidy

Five patients (Rob4, Rob8, Rec5, Rec23, Rec24) showing a high frequency of disomy for chromosomes X, Y and 8, and seven patients (Rob7, Rec1, Rec2, Rec3, Rec20, Rec29, Rec35) showing a low frequency of disomy for these chromosomes in the sperm-FISH analysis were enrolled in the somatic aneuploidy study. Slides with phytohaemagglutinin stimulated cultured lymphocytes from venous blood samples

Table 3 Results of the meiotic segregation analysis (%)

Patient	Karyotype	Probes	Adjacent	Diploid/3:0	Others	Total abnormal		
Group A								
Rob1	45,XY,der(13;14)(q10;q10)	13q14 Ls, 14q Tel	9.4	0.3	0.1	9.8		
Rob2	45,XY,der(13;14)(q10;q10)	13q14 Ls, 14q Tel	11.8	0.3	0.2	12.3		
Rob3	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	8.8	0.4	0.0	9.2		
Rob4	45,XY,der(15;22)(q11;q11)	15q Tel, 22q Tel	17.7	1.0	0.5	19.1		
Median (Q1;Q3)			10.6 (8.9;16.2)	0.4 (0.3;0.8)	0.1 (0.0;0.4)	11.1 (9.4;17.4)		
Group B								
Rob5	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	6.1	1.4	0.3	7.8		
Rob6	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	7.6	0.6	0.3	8.4		
Rob7	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	7.8	0.2	0.7	8.7		
Rob8	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	5.3	0.4	0.1	5.8		
Rob9	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	22.4	0.4	0.8	23.5		
Rob10	45,XY,der(13;14)(q10;q10)	13q14 Ls, 14q Tel	13.0	0.2	0.4	13.6		
Rob11	45,XY,der(13;14)(q10;q10)	13q Tel, 14q Tel	13.0	0.3	0.3	13.5		
Rob12	45,XY,der(13;14)(q10;q10)	13q14 Ls, 14q Tel	14.8	0.1	1.0	15.9		
Rob13	45,XY,der(14;21)(q10;q10)	14q Tel, 21q22 Ls	6.7	1.6	0.6	8.8		
Median (Q1;Q3)			7.8 (6.4;13.9)	0.4 (0.2;1.0)	0.4 (0.3;0.7)	8.8 (8.1;14.7)		
			Adjacent 1	Adjacent 2	3:1	Diploid or 4:0	Others	Total Abnormal
Group C								
Rec1	46,XY,t(1;3)(p32.1;q29)	1 cen, 1q Tel, 3 cen	34.1	8.7	9.7	0.1	0.4	52.9
Rec2	46,XY,t(1;6)(q44;p11)	1cen, 1q Tel, 6p Tel	32.6	10.1	13.2	0.0	1.6	57.5
Rec3	46,XY,t(2;7)(p13;q34)	7 cen, 7q Tel, 2p Tel	34.0	9.5	12.4	0.5	1.4	58.0
Rec4	46,XY,t(3;4)(p25.2;q25)	3cen, 3p Tel, 4q Tel	33.2	14.5	6.2	0.2	0.1	54.2
Rec5	46,XY,t(3;5)(q27;p15)	5p Tel, 5q Tel, 3cen	26.9	12.9	7.8	0.4	1.2	50.1
Rec6	46,XY,t(4;7)(q2.7;p1.4)	7cen, 7p Tel, 4q Tel	40.3	8.6	8.8	0.1	0.2	57.9
Rec7	46,XY,t(4;7)(q2.7;p1.4)	7cen, 7p Tel, 4q Tel	39.7	10.1	9.2	0.0	0.6	59.6
Rec8	46,XY,t(4;8)(q35;p11.2)	4 cen, 4q Tel, 8 cen	28.9	9.7	8.5	0.0	2.6	50.3
Rec9	46,XY,t(4;11)(q33;p11.2)	11cen, 11p Tel, 4q Tel	30.8	6.0	13.8	0.2	3.7	54.5
Rec10	46,XY,t(4;16)(p13;q21)	16cen, 16q Tel, 4p Tel	34.0	12.1	2.5	0.1	0.3	49.0
Rec11	46,XY,t(5;9)(p15.1;q22.1)	5p Tel, 5q Tel, 9q Tel	34.8	8.0	4.4	0.3	0.2	47.5
Rec12	46,XY,t(5;19)(q15;p12)	5p Tel, 5q Tel, 19p Tel	13.7	14.6	19.4	0.0	2.0	50.0
Rec13	46,XY,t(6;7)(q25;q34)	7cen, 7q Tel, 6cen	40.2	5.9	3.3	0.3	0.4	50.0
Rec14	46,XY,t(6;14)(q24.2;q24.2)	6cen, 6q Tel,14q Tel	19.8	27.6	14.4	0.3	6.8	68.9
Rec15	46,XY,t(7;9)(p15.2;q34.1)	7cen, 7p Tel, 9cen	39.4	7.4	5.6	0.7	0.4	53.7
Rec16	46,XY,t(7;10)(p15.1;q23.2)	10cen, 10q Tel, 7cen	21.9	9.5	12.5	0.0	2.5	46.8

