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The paternal effect of chromosome translocation carriers observed from meiotic segregation in embryos

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BACKGROUND: Human translocation carriers may present alterations in meiosis. Understanding the mechanism of meiotic segregation of reciprocal translocations is important for estimation of the risk of either pregnancy loss or birth defects. The objective of this work was to estimate meiotic segregation rates in preimplantation embryos from preimplantation genetic diagnosis (PGD) cycles of female and male reciprocal translocation carriers.

METHODS: In 20 cycles for 14 couples, PGD was performed on 118 day three embryos using fluorescence *in situ* hybridization (FISH) with specific probes for each translocation. The meiotic segregation modes and the effect of the paternal origin of translocated carrier were estimated.

RESULTS: Overall, the proportions of alternate segregation for normal or balanced chromosome contents in preimplantation embryos from PGD cycles in reciprocal male and female carriers were not significantly different (35.5 versus 23.8%). However, the frequencies of adjacent-1 and adjacent-2 segregation were lower in embryos from female reciprocal translocation carriers than from male carriers. For male translocations, alternate segregation was the most frequent mode. The proportion of 3:1 segregation was the most frequent in female translocations carriers.

CONCLUSIONS: We report differences in segregation modes in embryos obtained from PGD cycles according to the gender of reciprocal translocation carrier. However, these differences did not affect the proportion of balanced embryos and the take home baby rate. The analysis of the meiotic behaviour of chromosomes and the differences between the meiotic products of female and male for a chromosomal rearrangement could help predict the outcome of PGD for translocation carriers.

Key words: reciprocal translocation / preimplantation genetic diagnosis / fluorescence *in situ* hybridization / meiotic segregation

Introduction

Chromosomal translocations are among the most common genetic abnormalities in humans. Balanced translocations occur in 0.2% of the neonatal population, in 0.6% of infertile couples and in up to 9.2% of patients with recurrent abortions (Stern *et al.*, 1999).

Carriers of balanced reciprocal translocations have a high reproductive risk of conceiving chromosomally abnormal embryos as a result of chromosomal imbalances from segregation during meiosis, leading to recurrent pregnancy loss or birth of affected offspring. For male carriers of balanced reciprocal translocations, the chances of generating chromosomally abnormal sperm varies from 20 to 80%, depending on the type of translocation, the chromosomes involved in the translocation and the position of the breakpoints (Escudero *et al.*, 2003).

Couples with genetic disorders including single gene defects, sex-linked conditions or chromosome rearrangements face a reproductive risk. Preimplantation genetic diagnosis (PGD) is a diagnostic tool to avoid inheritance of genetic/chromosomal disease by transferring unaffected/balanced IVF embryos and represents an alternative to prenatal diagnosis (Verlinsky and Kuliev, 2003). IVF with PGD has been offered to carriers of balanced translocations in order to increase the chance of conceiving and decrease the probability of miscarriages and chromosomally unbalanced offspring. Indeed, PGD results for chromosomal translocation carriers are comparable with regular IVF without PGD patients and allow a very significant reduction in the frequency of early pregnancy loss (Otani *et al.*, 2006).

During meiosis I of reciprocal translocations, chromosomes involved in the rearrangement form a quadrivalent. Centromeres

from different chromosomes separate properly and migrate in alternate or adjacent-1 segregation, whereas homologous centromeres migrate in adjacent-2 segregation. Partial or complete non-disjunction of the quadrivalent leads to 3:1 or 4:0 segregation (Scriven *et al*., 1998). Out of the 32 possible gametes, only two are genetically balanced (those with alternate segregation), one having normal chromosomes and the other carrying the balanced form of the translocation.

Each reciprocal translocation has various segregation modes in meiosis and, it is therefore difficult to predict the number of normal embryos in any single PGD case. Understanding the mechanism of meiotic segregation of reciprocal translocation is important for estimation of the risk of pregnancy loss and birth defects.

The behaviour and segregation mode frequencies of these chromosome rearrangements has been studied in male carriers by meiotic analysis in testicular biopsies (Pinton *et al*., 2008), in metaphase chromosomes obtained by sperm fusion (Estop *et al*., 1995) and by using fluorescence *in situ* hybridization (FISH) of decondensed sperm (Vozdova *et al*., 2008).

Segregation modes in female translocation carriers had not been easy to collect and had been restricted to studies on fetal ovarian tissue due to the lack of direct access to female gametes (Hartshorne *et al*., 1999). PGD for chromosome rearrangements now allows the study of female gamete segregation.

The first studies of segregation modes employed post-zygotic material and formulated rules to predict unbalanced offspring (Smith and Gaha, 1990). However, the specimens used in these studies came from late stage embryos (spontaneous miscarriages) in which some selective process would have occurred showing only the most viable segregation types.

