Sperm FISH and chromatin integrity in spermatozoa from a t(6;10;11) carrier

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Abstract

Complex chromosome rearrangements (CCRs) are structurally balanced or unbalanced aberrations involving more than two breakpoints on two or more chromosomes. CCRs can be a potential reason for genomic imbalance in gametes, which leads to a drastic reduction in fertility. In this study, the meiotic segregation pattern, aneuploidy of seven chromosomes uninvolved in the CCR and chromatin integrity were analysed in the ejaculated spermatozoa of a 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat carrier with asthenozoospermia and a lack of conception. The frequency of genetically unbalanced spermatozoa was 78.8% with a prevalence of 4:2 segregants of 38.2%, while the prevalence of the adjacent 3:3 mode was 35.3%. Analysis of the aneuploidy of chromosomes 13, 15, 18, 21, 22, X and Y revealed an approximately fivefold increased level in comparison with that of the control group, indicating the presence of an interchromosomal effect. Sperm chromatin integrity status was evaluated using chromomycin A3 and aniline blue staining (deprotamination), acridine orange test and TUNEL assay (sperm DNA fragmentation). No differences were found when comparisons were made with a control group. We suggest that the accumulation of genetically unbalanced spermatozoa, significantly increased sperm aneuploidy level and decreased sperm motility (20%, progressive) were not responsible for the observed lack of reproductive success in the analysed infertile t(6;10;11) carrier. Interestingly, in the case described herein, a high level of sperm chromosomal imbalance appears not to be linked to sperm chromatin integrity status.

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Introduction

Complex chromosome rearrangements (CCRs) are structurally balanced or unbalanced aberrations involving more than two breakpoints on two or more chromosomes, which have been described in more than 250 papers (Ergul *et al.* 2009, Madan 2012). The majority of cases involve CCRs with three (30%) or four (29%) chromosome breakpoints. It is well understood that a higher number of breakpoints is related to a higher risk of abnormal phenotype in a carrier (30–50%) or the offspring (20–90%) (Gorski *et al.* 1988, Madan *et al.* 1997, Madan 2012). Approximately 75% of CCRs are *de novo* or are maternally inherited (70% of the familial cases) (Gruchy *et al.* 2010, Pellestor *et al.* 2011a).

According to the structure of the rearrangement, CCRs can be categorised into four main types (Escudero *et al.* 2008, Gruchy *et al.* 2010, Kang *et al.* 2010, Pellestor *et al.* 2011a, Madan 2012). The first type is the simplest in structure and includes an equal number of breakpoints and of chromosomes involved in the CCR, a so-called

three-way rearrangement with three non-homologous chromosomes with one breakpoint each. This type is mostly observed among CCR cases and generally inherited from the mother. The second type, ‘exceptional complex chromosomal rearrangement’, typically originates *de novo*. Its distinctive feature is a minimum of two breakpoints on one of the chromosomes involved. An example of this type of CCR is the presence of a chromosome inversion or insertion beside the reciprocal chromosome translocation (RCT). At present, the maximum number of observed breakpoints is 15 on seven chromosomes involved. The third type, ‘double/triple two-way translocation’, is not exactly a ‘true’ CCR because it consists of two or three separate, simultaneous simple RCTs or Robertsonian translocations in one carrier. All the types listed above are CCRs with balanced genetic content and can be recognised by the microscopic observation of the karyotype. There is one more CCR type, ‘insertional translocation’ (IT), that leads to genetic imbalance (Kang *et al.* 2010). Identification of IT is possible only in array comparative genomic

hybridisation (CGH) resolution and not by microscopy. The presence of IT is strictly associated with the formation of a deletion, duplication or a gene disruption (Kang *et al.* 2010).

CCRs can be a potential cause of genomic imbalance, which can lead to drastic fertility reduction, mostly reaching the gametogenesis arrest (Lee *et al.* 2006, Ergul *et al.* 2009). Currently, ~130 of the described cases involve male CCR carriers and <20 of them are able to fertilise (Saadallah & Hulten 1985, Burns *et al.* 1986, Johannisson *et al.* 1988, Madan *et al.* 1997, Cifuentes *et al.* 1998, Cai *et al.* 2001, Grasshoff *et al.* 2003, Soler *et al.* 2005, Basinko *et al.* 2010, Loup *et al.* 2010, Ferfour *et al.* 2013, 2014). Increased infertility rates among male CCR carriers result from decreased and/or even arrested spermatogenesis, which develops due to disrupted chromosome pairing during the pachytene stage. Similar to simple RCTs, CCRs are highly connected to the meiotic multivalent formation and possible impairment of chromosome ends. Such unpaired fragments allow the conjugation of other chromosomes, not involved in CCRs, mainly X and Y (Oliver-Bonet *et al.* 2005). Diminished spermatogenesis could lead to a decrease in the sperm parameters according to the elimination of genetically unbalanced gametes. Unbalanced sperm cells result from aberrant meiotic segregation, the frequency of which increases with the number of chromosomes involved in rearrangement. Similar to RCTs, the behaviour of meiotic multivalent formation depends on the character of chromosomes involved, the size of interstitial and translocated fragments, the proximity of the centromeres to the breakpoint, and the presence and number of interstitial chiasmata. In cases of CCRs, a multivalent meiotic configuration generates a minimum of 64 different types of meiotic segregants: three-way CCR, hexavalent structure and reduced recombination (Saadallah & Hulten 1985, Johannisson *et al.* 1988, Loup *et al.* 2010, Pellestor *et al.* 2011b). To date, only seven reports examining meiotic segregation in ejaculated spermatozoa in CCR cases have been published (Loup *et al.* 2010, Pellestor *et al.* 2011b, Ferfour *et al.* 2012, 2013, 2014, Kirkpatrick & Ma 2012, Godo *et al.* 2013), two of which have evaluated the genetic content of sperm complements in the human–hamster oocyte penetration assay (Burns *et al.* 1986, Cifuentes *et al.* 1998). The percentage of unbalanced chromosome complements has been found to range from 69.4 to 88.3%. Such a high frequency indicates an increased risk of reproductive failure, such as miscarriages (50–100%) (Batista *et al.* 1994). Moreover, only 5–10% of CCRs are recognised *de novo* in prenatal diagnosis (Giardino *et al.* 2006, Escudero *et al.* 2008, Lim *et al.* 2008). In such cases, an estimated value of 3.5% per every chromosome breakpoint in CCRs has been adapted as a reliable and predictable value for the determination of the risk factor in offspring (Madan 2012).

The purpose of this study was to determine the meiotic segregation pattern in the ejaculated spermatozoa of a balanced CCR carrier 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat undescribed previously, as well as the sperm aneuploidy level of chromosomes 13, 15, 18, 21, 22, X and Y. Additionally, chromatin integrity status was evaluated by chromatin deprotonation and sperm DNA fragmentation analyses. These results will greatly contribute to the reported literature regarding infertile male carriers of CCRs with known genetic content of gametes, which may be helpful in genetic counselling.