Table 3 (continued)

Patient	Karyotype	Probes	Adjacent 1	Adjacent 2	3:1	Diploid or 4:0	Others	Total Abnormal
Rec17	46,XY,t(7;10)(q34;q24)	7cen, 7q Tel, 10cen	31.4	6.5	7.1	0.3	4.0	49.4
Rec18	46,XY,t(7;12)(p13;q15)	7cen, 7p Tel, 12q Tel	12.3	14.3	31.8	0.1	0.9	59.3
Rec19	46,XY,t(10;15)(q25.2;q12)	10cen, 10q Tel, 15q Tel	28.1	16.3	9.9	1.1	4.0	59.2
Rec20	46,XY,t(11;18)(q22;q21.3)	11cen, 11q Tel, 18cen	28.4	10.1	10.2	0.2	6.6	55.5
Rec21	46,XY,t(11;18)(q22;q21.3)	11cen, 11q Tel, 18cen	30.0	15.3	8.3	0.3	1.5	55.4
Rec22	46,XY,t(13;15)(q14.1;q26.3)	15cen, 15q Tel, 13q Tel	41.9	4.1	3.1	0.2	0.0	50.3
Median (Q1;Q3)			32.0 (27.8;36.0)	9.9 (7.9;14.3)	9.0 (6.0;12.7)	0.2 (0.1;0.3)	1.3 (0.3;2.8)	53.9 (50.0;57.9)
Group D								
Rec23	46,XY,t(1;2)(q321;q11.2)	1cen, 1q Tel, 2q Tel	24.6	13.5	27.4	0.1	2.8	68.4
Rec24	46,XY,t(1;7)(p32;q22)	1cen, 1p Tel, 7cen	27.9	6.0	14.4	0.2	3.0	51.5
Rec25	46,XY,t(1;13)(q42;q14)	1cen, 1q Tel, 13q Tel	33.9	6.3	10.7	1.1	0.5	52.5
Rec26	46,XY,t(1;14)(q44;q22)	1cen, 1q Tel, 14q Tel	36.8	13.6	7.3	0.2	0.7	58.7
Rec27	46,XY,t(2;7)(p13;q32)	7cen, 7q Tel, 2p Tel	26.2	9.3	21.0	3.5	3.8	63.9
Rec28	46,XY,t(2;7)(q31;q34)	7cen, 7q Tel, 2q Tel	25.0	12.3	28.5	0.1	3.3	69.2
Rec29	46,XY,t(4;6)(q33;q27)	4cen, 4q Tel, 6cen	29.6	6.2	8.9	0.3	3.1	48.1
Rec30	46,XY,t(4;10)(p16.2;p11.1)	10cen, 10p Tel, 4p Tel	28.9	15.2	12.8	0.4	2.6	59.9
Rec31	46,XY,t(5;15)(q35;q26.2)	15cen, 15q Tel, 5q Tel	46.7	2.3	1.6	0.7	0.4	51.6
Rec32	46,XY,t(7;10)(q36;q24.3)	7cen, 7q Tel, 10 cen	36.0	10.0	5.4	0.2	0.3	51.9
Rec33	46,XY,t(7;13)(q11.22;q21.3)	7cen, 7q Tel, 13q Tel	20.3	17.2	28.8	0.5	1.3	68.0
Rec34	46,X,Y,t(8;13)(q22;q22)	8cen, 8q Tel, 13q Tel	35.0	8.6	10.1	0.1	0.7	54.5
Rec35	46,XY,t(11;22)(q25;q13.1)	11cen,11q Tel, 22q Tel	26.6	24.6	8.8	0.8	1.4	62.3
Rec36	46,XY,t(13;15)(q32;p11.2)	13q14 Ls, 13q Tel, 15cen	41.8	0.6	1.9	0.2	0.1	44.6
Rec37	46,XY,t(16;17)(p13.13;q11.2)	16cen, 16p Tel, 17q Tel	25.6	16.7	2.5	0.0	0.7	45.5
Median (Q1;Q3)			28.9 (25.6;36.0)	1.0 (6.2;15.2)	10.1 (5.4;21.0)	0.2 (0.1;0.7)	1.3 (0.5;3.0)	54.5 (51.5;63.9)

Q1-first quartile; Q3-third quartile

obtained from each of the patients at the time of sperm collection for karyotype verification were used for the somatic aneuploidy analysis. FISH using the same X, Y, 8 probe mixture as in the sperm FISH was performed according to the manufacturers' instructions and the preparations were finally mounted in the antifade solution containing 0.24 μg/ml DAPI.

Statistical analysis

Statistical analysis was performed by nonparametric Mann–Whitney exact tests, Pearson bivariate correlation and one-sample *t*-test for the ICE study using the SPSS software package, version 18 for Windows (SPSS, Inc. Chicago, IL, USA). The results were considered statistically significant when $P<0.05$.

Results

Semen analysis

Semen characteristics of the study participants are summarized in Tables 1 and 2. The translocation carriers were divided into four groups (Group A–D) according to their translocation type and results of the semen analysis. The concentration at least 15 mil/ml, more than 40 % of motile sperm and more than 4 % of morphologically normal sperm were considered normal. Normal semen parameters were observed in 30.8 % of the Robertsonian and 59.5 % of the reciprocal translocation carriers. Asthenozoospermia was the most common abnormality (32 % of the translocation carriers). The control donors were normospermic.

SCSA

At least 5,000 sperm cells were measured by SCSA in each sample. Results are summarized in Tables 1 and 2. There were significant differences in the percentage of HDS cells between normospermic translocation carriers and those showing abnormal semen parameters in both Robertsonian ($P=0.024$) and reciprocal ($P=0.001$) translocation carriers. When compared with control donors, significantly increased DFI and percentage of HDS cells were detected in patients with abnormal semen parameters (Group B, DFI and HDS both $P<0.001$; Group D, DFI: $P=0.003$, HDS: $P<0.001$) but not in the normospermic translocation carriers (Groups A and C). Still, the 30 % DFI threshold value for normal fertility [14, 49] was exceeded in 28 % of the normospermic translocation carriers and in 63.2 % of the carriers with abnormal semen parameters. Concerning the percentage of HDS cells, the 15 % threshold value [49] was exceeded in 12 % of the normospermic carriers and in 84.2 % of the carriers with abnormal semen parameters.

Meiotic segregation of translocations

At least 3000 spermatozoa were scored from each Robertsonian translocation carrier (range 3001–3058) and the frequencies of chromosomally unbalanced gametes ranged from 5.8 % to 23.5 %. Concerning reciprocal translocation carriers ~1,000 spermatozoa (range 887–1111) were scored and the unbalanced gametes were observed in frequencies from 40.1 % to 69.2 %. Results are summarized in Table 3. Most of the gametes resulted from alternate segregation. Unbalanced gametes in reciprocal translocation carriers were most frequently formed through adjacent 1 segregation, while the adjacent 2 and 3:1 segregation modes were less abundant and comparably frequent. Other segregants were rare. The frequencies of chromosomally unbalanced gametes were not significantly different in normospermic translocation carriers and those showing abnormal semen parameters.

Interchromosomal effect

At least 10,000 spermatozoa (range 10,002 to 10,511) were scored from each of the men by sperm-FISH for each probe combination in the interchromosomal effect (ICE) study. The results are summarized in Table 4. Only disomic and diploid sperm were considered for the statistical evaluation as it is generally accepted in sperm aneuploidy studies [46]. The frequencies of diploidy observed in the ICE study corresponded to the diploidy/3:0 and diploidy/4:0 rates in the meiotic segregation study.