Materials and methods

Patient

Figure 1 shows the family pedigree of the evaluated case. The samples analysed were lymphocytes and spermatozoa obtained from a 30-year-old infertile man (II:3, proband) with a 7-year period of a lack of conception and with a karyotype of 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat. The wife of the proband (II:4; 30 years old) had a normal karyotype and no reproductive problems (son III:1 born in 1994 with another partner). Both the proband and his wife were healthy, with normal levels of hormones (monitored regularly) and without any previously diagnosed diseases. In the last 3 years, the couple was subjected to IVF three times with three embryos successfully reaching only the first round of treatment. Using a morphological criterion, two embryos were selected to be transferred into the uterus. Unfortunately, there was no implantation or pregnancy. The lack of implantation most probably resulted from unbalanced chromosomal content of the embryo, but there was no preimplantation genetic diagnosis offered.

The chromosomes involved in the CCR and their breakpoints were analysed by GTG banding, a classical cytogenetic

Figure 1 Family pedigree. The proband (II:3) carried a 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat CCR and is indicated by an arrow at the partially filled square.

method. Lymphocytes and sperm samples were fixed according to a classical cytogenetic method with 3:1 ice-cold (-20°C) methanol:acetic acid fixative. Evaluation of sperm parameters indicated asthenozoospermia – decreased motility: progressive – 20%, non-progressive – 40%, and immotile – 40%, with other normal parameters: concentration 18×10^6 cells/ml, semen volume 2.5 ml, typical sperm morphology 50%, and sperm viability 60% (according to the criteria of the WHO 2010).

Control

The control group consisted of healthy male donors, 25–30 years old with normozoospermia (according to the criteria of the WHO 2010). Seven individuals were evaluated for aneuploidy and 15 individuals were evaluated for all the chromatin integrity observations. Ejaculated sperm samples were collected after 3–5 days of sexual abstinence. After liquefaction and washing with PBS (pH 7.4; Biomed, Lublin, Poland), sperm samples were fixed with a fresh fixative solution (methanol:acetic acid, 3:1 v/v, -20°C) and then stored at -20°C until further use. All men were notified of the purpose of the planned research and written consent was obtained from them, according to the guidelines of the Local Bioethical Committee, Medical University, Lviv and Poznan.

Fluorescence hybridisation *in situ*

Fluorescence hybridisation *in situ* (FISH) experiments with combinations of directly labelled probes were carried out to study the following:

- i) chromosomes from metaphase lymphocytes involved in the CCR – five-colour combination of α -satellite and subtelomeric probes: 10cen (D10Z1): red and blue (=violet); 11cen (D11Z1): red and green (=yellow); 6q (clone 57H24): red; 10q (137E24): green; and 11q (dj770G7): blue (Kreatech, Amsterdam, NL, USA); mFISH (MetaSystems, Altussheim, Germany);
- ii) meiotic segregation pattern in sperm cells – five-colour combination of α -satellite and subtelomeric probes: 10cen (D10Z1): red and blue (=violet); 11cen (D11Z1): red and green (=yellow); 6q (57H24): red; 10q (137E24): green; and 11q (dj770G7): blue (Kreatech); $n=1388$; and
- iii) aneuploidy level in sperm cells – α -satellite probes: 18cen (D18Z1): blue; Xcen (DXZ1): green; and Ycen (DYZ3): red (Cytocell, Cambridge, UK); $n=3752$; whole-chromosome painting probes: 13wcp: red and 15wcp: green (MetaSystems); $n=3491$; and band-specific probes: 21q22: green and 22q12: red (Cytocell); $n=3622$. Such colour combinations of probes allowed for the differentiation of disomy from diploidy.

At least 5000 sperm cells were assessed for aneuploidy by counting every chromosome in every control male ($n=7$). The mean counts for chromosomes 13 and 15 in the control group accounted for our laboratory control group, as described previously by Wiland (2010).

Slide preparation

Fixed sperm samples (3:1 methanol:acetic acid) were spread onto slides, washed with PBS (pH 7.4; Biomed) and incubated in a decondensation solution (10 mM dithiothreitol (Merck) and 100 mM Tris-HCl; pH 8.5, 43°C) for 5–10 min. The slides were briefly rinsed in $2 \times$ SSC (pH 7.0), air-dried and then stored in a freezer at -20°C until the FISH procedure. Fixed lymphocyte cultures were spread onto slides directly before FISH.

Hybridisation

The FISH procedure was carried out according to the manufacturer's protocol (Cytocell) incorporating modifications described elsewhere (Olszewska *et al.* 2013). For sperm FISH experiments, only slides containing non-overlapping spermatozoa with preserved nuclear shape and tail were selected. For sperm meiotic segregation pattern analysis, a hybridisation mixture containing $2.0 \mu\text{l}$ of each α -satellite and subtelomeric probe made up with a hybridisation solution to a final volume of $20 \mu\text{l}$ was used. The same probe set was used for lymphocyte FISH experiments. Additionally, mFISH (MetaSystems) was performed on lymphocyte spreads to exclude any other chromosomal rearrangement in the genome. For sperm aneuploidy evaluation, a hybridisation mixture containing $2.5 \mu\text{l}$ of each α -satellite probe with a hybridisation solution was added to a final $10 \mu\text{l}$ volume or $8.0 \mu\text{l}$ of each wcp probe. When using probes specific for chromosome 21/22 bands, $10 \mu\text{l}$ of probe solution were used.

The success of the FISH experiments was $\sim 98\%$. For analysis, a Zeiss Axiolmager D1 microscope equipped with proper filter set (DAPI/FITC/SpO/TR/Cy5/DEAC/Triple) and objectives ($20\times$ and $100\times$ oil immersion) was used. Images were acquired with a CCD camera (Jenoptik, Jena, Germany) and processed using ISIS software (MetaSystems).

Sperm chromatin integrity

Sperm chromatin deprotection

The status of chromatin maturity was evaluated using two methods and dyes described elsewhere (Kazerooni *et al.* 2009, Olszewska *et al.* 2013). Briefly, chromomycin A3 (CMA3; Sigma-Aldrich) is a fluorochrome competing with protamines for the same binding GC sites of DNA. Therefore, CMA3 fluorescence can be an indicator of spermatozoa chromatin deprotection status. Two populations of sperm cells were visible under the fluorescent microscope using a $100\times$ oil immersion objective (Olympus BX41, FITC/DAPI filters): dark (unstained) – mature with a proper ratio of protamines and light-green (stained) – deprotected/immature. For CMA3 staining, 1020 CCR carrier sperm cells were counted. The second dye, aniline blue (AB; Water Blue, Fluka, Germany), binds to the lysine residues of histones, which gives the dark blue-stained appearance to the nucleus. Three subpopulations of spermatozoa can be distinguished: pink – mature sperm cells with a proper ratio of protamines, purple-pink – mature sperm cells with disturbed protamine quantity, and dark blue – immature sperm cells with a high amount of the remaining histones (lysine rich). For the CCR carrier, 6070 spermatozoa were assessed using a light

microscope (Olympus BX41, 100× oil immersion objective). Mean control values were obtained from our laboratory control group ($n=15$; at least 1000 spermatozoa for CMA3 staining and 5000 spermatozoa for AB staining were counted).

Sperm DNA fragmentation

Sperm DNA fragmentation status was evaluated using two methods: i) TUNEL assay to estimate the real level of double-stranded (ds) and single-stranded (ss) breaks of DNA and ii) an acridine orange (AO) test to quantify ss breaks of DNA.