Concerning the interchromosomal effect, 62.0 % (31/50) of all translocation carriers (61.5 % of Robertsonian and 62.2 % of reciprocal translocation carriers) showed significantly increased frequencies of total disomic and diploid gametes compared with the control group. More men showing increased disomy and diploidy frequencies were among patients with abnormal semen parameters (18/23 vs. 13/27 in normospermic patients; see Table 4).

The normospermic translocation carriers did not show any increase in sperm disomy and diploidy compared with the control donors with the exception of a higher level of disomy 13 ($P=0.019$) in normospermic reciprocal translocation carriers (Group C). There were no significant differences between Groups A and B of Robertsonian translocation carriers. However, significantly higher frequency of disomy 18 ($P=0.001$) and 21 ($P=0.034$) was detected in Group B with abnormal semen parameters than in the control donors. Concerning reciprocal translocation carriers, patients with abnormal semen parameters (Group D) showed significantly higher frequencies of disomy XY, disomy 18 and 21 and

Table 4 Results of the aneuploidy study (frequencies per 10,000 sperm)

	Disomy									Diploidy
	XX	YY	XY	sex	7	8	13	18	21	
Group A										
Rob1	2.0	1.0	5.0	8.0	1.0	0.0	t	3.0	5.0	8.0
Rob2	4.9	1.0	22.8	28.7	4.0	2.0	t	4.0	23.0	21.8
Rob3	1.0	5.9	15.8	22.8	n.a.	4.0	t	3.0	21.0	21.8
Rob4*	4.9	13.7	35.3	54.0	5.0	7.8	16.9	10.9	41.7	71.6
Median (Q1;Q3)	3.5 (1.2;4.9)	3.5 (1.0;11.8)	19.3 (7.7;32.2)	25.7 (11.7;47.6)	4.0 (1.0;)	3.0 (0.5;6.9)		3.5 (3.0;9.2)	22.0 (9.0;37.0)	21.8 (11.4;59.2)
Group B										
Rob5*	5.0	2.0	9.0	16.0	n.a.	4.0	t	12.0	11.0	127.8
Rob6*	1.0	10.0	48.9	59.9	2.0	4.0	t	11.0	33.0	44.9
Rob7*	5.0	7.0	3.0	15.0	3.0	3.0	t	6.0	36.8	54.9
Rob8*	1.0	57.8	115.7	174.5	n.a.	13.0	t	19.0	34.0	162.5
Rob9	2.0	9.9	4.9	16.8	8.0	7.9	t	10.0	9.0	30.6
Rob10	2.0	13.0	11.0	25.9	n.a.	2.0	t	5.0	26.0	12.0
Rob11*	2.0	22.9	25.8	50.7	4.0	10.9	t	6.0	34.9	21.9
Rob12*	2.0	17.9	9.0	28.9	2.0	3.0	t	3.0	21.0	30.9
Rob13*	2.0	8.0	27.8	37.8	7.0	7.0	n.a.	27.0	t	96.5
Median (Q1;Q3)	20.0 (1.5;3.5)	10.0 (7.5;20.4)	11.0 (7.0;38.4)	28.9 (16.4;55.3)	3.5 (2.0;7.2)	4.0 (3.0;9.4)		10.0 (5.5;15.5)	29.5 (13.5;34.7)	44.9 (26.2;112.1)
Group C										
Rec1*	4.0	2.0	14.9	20.8	1.0	2.0	5.0	3.0	13.0	24.8
Rec2*	4.0	5.0	10.0	19.0	1.0	2.0	25.9	2.0	21.0	23.0
Rec3	4.9	7.9	19.7	32.5	t	9.8	n.a.	7.9	48.7	70.9
Rec4*	1.0	1.0	4.0	6.0	1.0	1.0	5.0	3.0	8.0	10.0
Rec5	1.0	7.0	42.9	50.9	3.0	2.0	11.9	5.0	15.0	31.9
Rec6	16.8	3.0	5.9	25.7	t	4.0	14.0	5.0	14.9	9.9
Rec7	3.0	3.0	8.0	14.0	t	0.0	1.0	2.9	4.0	12.0
Rec8*	3.0	4.0	2.0	9.0	1.0	t	6.9	1.0	14.0	17.0
Rec9	1.0	11.8	29.6	42.4	3.0	7.9	8.0	6.0	11.9	40.5
Rec10	0.0	0.0	5.0	5.0	0.0	0.0	3.0	4.0	6.0	21.0
Rec11	1.0	7.0	6.0	13.9	2.0	4.0	10.9	2.0	13.9	7.0
Rec12	3.0	6.0	4.0	12.9	1.0	4.0	6.0	2.0	11.0	13.9
Rec13	2.0	0.0	4.0	6.0	t	1.0	4.0	10.0	4.0	15.0
Rec14*	2.0	6.0	6.9	14.9	1.0	0.0	4.0	9.0	21.0	24.8
Rec15*	4.0	7.0	19.0	29.9	t	11.0	10.0	4.0	13.0	45.9
Rec16*	6.0	8.0	7.0	20.9	t	7.0	12.0	8.7	33.9	29.8

Table 4 (continued)

	Disomy									Diploidy
	XX	YY	XY	sex	7	8	13	18	21	
Rec17*	1.0	11.8	25.6	38.4	t	5.9	7.9	1.0	21.8	19.7
Rec18*	2.0	8.0	22.0	31.9	t	3.0	8.9	1.0	13.9	29.9
Rec19*	1.0	3.0	17.0	21.0	4.0	6.0	7.0	8.9	31.6	24.0
Rec20	2.0	20.7	47.4	70.1	6.0	7.9	17.9	t	17.9	58.2
Rec21*	1.0	11.6	11.6	24.2	2.0	1.0	4.0	t	16.8	28.1
Rec22*	1.0	5.9	21.7	28.6	3.0	2.0	t	1.0	13.9	59.3
Median (Q1;Q3)	2.0 (1.0;4.0)	6.0 (3.0;8.0)	10.8 (5.7;21.8)	20.9 (13.7;32.1)	1.5 (1.0;3.0)	3.0 (1.0;6.5)	7.4 (4.2;11.7)	3.5 (2.0;7.5)	14.0 (11.7;14.0)	24.4 (14.7;34.1)
Group D										
Rec23	6.8	33.2	45.0	85.1	5.0	15.6	7.0	3.9	25.6	34.2
Rec24*	4.9	9.9	50.3	65.1	t	8.9	n.a.	4.9	44.4	73.0
Rec25*	2.0	4.0	19.9	25.9	14.0	3.0	t	2.0	19.9	59.8
Rec26*	2.9	6.7	19.0	28.5	n.a.	14.3	n.a.	13.0	22.9	59.0
Rec27*	5.0	5.0	22.0	31.9	t	28.0	7.0	20.0	25.0	471.2
Rec28*	5.0	5.9	8.9	19.8	t	5.0	0.0	5.0	15.9	22.8
Rec29	1.0	5.0	14.0	20.0	6.0	4.0	7.0	3.0	19.9	35.9
Rec30*	7.0	0.0	19.9	26.9	2.0	2.0	8.0	7.9	26.8	69.8
Rec31*	4.0	6.0	14.0	24.0	2.0	0.0	8.0	6.0	28.9	47.0
Rec32*	3.0	24.0	10.0	36.9	t	1.0	8.0	4.0	8.0	12.0
Rec33	2.0	24.6	46.3	72.9	t	4.9	t	8.9	46.7	52.2
Rec34*	2.0	7.0	35.9	44.9	11.0	t	t	8.0	22.0	26.9
Rec35*	2.0	11.0	7.0	20.0	1.0	6.0	5.0	14.0	11.0	58.0
Rec36*	5.0	8.9	26.8	40.6	2.0	8.9	t	10.0	22.9	71.4
Rec37	1.0	7.9	9.9	18.8	0.0	2.0	5.0	6.0	18.0	20.8
Median (Q1;Q3)	3.0 (2.0;5.0)	7.0 (5.0;11.0)	19.9 (10.0;35.9)	28.5 (20.0;44.9)	2.0 (1.5;8.5)	4.9 (2.0;10.3)	7.0 (5.0;8.0)	6.0 (4.0;10.0)	22.9 (18.0;26.8)	52.2 (26.9;69.8)
Controls Median (Q1;Q3)	3.0 (1.7;5.9)	5.4 (3.7;7.9)	8.9 (4.4;11.7)	17.2 (15.6;22.1)	3.0 (1.7;3.0)	4.5 (2.7;6.5)	4.0 (2.0;6.4)	4.0 (3.0;4.9)	12.9 (8.9;21.0)	26.6 (21.6;33.8)

t chromosome involved in translocation; *n.a.* not analysed

*significant ($P<0.05$) increase in the frequency of total aneuploidy and diploidy compared to controls

diploidy than normospermic carriers (P=0.042, P=0.021, P=0.011 and P=0.006) and control donors (P=0.004, P=0.01, P=0.023 and P=0.023).