The TUNEL assay was carried out using the FlowTACS Apoptosis Detection Kit (R&D Systems, Minneapolis, MN, USA). The assay was carried out to identify sperm cells with fragmented DNA (presence of nicks) by creation of a complex between biotinylated DNA fragments and streptavidin-conjugated fluorescein (FITC) in the presence of terminal deoxynucleotidyl transferase (TdT). Briefly, after permeabilisation in 0.1% Triton/sodium citrate solution (15 min at room temperature), slides were washed with PBS (pH 7.4; Biomed). Then, the slides were incubated with TdT and a labelling buffer (1 h at 37 °C in the dark). Next, the slides were washed with PBS twice and air-dried. The slides were counterstained with 15 µl of DAPI. TUNEL-positive cells (with fragmented DNA) were fluorescently labelled (green colour) and visualised and counted under a fluorescent microscope with a 100× oil immersion objective (Olympus BX41, FITC/DAPI filters). For the CCR carrier, 1142 sperm cells were analysed. Mean control value was obtained from the laboratory control group ($n=15$; at least 1056 spermatozoa).

An AO test was carried on microscope slides with fixed spermatozoa according to a standard protocol (Chohan *et al.* 2004, Varghese *et al.* 2011). Briefly, dry slides were stained using a freshly prepared AO solution: 10 ml of 1% AO in distilled water added to a mixture of 40 ml 0.1 M citric acid (Sigma–Aldrich) and 2.5 ml 0.3 M $\text{Na}_2\text{HPO}_4 \times 7\text{H}_2\text{O}$ (Sigma–Aldrich; pH 2.5). After 5 min of staining (in the dark at room temperature), the slides were gently washed with distilled water and slowly dried. Next, a coverslip was applied and sealed with nail polish. The slides were immediately evaluated under a fluorescent microscope using a 60× objective (Olympus BX41, triple filter within the range of 490 and 530 nm wavelengths of excitation and barrier respectively). Observation time of each field of view did not exceed 20–25 s. Three populations of cells were observed: green – without ssDNA fragmentation and yellow or red – with ssDNA fragmentation. At least 1000 sperm cells were counted for the CCR carrier and each of the 15 control males.

Statistical analysis

Statistical significance between the mean values of the CCR carrier and those of the control group was checked from the results obtained for sperm aneuploidy level, sperm chromatin deprotonation and sperm DNA fragmentation. The χ^2 and one-sample t -test were used, with the level of significance being set at $\alpha=0.05$. $P<0.05$ indicated statistically significant differences. Microsoft Excel and GraphPad software (www.graphpad.com) were employed for statistical examinations.

Results

Partial karyotypes from GTG banding with ideograms of the chromosomes involved in the CCR are shown in Fig. 2A. A scheme of the analysed CCR with a chromosome labelling system for metaphase lymphocytes is shown in Fig. 2B. FISH results characterising chromosomes involved in the CCR, obtained from metaphase lymphocytes, are shown in Fig. 2C, D, and E.

Sperm FISH analysis

Meiotic segregation pattern

Figure 3 shows a schematic representation of the theoretically predicted hexavalent structure (pachytene stage of meiosis I) with a chromosome labelling system in the analysed carrier. Figure 4 presents the summarised results of the meiotic segregation pattern. Only 21.18% of the sperm cells were genetically normal/balanced, while frequencies of unbalanced gametes were as follows: segregation type 3:3 – 35.30%; 4:2 – 38.18%; 5:1 – 2.88%; and 6:0 – 0.14%. In total, 48 of the 64 possible segregants were observed. Figure 5 shows representative examples of different spermatozoa FISH phenotypes that occur as a result of meiotic segregation patterns.

Sperm aneuploidy

Evaluation of the disomy level of chromosomes 13, 15, 18, 21, 22, X and Y in sperm cells revealed the following rates:

- i) for the CCR carrier: 0.32% (13), 0.28% (15), 0.72% (18), 0.55% (21), 0.41% (22), 0.27% (XX), 0.88% (XY) and 0.21% (YY); and
- ii) for the control group: $0.07 \pm 0.04\%$ (13), 0.07 ± 0.05 (15), $0.09 \pm 0.05\%$ (18), $0.11 \pm 0.07\%$ (21), $0.08 \pm 0.06\%$ (22), $0.11 \pm 0.09\%$ (XX), $0.08 \pm 0.02\%$ (XY) and $0.10 \pm 0.05\%$ (YY).

The diploid rate observed in the CCR carrier spermatozoa was 0.12% (control: $0.07 \pm 0.02\%$). All disomy and diploidy frequencies were statistically significant according to the mean control values (χ^2 : $P=0.0149$; t -test: $P<0.0001$ – 0.0027 – disomy and $P=0.0131$ – diploidy). Representative examples of disomic and diploidic spermatozoa are shown in Fig. 6.

Sperm chromatin integrity

Examples of sperm cells after staining for chromatin integrity evaluation are shown in Fig. 7.

Sperm chromatin deprotonation

CMA3 staining results revealed deprotonation in 20.0% of the CCR carrier spermatozoa and AB

Figure 2 (A) Partial karyotypes after GTG banding and ideograms of chromosomes involved in a 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat CCR carrier. (B) Labelling scheme for FISH (five-colour combination of α -satellite and subtelomeric probes). (C, D, and E). Results of FISH analysis on lymphocytes at the metaphase stage: C – five-colour probe combination, 'inverted grey' mode and D – mFISH with 24-colour pattern for all chromosomes, including (E) single colour gallery for chromosomes 6, 10 and 11 involved in the CCR. Fluorescent microscopy, magnification 1000 $\times$, and software – ISIS (MetaSystems).

staining results revealed deprotonation in 16.47%. No statistical significance ($P > 0.05$) was found when compared with the mean control values (CMA3 staining – mean $21.79 \pm 7.79\%$, range: 8.57–31.85%, and AB staining – mean $16.53 \pm 7.89\%$, range: 6.2–32.3%).

Sperm DNA fragmentation

TUNEL assay results revealed sperm DNA fragmentation in 5.43% of the CCR carrier spermatozoa. No statistical significance ($P > 0.05$) was found in comparison with the mean control value of $8.41 \pm 3.39\%$ (range: 4.58–13.58%).

Figure 3 Schematic model of the suggested hexavalent configuration during the pachytene stage in meiosis I in a 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat carrier. Combination of α -satellite and subtelomeric probes used for chromosome labelling in the meiotic segregation pattern analysis.

AO test results revealed ssDNA fragmentation in 7.46% of the CCR carrier spermatozoa. No statistical significance ($P > 0.05$) was observed when compared with the mean control value of $5.91 \pm 2.52\%$ (range: 3.18–11.27%).

Discussion

In this study, we evaluated the chromosome and chromatin characteristics in the spermatozoa of a balanced three-way CCR carrier t(6;10;11) with asthenozoospermia and a lack of conception.