Somatic aneuploidy

At least 5000 interphase lymphocytes per patient were scored in two groups of patients differing significantly (P=0.003) in disomy frequency of chromosomes X, Y and 8 in sperm. The results are summarized in Fig. 1. No significant differences in the frequency of somatic aneuploidy were observed between the two groups. The aneuploidy rates were significantly higher (P=0.008) in spermatozoa than in lymphocytes in the group of patients with high sperm aneuploidy frequencies.

Correlations

Correlation and regression analyses, including available data from conventional semen analysis, SCSA, meiotic segregation and interchromosomal effect studies, were carried out. The frequency of motile sperm was found to be correlated with sperm concentration, total autosomal and sex disomies, diploidy and the frequency of HDS cells. The frequency of HDS cells was further correlated with DFI and diploidy. The results are displayed in Fig. 2. No other significant correlations were detected.

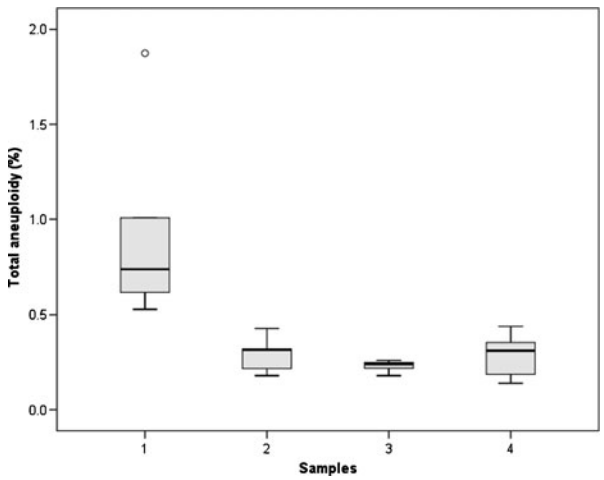

Fig. 1 Distributions of frequencies of sperm and lymfocytes aneuploid for chromosomes X, Y and 8 in the studied subgroups. The frequency of aneuploid sperm (Sample 1) and lymphocytes (Sample 2) in the group of patients showing high sperm aneuploidy was compared with the frequency of aneuploid sperm (Sample 3) and lymphocytes (Sample 4) in the group of patients with low sperm aneuploidy. The height of each box represents the 25 %–75 % data range, the *horizontal line* within each box represents the median value, and the upper and lower extensions represent the largest and smallest values. The donor Rob8 was considered a simple outlier (o), because his frequency of aneuploid sperm fell more than 1.5 box-lengths from the 25th percentile of the distribution for the ten donors

Discussion

Most of the patients included in this study were ascertained as translocation carriers while attending assisted reproduction centres for primary infertility. As the treatment by in vitro fertilization (IVF) was unsuccessful in most of them, a study combining conventional semen analysis, SCSA and sperm FISH analysis for meiotic segregation of translocations and aneuploidy was initiated to improve reproductive counselling. The value of this study lies primarily in the complex analysis of data on high numbers of sperm, obtained from a large group of patients, performed in the same laboratory by highly experienced scorers.

Conventional semen analysis is the first approach taken when searching for the cause of male infertility. In this study, normal semen parameters were found in 30.8 % of Robertsonian and 59.5 % of reciprocal translocation carriers. These rates are most likely lower than in a normal population, because semen samples from 226 donors were recently analyzed in our laboratory and normal semen parameters were observed in 82 % of them (unpublished results). In this study, all control donors were normospermic.