Historically, meiotic segregation pattern analysis in male CCR carriers was carried out nearly 30 years ago using a hamster sperm–oocyte penetration method for a double two-way translocation t(5;11),t(7;14) (Burns *et al.* 1986). It was found that 87% (19/23) of sperm karyotypes were genetically unbalanced. A similar result (86.5%) (180/208) was obtained for a three-way CCR t(2;11;22) carrier analysed using the same technique (Cifuentes *et al.* 1998). The disadvantage of the hamster sperm–oocyte penetration assay is the limited (small) number of sperm karyotypes available for examination, which can be crucial for the proper prediction of reproduction efficacy. The development of FISH techniques with the use of probes specific for particular chromosome fragments has allowed the evaluation of meiotic segregation patterns in a much higher number of spermatozoa, thus improving statistical power (Anton *et al.* 2007). To date, only seven studies have examined meiotic segregants by FISH in the ejaculated spermatozoa of CCR carriers. Only three were classical three-way CCRs: t(1;19;13) (Loup *et al.* 2010), t(5;13;14) (Pellestor *et al.* 2011b) and t(1;8;2) (Godo *et al.* 2013); two were

exceptional CCRs: (1;2;10),inv(10) (Kirkpatrick & Ma 2012) and t(3;6),inv(8) (Ferfour *et al.* 2012); and two had double two-way translocations t(8;9),t(1;16) (Ferfour *et al.* 2013) and t(Y;15),rob(13;14) (Ferfour *et al.* 2014). In all the three-way studies, the percentage of unbalanced segregants ranged from 69.4 to 88.3%. In this study, we observed unbalanced gametes at a rate of 78.82%, with 4:2 being the most common segregation type observed (38.18%), followed by adjacent 3:3 (35.30%) (Fig. 4). This was similar to previously reported data (4:2 – 38.2, 35.69, and 28.9%; adjacent 3:3 – 34.14, 31.03, and 43.1% as reported by Loup *et al.* (2010), Pellestor *et al.* (2011b) and Godo *et al.* (2013) respectively). This is also in accordance with data obtained from familial CCR cases that indicate that most of the observed live births have resulted from these two segregation modes (Batista *et al.* 1994, Lespinasse *et al.* 2003). Moreover, Madan (2012) suggested that there are some similarities between balanced CCRs and RCTs, enabling the prediction of the type of segregants produced in relevance to the ratios of the chromosome segments involved in a particular rearrangement. It has been reported that in cases of three-way CCRs, where the hexavalent configuration is being created, segregation 4:2 behaves very similarly to segregation 3:1 in simple RCTs, because the occurrence of the quadrivalent chain configuration depends primarily on chiasma numbers and asymmetry of multivalent, leading to unbalanced 4:2, 5:1 (CCR) and 3:1 (RCT) segregants (Jalbert *et al.* 1980, Batista *et al.* 1994, Madan *et al.* 1997, Faraut *et al.* 2000, Gardner & Sutherland 2004, Pellestor *et al.* 2011a, Madan 2012). In the case of the 3:3 segregation type, it has been postulated that chromosomes behave similarly to the 2:2 adjacent I mode, in which short translocated segments and quadrivalent symmetry are favourable factors for ring configuration. Madan (2012) also reviewed the present literature and reported a theoretical prediction of segregation patterns in 61 balanced CCR carriers, in both infertile males and females with recurrent abortions and no unbalanced progeny. This theoretical evaluation indicates the dominance of the segregation modes 4:2 and adjacent 3:3.

It must be noted that in meiotic segregation pattern analysis, some percentage of unexpected fluorescent signals in spermatozoa do not correspond to any of the established FISH segregation patterns. Our results indicated such a frequency at 2.31%, while three other cases of three-way CCR analyses found it to be 9.3% (Loup *et al.* 2010), 3.15% (Pellestor *et al.* 2011b) and 15.6% (Godo *et al.* 2013). Unexpected FISH phenotypes can be the consequence of three factors: i) non-disjunction at the second meiotic division, ii) increasing difficulty in FISH result interpretation with increasing number of chromosomes involved, and iii) possible chiasma occurrence, leading to the formation of additional recombinant chromosomes (Anton *et al.* 2007, Uroz & Templado 2012). In cases of three-way

Segregation type	Sperm genotype frequency (n/%)	Sum (n)	Sum (%)
3:3	23 ; 23,-6,-10,-11,+der(6),+der(10),+der(11)	294	21.18
	23,-11,+der(10) (0/0%) ; 23,-6,-10,+der(6),+der(11) (0/0%) ; 23,-6,-11,+der(10),+der(11) (7/0.50%) ; 23,-10,+der(6) (13/0.94%) ; 23,-6,+der(11) (0/0%) ; 23,-10,-11,+der(6),+der(10) (4/0.29%) ; 23,-10,-11,+der(10),+der(11) (35/2.52%) ; 23,-6,+der(6) (34/2.45%) ; 23,-10,+der(10) (57/4.11%) ; 23,-6,-11,+der(6),+der(11) (2/0.14%) ; 23,-6,-11,+der(6),+der(10) (13/0.94%) ; 23,-10,+der(11) (6/0.43%) ; 23,-11,+der(11) (11/0.79%) ; 23,-6,-10,+der(6),+der(10) (24/1.73%) ; 23,-6,+der(10) (33/2.38%) ; 23,-10,-11,+der(6),+der(11) (112/8.07%) ; 23,-11,+der(6) (109/7.85%) ; 23,-6,-10,+der(10),+der(11) (30/2.16%)	490	35.30
4:2	24,-11,+der(10),+der(11) (0/0%) ; 22,-6,-10,+der(6) (4/0.29%) ; 24,-6,+der(10),+der(11) (0/0%) ; 22,-10,-11,+der(6) (13/0.94%) ; 24,-6,+der(6),+der(11) (0/0%) ; 22,-10,-11,+der(10) (0/0%) ; 24,-10,+der(6),+der(11) (2/0.14%) ; 22,-6,-11,+der(10) (0/0%) ; 24,-10,+der(6),+der(10) (15/1.08%) ; 22,-6,-11,+der(11) (2/0.14%) ; 24,-11,+der(6),+der(10) (10/0.72%) ; 22,-6,-10,+der(11) (0/0%) ; 24,-6,+der(6),+der(10) (8/0.58%) ; 22,-10,-11,+der(11) (8/0.58%) ; 24,-11,+der(6),+der(11) (39/2.81%) ; 22,-6,-10,+der(10) (78/5.62%) ; 24,-10,+der(10),+der(11) (13/0.94%) ; 22,-6,-11,+der(6) (20/1.44%) ; 24,-6,-10,+der(6),+der(10),+der(11) (10/0.72%) ; 22,-11 (24/1.73%) ; 24,-6,-11,+der(6),+der(10),+der(11) (3/0.22%) ; 22,-10 (37/2.74%) ; 24,+der(6) (44/3.17%) ; 22,-6,-10,-11,+der(10),+der(11) (36/2.66%) ; 24,+der(11) (0/0%) ; 22,-6,-10,-11,+der(6),+der(10) (12/0.86%) ; 24,-10,-11,+der(6),+der(10),+der(11) (93/6.70%) ; 22,-6 (31/2.23%) ; 24,+der(10) (8/0.16%) ; 22,-6,-10,-11,+der(6),+der(11) (20/1.44%)	530	38.18
5:1	25,-6,+der(6),+der(10),+der(11) (0/0%) ; 21,-10,-11 (2/0.14%) ; 25,-10,+der(6),+der(10),+der(11) (4/0.29%) ; 21,-6,-11 (4/0.29%) ; 25,-11,+der(6),+der(10),+der(11) (0/0%) ; 21,-6,-10 (0/0%) ; 25,+der(10),+der(11) (2/0.14%) ; 21,-6,-10,-11,+der(6) (23/1.65%) ; 25,+der(6),+der(11) (0/0%) ; 21,-6,-10,-11,+der(10) (0/0%) ; 25,+der(6),+der(10) (3/0.22%) ; 21,-6,-10,-11,+der(11) (2/0.14%)	40	2.88
6:0	26,+der(6),+der(10),+der(11) (2/0.14%) ; 20,-6,-10,-11 (0/0%) -	2	0.14
Unexpected		32	2.31
		1388	100.0

Figure 4 Meiotic segregation pattern of chromosomes in the sperm cells of the 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat CCR carrier obtained by five-colour FISH analysis. Sixty-four theoretical segregants (without recombination) are listed with the number (n) and frequency (%) of gametes observed and schematic FISH colour pattern.

CCRs, it has been documented that the hexavalent configuration has a reduced number of chiasmata, indicating that the incidence of recombination events is rare in three-way CCRs (Berend *et al.* 2002). It has been suggested that this is a consequence of the small size of chromosome fragments remaining unpaired during the pachytene stage in meiosis I (Saadallah & Hulten 1985, Johannisson *et al.* 1988). In this regard, the presence of unexpected FISH phenotypes appears to be explained primarily by the first two points listed above. However, results obtained for a case reported by Godo *et al.* (2013) (15.6%) suggest that some occurrence of

chiasmata and formation of additional recombinant chromosomes cannot be excluded.

The estimation of aneuploidy level in sperm cells of the analysed t(6;10;11) carrier indicates the presence of an interchromosomal effect (ICE). All the seven chromosomes evaluated (13, 15, 18, 21, 22, X and Y) had a significantly increased rate of disomic (2.4- to 11.0-fold, mean: 5.3) and diploid (1.7-fold) gametes compared with the chromosomes of the control group ($P < 0.05$). This result is in accordance with the previous findings of simple translocation carriers and confirmed ICE occurrence (Pellestor *et al.* 2001, Kirkpatrick *et al.* 2008).

Figure 5 Examples of sperm cell phenotypes observed after FISH analysis in a 46,XY,t(6;11)(q25.1;q24.3;q23.1)mat carrier (five-colour combination of α -satellite and subtelomeric probes). (A) Main view with marked segregant types. (B) Representative examples of segregants listed in Fig. 1. Fluorescent microscopy, magnification 1000 $\times$, and software – ISIS (MetaSystems).

It should be noted that the ICE phenomenon remains ambiguous because of its presence in not all, but in $\sim 50\%$ of simple and complex translocation carriers (Douet-Guilbert *et al.* 2005, Machev *et al.* 2005, Kirkpatrick *et al.* 2008, Moretti *et al.* 2009, Anton *et al.* 2011, Pellestor *et al.* 2011b, Ferfour *et al.* 2012, 2013, 2014, Kirkpatrick & Ma 2012, Godo *et al.* 2013, Vozdova *et al.* 2013). However, it cannot be excluded that CCR carriers are more prone to exhibit ICE because of the substantially high numbers of chromosomes involved in rearrangement, especially with regard to the ongoing debate on the influence of chromosomal rearrangements on the proper meiotic behaviour of other chromosomes. It has also been documented that

increased aneuploidy rates can be present in spermatozoa of males with decreased sperm seminological parameters (OAT – oligo-, astheno-, teratozoospermia) (Hristova *et al.* 2002, Tempest & Griffin 2004, Machev *et al.* 2005, Miharu 2005, Rives 2005, Tang *et al.* 2010). A large majority of findings indicate a well-defined link between ICE presence and oligozoospermia, while no such clear association has been observed in cases of astheno- or teratozoospermia (Tempest & Griffin 2004, Machev *et al.* 2005, Miharu 2005, Rives 2005). The fivefold increased aneuploidy level observed in our t(6;10;11) carrier is in accordance with previously reported data linking ICE with sperm quality. We observed a reduced motility at a value of 20% of

Figure 6 Examples of disomic and diploidic spermatozoa observed after FISH analysis in a 46,XY,t(6;10;11)(q25.1;q24.3;q23.1)mat carrier with (A) centromere-specific FISH probes for chromosomes 18 (blue), X (green) and Y (red); (B) whole-chromosome painting probes for chromosomes 13 (red) and 15 (green); and (C) band-specific FISH probes for chromosomes 21q22 (green) and 22q12 (red). Fluorescent microscopy, magnification 1000 $\times$, and software – ISIS (MetaSystems).

Figure 7 Representative examples of typical staining results for spermatozoa with intact and affected DNA or chromatin. (A) Chromomycin A3 (CMA3) staining: dark (only DAPI as a counterstain) – mature and light-green (deprotaminated) – immature. (B) Aniline blue (AB) staining: pink – mature (a); purple-pink (b) – semi-mature (moderate level of remaining histones); and dark blue (c) – immature (remaining histones). (C) TUNEL assay: dark (only DAPI as a counterstain) – without DNA fragmentation and light-green – with ssDNA + dsDNA fragmentation. (D) Acridine orange (AO) test: green – without ssDNA fragmentation and yellow or red – with ssDNA fragmentation. Microscopy: fluorescent (A, C, and D) and UV-VIS (B) with magnifications: 200 $\times$ (A) and 1000 $\times$ (B, C, and D) and software – ISIS (MetaSystems) (A, C, and D) and CellB (B) (Olympus).

progressively motile gametes in our CCR carrier, classifying it as asthenozoospermia. The prevalence of isolated asthenozoospermia occurrence is $\sim 19\%$ of seminological values documented in male infertility cases (Curi *et al.* 2003). Mostly, asthenozoospermia is specifically associated with oligo- and/or teratozoospermia. There are several factors influencing sperm motility, including respective gene expression, anomalies in flagellum (i.e. mitochondria) and environmental factors (Terai *et al.* 2010, Moretti *et al.* 2011, Pelliccione *et al.* 2011, Visser *et al.* 2011, Ferramosca *et al.* 2012). Thus, it cannot be ruled out that some of these genes were interrupted or mutated and their expression change constituted an indirect factor that decreased the motility of sperm cells. It can also be noted that normal concentrations of spermatozoa in the analysed CCR cases indicate a lack of efficient elimination of the genetically unbalanced gametes in meiotic checkpoints, which are strongly associated with breakpoint

localisation. It appears that these breakpoints have not been very efficient in the analysed CCR cases.

Interesting observations were made during sperm chromatin integrity evaluation in the t(6;10;11) carrier. All assays used, chromatin deprotamination and sperm DNA fragmentation, yielded results similar to those obtained for the control group (frequency of mature gametes in the CCR carrier 81.8%, mean control value 80.9%, $P > 0.05$; frequency of gametes with fragmented DNA in the CCR carrier 5.43%/7.46% (TUNEL/AO), mean control value 8.41%/5.91%, $P > 0.05$). Such results indicate a lack of direct linkage between sperm chromatin integrity and the presence of CCRs. Interestingly, although the percentages obtained for the CCR carrier using both methods of DNA fragmentation gave rise to rather low values and statistically insignificant differences when compared with the control values, the frequency of spermatozoa after the AO test was slightly higher than that obtained after the TUNEL assay ($AO > T$) and in opposition to the value of the control mean ($AO < T$). We can postulate that this discrepancy can be explained by the following: i) the specificity of measurement technique used for ssDNA and dsDNA fragmentation (TUNEL) and of the ability of induction of ssDNA breaks in the acidic conditions of the AO test and/or ii) unclear role/influence of the structural aberration on chromatin stability. It cannot be excluded that CCR itself may be responsible for the higher susceptibility of sperm chromatin to ssDNA induction. However, results obtained in this study did not differ from control values, so this hypothesis will remain purely speculative. It is well known that spermatozoa are lacking the endogenous mechanisms of DNA repair, so any increased status of impaired chromatin integrity may influence the quality of the zygote (García-Peiró *et al.* 2011, Varghese *et al.* 2011). It has been established previously that the fertilisation rate in IVF is low in the majority of patients with high DNA fragmentation (Virant-Klun *et al.* 2002, Kazerooni *et al.* 2009). It has also been shown in such cases that not only pregnancy termination occurs frequently but also more embryos are of 'not transferable' quality (Virant-Klun *et al.* 2002). The influence of DNA fragmentation observed in the spermatozoa of the analysed CCR carrier on his fertility status cannot be entirely excluded, even if this parameter was of relatively small gravity. Taken together with other factors, such as the high proportion of chromosomally unbalanced sperm and their decreased motility, we can suppose that DNA fragmentation contributed to the issue at some level. We can suppose that an accumulation of all disturbances in the evaluated CCR carrier may play a more important role than the single values of each parameter alone. Moreover, this appears to be supported by the fact that only two embryos with good morphology (quality) have been obtained for the IVF of this couple.