Among other methods of sperm analysis, SCSA provides useful data for predicting reproductive success. Virro et al. [49] reported a correlation of high DFI and increased frequency of immature (HDS) cells with a reduction of fertilization rates. Significantly higher DFI and frequency of HDS cells than in the control donors were described in translocation carriers previously studied by SCSA or TUNEL analyses [5, 34, 53]. In this study, a significant increase in DFI and HDS cells levels was detected only in Groups B and D (patients with abnormal semen characteristics). Still, the DFI and percentage of HDS cells exceeded the threshold values in 28 % and 12 %, respectively, of all normospermic translocation carriers. A correlation of the percentage of HDS cells with the frequencies of diploid cells was found in accordance with previously published papers [44, 53].

The sperm FISH method was adopted for analysis of chromosomes in sperm. The frequencies of gametes unbalanced for chromosomes involved in translocations were 5.8 %–23.5 % and 40.1 %–69.2 % in Robertsonian and reciprocal translocation carriers, respectively, which is within the range of previously published results [3, 27, 38]. The results were more heterogenous in reciprocal translocation carriers, which can be attributed to different meiotic configurations dependent on the character of individual translocations. There were no differences in the frequency of chromosomally unbalanced gametes between normospermic translocation carriers and carriers with abnormal semen parameters.

Concerning numerical aberrations of chromosomes not involved in translocations, 62.0 % of all translocation carriers

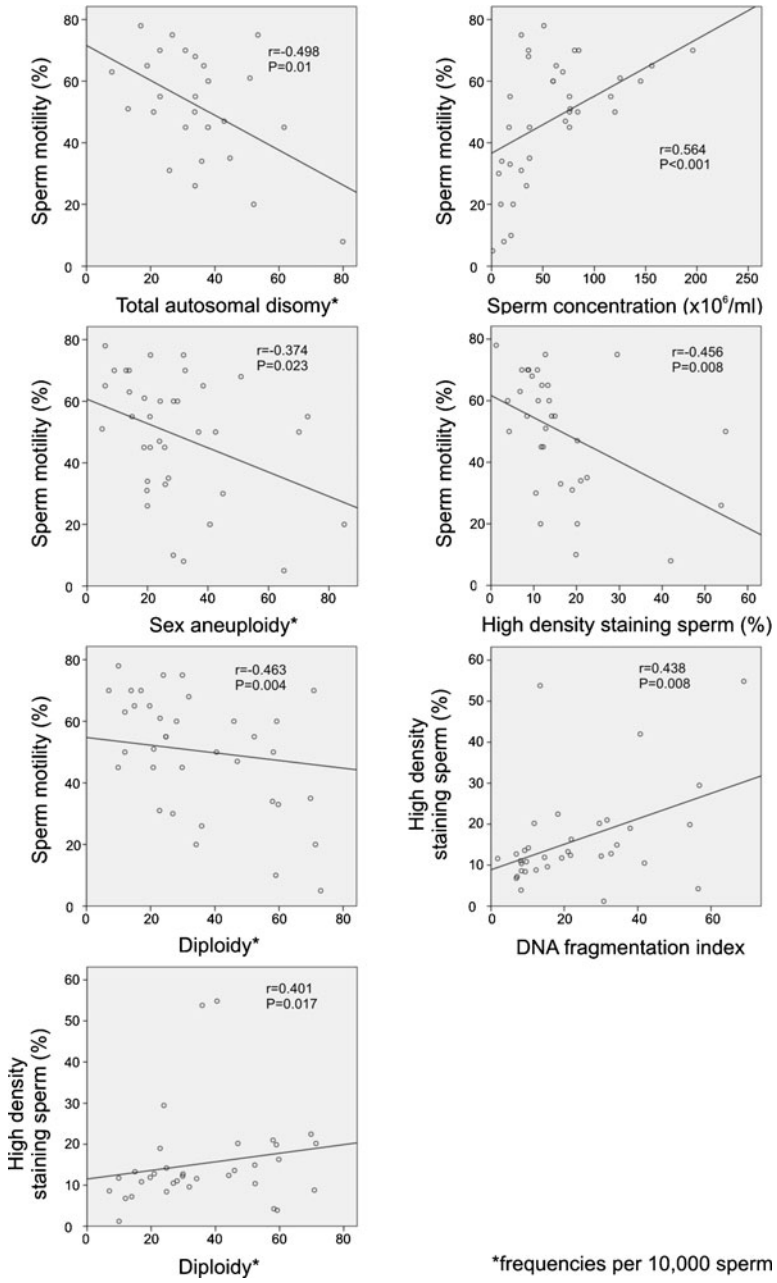

Fig. 2 Significant relationships between semen characteristics, SCSA and sperm FISH results. Two-tailed Pearson correlation coefficients and P values are displayed