It must also be noted that results regarding sperm chromatin integrity obtained in this study cannot be

compared with those of other CCR cases because of the lack of such analyses elsewhere. We can compare results only with fragmentary data available from RCT carrier studies. For example, our previous data from a RCT carrier group indicated that in four of eight males analysed, the sperm chromatin maturity assay values obtained were similar to those of a control group (Olszewska *et al.* 2013). Parallel observations were reported when analysing sperm DNA fragmentation by a TUNEL assay, in which ~15–40% of the RCT carriers exhibited sperm DNA fragmentation levels similar to those of the controls (Brugnon *et al.* 2006, García-Peiró *et al.* 2011, Ferfour *et al.* 2012, Olszewska *et al.* 2013, Perrin *et al.* 2013, Vozdova *et al.* 2013). To date, sperm DNA fragmentation evaluated by the TUNEL assay has yielded a result of a threefold higher level only in one CCR case, when compared with that of the controls (Ferfour *et al.* 2012). All results obtained thus far indicate a rather heterogeneous nature of sperm chromatin integrity in carriers of chromosome structural aberrations.

On account of the rare presence of spermatozoa in ejaculates of male CCR carriers and their subfertility/sterility status, any information about the genetic content of gametes (meiotic segregation pattern and aneuploidy) appears to be particularly important for understanding the behaviour of chromosomes involved in CCRs (Pellestor *et al.* 2011a, Madan 2012). Genetic counselling may potentially be improved by assessing the empirical probability rate of the risk of abnormal offspring resulting from genetically unbalanced spermatozoa. We suggest that in an analysed t(6;10;11) carrier, the coexistence of three elements observed, low frequency of genetically normal/balanced spermatozoa (21%), an approximately fivefold higher sperm aneuploidy level and decreased sperm motility, may be together responsible for the observed lack of reproductive success. This simply underlines the low probability of having a progeny in natural procreation. It should be further emphasised that the male CCR carrier described herein is clearly included in the group of carriers of structural chromosome aberrations with unaffected chromatin integrity status.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Anton E, Vidal F & Blanco J 2007 Role of sperm FISH studies in the genetic reproductive advice of structural reorganization carriers. *Human Reproduction* **22** 2088–2092. (doi:10.1093/humrep/dem152)
- Anton E, Vidal F & Blanco J 2011 Interchromosomal effect analyses by sperm FISH: incidence and distribution among reorganization carriers. *Systems Biology in Reproductive Medicine* **57** 268–278. (doi:10.3109/19396368.2011.633682)
- Basinko A, Perrin A, Nguyen HA, Morel F, Le Bris M-J, Saliou A-H, Collet M, Parent P, Benech C, Quemener S *et al.* 2010 Balanced transmission of a paternal complex chromosomal rearrangement involving chromosomes 2, 3, and 18. *American Journal of Medical Genetics* **152A** 2646–2650. (doi:10.1002/ajmg.a.32982)
- Batista DA, Pai GS & Stetten G 1994 Molecular analysis of a complex chromosomal rearrangement and a review of familial cases. *American Journal of Medical Genetics* **53** 255–263. (doi:10.1002/ajmg.1320530311)
- Berend SA, Bodamer OA, Shapira SK, Shaffer LG & Bacino CA 2002 Familial complex chromosomal rearrangement resulting in a recombinant chromosome. *American Journal of Medical Genetics* **109A** 311–317. (doi:10.1002/ajmg.10334)
- Brugnon F, Van Assche E, Verheyen G, Sion B, Boucher D, Pouly JL, Janny L, Devroey P, Liebaers I & Van Steirteghem A 2006 Study of two markers of apoptosis and meiotic segregation in ejaculated sperm of chromosomal translocation carriers patients. *Human Reproduction* **21** 685–693. (doi:10.1093/humrep/dei401)
- Burns JP, Koduru PRK, Alonso ML & Chaganti RSK 1986 Analysis of meiotic segregation in a man heterozygous for two reciprocal translocations using the hamster *in vitro* penetration system. *American Journal of Human Genetics* **38** 954–964.
- Cai T, Yu P, Tagle DA, Lu D, Chen Y & Xia J 2001 A *de novo* complex chromosomal rearrangement with a translocation 7;9 and 8q insertion in a male carrier with no infertility. *Human Reproduction* **16** 59–62. (doi:10.1093/humrep/16.1.59)
- Chohan KR, Griffin JT & Carrell DT 2004 Evaluation of chromatin integrity in human sperm using acridine orange staining with different fixatives and after cryopreservation. *Andrologia* **36** 321–326. (doi:10.1111/j.1439-0272.2004.00626.x)
- Cifuentes P, Navarro J, Miguez L, Egozcue J & Benet J 1998 Sperm segregation analysis of a complex chromosome rearrangement, 2;22;11, by whole chromosome painting. *Cytogenetics and Cell Genetics* **82** 204–209. (doi:10.1159/000015101)
- Curi SM, Ariagno JL, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi Segovia LM, Repetto HE & Blanco AM 2003 Asthenozoospermia: analysis of a large population. *Archives of Andrology* **49** 343–349. (doi:10.1080/713828220)
- Douet-Guilbert N, Bris MJ, Amice V, Marchetti C, Delobel B, Amice J, Braekeleer MD & Morel F 2005 Interchromosomal effect in sperm of males with translocations: report of 6 cases and review of the literature. *International Journal of Andrology* **28** 372–379. (doi:10.1111/j.1365-2605.2005.00571.x)
- Ergul E, Liehr T, Mrasek K & Sazci A 2009 A *de novo* complex chromosome rearrangement involving three chromosomes (2, 13, and 18) in an oligozoospermic male. *Fertility and Sterility* **92** 391.e9–391.e12. (doi:10.1016/j.fertnstert.2009.02.042)
- Escudero T, Estop A, Fischer J & Munne S 2008 Preimplantation genetic diagnosis for complex chromosome rearrangements. *American Journal of Medical Genetics* **146A** 1662–1669. (doi:10.1002/ajmg.a.32286)
- Faraut T, Mermet MA, Demongeot J & Cohen O 2000 Cooperation of selection and meiotic mechanisms in the production of imbalances in reciprocal translocations. *Cytogenetics and Cell Genetics* **88** 15–21. (doi:10.1159/000015476)
- Ferfour F, Biotrelle F, Tapia S, Gomes DM, Selva J & Vialard F 2012 Sperm FISH analysis of a 46,XY,t(3;6)(p24;p21.2), inv(8)(p11;2q21.2) double chromosomal rearrangement. *Reproductive Biomedicine Online* **24** 219–223. (doi:10.1016/j.rbmo.2011.10.009)

- Ferfour F, Boitrelle F, Clement P, Gomes DM, Selva J & Vialard F 2013 Can one translocation impact the meiotic segregation of another translocation? A sperm-FISH analysis of a 46,XY,t(1;16)(q21;p11.2), t(8;9)(q24.3;p24) patient and his 46,XY,t(8;9)(q24.3;p24) brother and cousin. *Molecular Human Reproduction* **19** 109–117. (doi:10.1093/molehr/gas048)
- Ferfour F, Boitrelle F, Clement P, Molina Gomes D, Selva J & Vialard F 2014 Sperm FISH analysis of a 44,X,der(Y),t(Y;15)(q12;q10)pat, rob (13;14)(q10;q10)mat complex chromosome rearrangement. *Andrologia*. In press. (doi:10.1111/and.12112)
- Ferramosca A, Provenzano SP, Coppola L & Zara V 2012 Mitochondrial respiratory efficiency is positively correlated with human sperm motility. *Urology* **78** 809–814. (doi:10.1016/j.urolgy.2011.12.042)
- García-Peirão A, Oliver-Bonet M, Navarro J, Abad C, Guitart M, Amengual MJ, Gosalvez J & Benet J 2011 Dynamics of sperm DNA fragmentation in patients carrying structurally rearranged chromosomes. *International Journal of Andrology* **34** e546–e553. (doi:10.1111/j.1365-2605.2011.01153.x)
- Gardner RJM & Sutherland GR 2004 Chromosome abnormalities and genetic counseling. 3rd edn, pp 163–177. New York: Oxford University Press.
- Giardino D, Corti C, Ballarati L, Finelli P, Valtorta C, Botta G, Giudici M, Grosso E & Larizza L 2006 Prenatal diagnosis of a *de novo* complex chromosome rearrangement (CCR) mediated by six breakpoints, and a review of 20 prenatally ascertained CCRs. *Prenatal Diagnosis* **26** 565–570. (doi:10.1002/pd.1460)
- Godo A, Blanco J, Vidal F, Parriego M, Boada M & Anton E 2013 Sequential FISH allows the determination of the segregation outcome and the presence of numerical anomalies in spermatozoa from a t(1;8;2)(q42;p21;p15) carrier. *Journal of Assisted Reproduction and Genetics* **30** 1115–1123. (doi:10.1007/s10815-013-0063-5)
- Gorski JL, Kistenmacher MN, Punnett HH, Zackai EH & Emanuel BS 1988 Reproductive risks for carriers of complex chromosome rearrangements: analysis of 25 families. *American Journal of Medical Genetics* **29A** 247–261. (doi:10.1002/ajmg.1320290202)
- Grasshoff U, Singer S, Liehr T, Starke H, Fode B, Schoenig M & Dufke A 2003 A complex chromosomal rearrangement with a translocation 4;10;14 in a fertile carrier: ascertainment through an offspring with partial trisomy 14q13→q21.1 and partial monosomy 4q27→q28. *Cytogenetic and Genome Research* **103** 17–23. (doi:10.1159/000076282)
- Gruchy N, Barreau M, Kessler K, Gourdier D & Leporrier N 2010 A paternally transmitted complex chromosomal rearrangement (CCR) involving chromosomes 2, 6, and 18 includes eight breakpoints and five insertional translocations (ITs) through three generations. *American Journal of Medical Genetics* **152A** 185–190. (doi:10.1002/ajmg.a.33154)
- Hristova R, Ko E, Greene C, Rademaker A, Chernos J & Martin R 2002 Chromosome abnormalities in sperm from infertile men with asthenoteratozoospermia. *Biology of Reproduction* **66** 1781–1783. (doi:10.1095/biolreprod66.6.1781)
- Jalbert P, Sele B & Jalbert H 1980 Reciprocal translocations: a way to predict the mode of imbalanced segregation by pachytene-diagram drawing. *Human Genetics* **55** 209–222. (doi:10.1007/BF00291769)
- Johannisson R, Löhns U & Passarge E 1988 Pachytene analysis in males heterozygous for a familial translocation (9;12;13)(q22;q22;q32) ascertained through a child with partial trisomy 9. *Cytogenetics and Cell Genetics* **47** 160–166. (doi:10.1159/000132537)
- Kang S-HL, Shaw C, Ou Z, Eng PA, Cooper ML, Pursley AN, Sahoo T, Bacino CA, Chinaut C, Stankiewicz P *et al.* 2010 Insertional translocation detected using FISH confirmation of array-comparative genomic hybridization (aCGH) results. *American Journal of Medical Genetics* **152A** 1111–1126. (doi:10.1002/ajmg.a.33278)
- Kazerooni T, Asadi N, Jadid L, Kazerooni M, Ghanadi A, Ghaffaripasad F, Kazerooni Y & Zolghadr J 2009 Evaluation of sperm's chromatin quality with acridine orange test, chromomycin A3 and aniline blue staining in couples with unexplained recurrent abortion. *Journal of Assisted Reproduction and Genetics* **26** 591–596. (doi:10.1007/s10815-009-9361-3)
- Kirkpatrick G & Ma S 2012 Meiotic segregation and interchromosomal effects in a rare (1:2:10) complex chromosomal rearrangement. *Journal of Assisted Reproduction and Genetics* **29** 77–81. (doi:10.1007/s10815-011-9655-0)
- Kirkpatrick G, Ferguson KA, Gao H, Tang S, Chow V, Yuen BH & Ma S 2008 A comparison of sperm aneuploidy rates between infertile men with normal and abnormal karyotypes. *Human Reproduction* **23** 1679–1683. (doi:10.1093/humrep/den126)
- Lee IW, Su MT, Hsu CC, Lin YH, Chen PY & Kuo PL 2006 Constitutional complex chromosomal rearrangements in azoospermic men – case report and literature review. *Urology* **68** 1343.e5–1343.e8. (doi:10.1016/j.urolgy.2006.09.007)
- Lespinasse J, North MO, Paravy C, Brunel MJ, Malzac P & Blouin JL 2003 A balanced complex chromosomal rearrangement (BCCR) in a family with reproductive failure. *Human Reproduction* **18** 2058–2066. (doi:10.1093/humrep/deg424)
- Lim CK, Cho JW, Kim JY, Kang IS, Shim SH & Jun JH 2008 A healthy live birth after successful preimplantation diagnosis for carriers of complex chromosome rearrangements. *Fertility and Sterility* **90** 1680–1684. (doi:10.1016/j.fertnstert.2007.08.016)
- Loup V, Bernicot I, Janssens P, Hedon B, Hamamah S, Pellestor F & Anahory T 2010 Combined FISH and PRINS sperm analysis of complex chromosome rearrangement t(1;19;13): an approach facilitating PGD. *Molecular Human Reproduction* **16** 111–116. (doi:10.1093/molehr/gap105)
- Machev N, Gosset P, Warter S, Treger M, Schillinger M & Viville S 2005 Fluorescence *in situ* hybridization sperm analysis of six translocation carriers provides evidence of an interchromosomal effect. *Fertility and Sterility* **84** 365–373. (doi:10.1016/j.fertnstert.2005.03.026)
- Madan K 2012 Balanced complex chromosome rearrangements: reproductive aspects. A review. *American Journal of Medical Genetics* **158A** 947–963. (doi:10.1002/ajmg.a.35220)
- Madan K, Nieuwint AWM & van Bever Y 1997 Recombination in a balanced complex translocation of a mother leading to a balanced reciprocal translocation in the child. Review of 60 cases of balanced complex translocations. *Human Genetics* **99** 806–815. (doi:10.1007/s004390050453)
- Miharu N 2005 Chromosome abnormalities in sperm from infertile men with normal somatic karyotypes: oligozoospermia. *Cytogenetic and Genome Research* **111** 347–351. (doi:10.1159/000086909)
- Moretti E, Pascarelli NA, Giannerini V, Geminiani M, Anichini C & Collodel G 2009 18,X,Y aneuploidies and transmission electron microscopy studies in spermatozoa from five carriers of different reciprocal translocations. *Asian Journal of Andrology* **11** 325–332. (doi:10.1038/aja.2008.31)
- Moretti E, Geminiani M, Terzuoli G, Renieri T, Pascarelli N & Collodel G 2011 Two cases of sperm immotility: a mosaic of flagellar alterations related to dysplasia of the fibrous sheath and abnormalities of head–neck attachment. *Fertility and Sterility* **95** 1787.e19–1787.e23. (doi:10.1016/j.fertnstert.2010.11.027)
- Oliver-Bonet M, Ko E & Martin RH 2005 Male infertility in reciprocal translocation carriers: the sex body affair. *Cytogenetic and Genome Research* **111** 343–346. (doi:10.1159/000086908)
- Olszewska M, Fraczek M, Huleyuk N, Czernikiewicz A, Wiland E, Boksa M, Zastavna D, Panasiuk B, Midro AT & Kurpisz M 2013 Chromatin structure analysis of spermatozoa from reciprocal chromosome translocation carriers (RCT) with known meiotic segregation patterns. *Reproductive Biology* **13** 209–220. (doi:10.1016/j.repbio.2013.06.002)
- Pellestor F, Imbert I, Andréo B & Lefort G 2001 Study of the occurrence of interchromosomal effect in spermatozoa of chromosomal rearrangement carriers by fluorescence *in-situ* hybridization and primed *in-situ* labelling techniques. *Human Reproduction* **16** 1155–1164. (doi:10.1093/humrep/16.6.1155)
- Pellestor F, Anahory T, Lefort G, Puechberty J, Liehr T, Hedon B & Sarda P 2011a Complex chromosomal rearrangements: origin and meiotic behavior. *Human Reproduction Update* **17** 479–494. (doi:10.1093/humupd/dmr010)
- Pellestor F, Puechberty J, Weise A, Lefort G, Anahory T, Liehr T & Sarda P 2011b Meiotic segregation of complex reciprocal translocations: direct analysis of the spermatozoa of a t(5;13;14) carrier. *Fertility and Sterility* **95** e17–e22. (doi:10.1016/j.fertnstert.2011.01.159)