showed increased frequencies of disomic and diploid gametes compared to the control group, indicating the interchromosomal effect of translocations (ICE). Anton et al. [1] reviewed previously published data on ICE and reported an increase in chromosomal disomy in about half of the 110 studied translocation carriers (54.5 % of Robertsonian and 43.9 % of reciprocal translocation carriers). The situation is complicated by the fact that individual chromosomes seem to be differentially affected and there are significant differences among individual translocation carriers. Moreover, a relationship between aneuploidy and poor semen parameters observed previously even in men with normal karyotype must not be neglected [16, 21, 33, 41, 47]. Also in this study, significantly increased frequencies of total sperm disomy and diploidy were observed more frequently among translocation carriers with abnormal semen parameters. Groups B and D of translocation carriers with abnormal semen parameters showed increased frequencies of disomy 18 and 21 compared to control donors. Additionally, frequencies of sperm showing XY disomy and diploidy were significantly higher in reciprocal translocation carriers with abnormal semen parameters than in the control group. These data, as well as the results of SCSA showed relationships between the spermatogenic outcome detectable by conventional techniques and internal sperm anomalies and indicated an increased reproductive genetic risk in translocation carriers with abnormal semen parameters.

However, the disomy of chromosome 13 was significantly increased in the group of normospermic reciprocal

translocation carriers compared to control. This implies that the presence of a balanced translocation might affect segregation of other chromosomes (ICE) in some carriers without any abnormality detectable by spermatological examinations. The phenomenon of ICE is most probably based on interactions of the asynapsed regions of chromosomes involved in trivalent and quadrivalent structures with other partially asynapsed bivalents, especially with acrocentric and sex chromosomes, which might affect their equal segregation [29, 32, 43]. A considerable interindividual variability in disomy and diploidy rates observed in our group of translocation carriers might be explained by differences in meiotic configurations [4] and unique spatial proximity of non-homologous chromosomal pairs, which was previously shown to be non-random [24] and altered in translocation carriers [55]. The meiotic disturbances, if recognized by control mechanisms, lead to meiotic arrest and production of semen with altered conventional parameters [32]. This might be the case of Robertsonian translocation carriers in our group, as only 30.8 % of them were normospermic.

Associations between germinal and somatic aneuploidy were described previously in men showing abnormal semen parameters and in normospermic men [18, 39]. The underlying role of the genomic instability, altered function of the spindle apparatus and mitotic checkpoint control, as well as the individual susceptibility to the exposure to genotoxic agents was discussed in this context [2, 10, 18, 39]. In this study, no difference in somatic aneuploidy was detected between two subgroups of patients showing a significant difference in the frequency of sperm disomy (chromosomes X, Y and 8). This assumes a different mechanism of aneuploidy formation in gametes of chromosomal translocation carriers.

From a practical point of view, it has to be mentioned that in translocation carriers, most of the sperm with chromosomal abnormalities are sperm with unbalanced karyotypes arising from meiotic segregation of translocations. Frequencies of aneuploid sperm are low despite their statistically significant increase in some patients. However, in some Robertsonian translocation carriers, total aneuploidy rates can thus reach or even exceed the frequency of gametes with unbalanced karyotype. The information about the frequency of abnormal sperm, including internal anomalies detected by sperm FISH and sperm DNA fragmentation analysis, if implemented in genetic counselling preceding assisted reproduction, helps to specify the additional reproductive risk and predict the outcome of IVF cycle [13, 20, 22, 30, 41]. The results of preimplantation genetic diagnosis for translocations and preimplantation aneuploidy screening in embryos from translocation carriers were demonstrated by Gianaroli et al. [19], Pujol et al. [35] and Fiorentino et al. [17].

In this study, a relationship between sperm motility and frequencies of spermatozoa showing disomy, diploidy and defective chromatin condensation is reported. Translocation carriers with altered semen parameters were shown to be at increased risk of forming gametes aneuploid for chromosomes not involved in the translocation. This should be considered for reproductive genetic counselling and planning of preimplantation embryo testing in such patients.

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