- Pelliccione F, Micillo A, Cordeschi G, D'Angeli A, Necozone S, Gandini L, Lenzi A, Francavilla F & Francavilla S 2011 Altered ultrastructure of mitochondrial membranes is strongly associated with unexplained asthenozoospermia. *Fertility and Sterility* **95** 641–646. (doi:10.1016/j.fertnstert.2010.07.1086)
- Perrin A, Nguyen MH, Bujan L, Vialard F, Amice V, Guéganic N, Douet-Guilbert N, De Braekeleer M & Morel F 2013 DNA fragmentation is higher in spermatozoa with chromosomally unbalanced content in men with a structural chromosomal rearrangement. *Andrology* **1** 632–638. (doi:10.1111/j.2047-2927.2013.00100.x)
- Rives NMD 2005 Chromosome abnormalities in sperm from infertile men with normal somatic karyotypes: asthenozoospermia. *Cytogenetic and Genome Research* **111** 172–176. (doi:10.1159/000086911)
- Saadallah N & Hulten M 1985 A complex three breakpoint translocation involving chromosomes 2, 4, and 9 identified by meiotic investigations of a human male ascertained for subfertility. *Human Genetics* **71** 312–320. (doi:10.1007/BF00388456)
- Soler A, Sanchez A, Carrio A, Badenas C, Mila M, Margarit E & Borrell A 2005 Recombination in a male carrier of two reciprocal translocations involving chromosomes 14, 14', 15, and 21 leading to balanced and unbalanced rearrangements in offspring. *American Journal of Medical Genetics* **134A** 309–314. (doi:10.1002/ajmg.a.30640)
- Tang SS, Gao H, Zhao Y & Ma S 2010 Aneuploidy and DNA fragmentation in morphologically abnormal sperm. *International Journal of Andrology* **33** e163–e179. (doi:10.1111/j.1365-2605.2009.00982.x)
- Tempest HG & Griffin DK 2004 The relationship between male infertility and increased levels of sperm disomy. *Cytogenetic and Genome Research* **107** 83–94. (doi:10.1159/000079575)
- Terai K, Yoshida K, Yoshiike M, Ejima M & Iwamoto T 2010 Association of seminal plasma motility inhibitors/semenogelins with sperm in asthenozoospermia infertile men. *Urologia Internationalis* **85** 209–215. (doi:10.1159/000315976)
- Uroz L & Templado C 2012 Meiotic non-disjunction mechanisms in human fertile males. *Human Reproduction* **27** 1518–1524. (doi:10.1093/humrep/des051)
- Varghese AC, Fischer-Hammadeh C & Hammadeh ME 2011 Acridine orange test for assessment of human sperm DNA integrity. In *Sperm Chromatin: Biological and Clinical Applications in Male Infertility and Assisted Reproduction*, 1st edn, pp 189–199. Eds A Zini & A Agarwal. New York: Springer Science+Business Media LLC.
- Virant-Klun I, Tomazevic T & Meden-Vrtovc H 2002 Sperm single-stranded DNA, detected by acridine orange staining, reduces fertilization and quality of ICSI-derived embryos. *Journal of Assisted Reproduction and Genetics* **19** 319–328. (doi:10.1023/A:1016006509036)
- Visser L, Westerveld GH, Xie F, van Daalen SK, van der Veen F, Lombardi MP & Repping S 2011 A comprehensive gene mutation screen in men with asthenozoospermia. *Fertility and Sterility* **95** 1020–4.e1–9. (doi:10.1016/j.fertnstert.2010.11.067)
- Vozdova M, Oracova E, Kasikova K, Prinosilova P, Rybar R, Horinova V, Gaillyova R & Rubes J 2013 Balanced chromosomal translocations in men: relationships among semen parameters, chromatin integrity, sperm meiotic segregation and aneuploidy. *Journal of Assisted Reproduction and Genetics* **30** 391–4050. (doi:10.1007/s10815-012-9921-9)
- Wiland E 2010 Cytogenetic studies of spermatozoa and somatic cells of men with reproductive failure. *Medical News Suppl* **179** 44–49.
- World Health Organization 2010 Reference values and semen nomenclature. In *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 5th edn, pp 223–225. Geneva: WHO Press.

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