With the development of PGD for chromosome rearrangement, new data concerning segregation modes in female and male translocations carriers are emerging (Ogilvie and Scriven, 2002; Lim *et al*., 2008). The aim of this work is to increase this valuable data for understanding the behaviour of these chromosome abnormalities at meiosis. In this paper, we present clinical outcomes of PGD and evaluate the chromosomal imbalances of preimplantation embryos from reciprocal translocation carriers. We report from a large data series on segregation of reciprocal translocation in female and male carriers.

Materials and Methods

Patients

Fourteen couples with reciprocal translocations carried out 20 PGD–FISH cycles from January 2006 through July 2009. Karyotypes of each patient are present in Table I. The probes used were a combination of α-satellite and sub-telomeric probes to translocated segments (Table I). There were nine cycles from seven couples involving female carriers and 11 cycles from seven couples involved male carriers. The telomeric and centromeric probes were first tested on the fixed lymphocytes to make certain that every translocation was characterized correctly. FISH probe efficiencies were estimated on interphase nuclei and metaphase lymphocytes from each translocation carrier. The range of efficiencies for telomeric probes was 95–98% and 100% for centromeric probes. They fall within the limits set in the ESHRE PGD Consortium's guidelines of >95% (PGDIS, 2008). Informed consent was obtained from all couples before the PGD cycle started. The clinical results are summarized in Table II.

Stimulation protocol and ICSI procedure

In the previous cycle oral contraceptives were given. Ovarian stimulation was performed after down pituitary regulation using GnRH Depot agonist in 14 cycles. After pituitary desensitization was confirmed by absence of ovarian follicles bigger than 10 mm, a combined protocol, using human follicle stimulating hormone (Gonal F®; Serono, London, UK) and human menopausal gonadotrophin (HMG-Lepori®; Farma-Lepori, Barcelona, Spain) was given according the ovarian response.

In the six remaining cycles, follicular recruitment was achieved using human follicle stimulating hormone (Gonal F®; Serono, London, UK) and human menopausal gonadotrophin (HMG-Lepori®; Farma-Lepori, Barcelona, Spain), followed by a short regime of antagonist (Cetrotide® 0.25 mg/daily; Serono, London, UK), when the leading follicle raised 14 mm to prevent an LH surge.

Follicular growth was monitored by transvaginal ultrasound and plasmatic estradiol levels. Final oocyte maduration was induced with 250 μg of recombinant human chorionic gonadotrophin (Ovitrelle®; Serono, London, UK). Oocytes were aspirated 36 h after hCG administration by a transvaginal ultrasound-guided needle aspiration under sedation. Surrounding oocyte cumulus and corona radiata cells were removed by a brief exposure to 80 IU/ml of hyaluronidase (Hyase®; Vitrolife, Göteborg, Sweden) followed by gentle pipetting. ICSI was carried out 4 h after oocyte retrieval on a heated stage (Tokai Hit Thermoplate, Model MATS-U505R30, Japan) at 37°C, mounted on an inverted microscope (Nikon Eclipse TE200, Japan) equipped with Hoffmann modulation optics and Narishige (Narishige®, Japan) micromanipulation system. Microinjection was performed according to Van Steirteghem *et al*. (1995). Only metaphase II oocytes were injected and were then incubated individually in 30 μl droplets of G1.3™ medium (Vitrolife AB, Kungsbacka, Sweden) covered with sterile equilibrated mineral oil (Ovoil®; Vitrolife, Göteborg, Sweden) at 37°C in an atmosphere of 6% CO_2. Fertilization was assessed 16–18 h after ICSI. Further development was evaluated on the morning of Day 2 and again at Day 3, just before embryo biopsy.

Blastomere biopsy of cleavage embryos

Six-eight cells embryos were biopsied on the morning of Day 3. A non-contact, 200 mW diode laser system (Saturn, Research Instruments LTD, Cornwall, UK) coupled to an inverted microscope was used to deliver 2-4 laser pulses of 4.900 ms to the zona pellucida, creating a funnel-shaped hole. One clearly nucleated blastomere was then gently aspirated through the hole.

Blastomere spreading and FISH

Single blastomeres were collected, washed in G-MOPS (Vitrolife AB, Kungsbacka, Sweden) and transferred to hypotonic solution (0.56% KCl in 0.5 mg/ml bovine serum albumin). After few seconds of incubation in hypotonic solution, the blastomeres were transferred onto clean slides in a small drop of Carnoýs fixative. Fixative was added until cell lysis. The slides were allowed to dry completely. The probes used were a combination of α-satellite and sub-telomeric probes to translocated segments. Probe mixture containing a combination of probes was added to each slide. Target material and probes were co-denatured at 75°C for 5 min, then hybridized for a minimum of 4 h at 37°C. After hybridization, the slides were rinsed in 0.4× standard saline citrate solution and 0.3% NP-40 at 73°C for 30 s and then in 2× standard saline citrate solution and 0.1% NP-40 at room temperature for 30 s. Preparations were counterstained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI; Vysis) and visualized using an Olympus fluorescence microscope. All slides were evaluated and interpreted at least by two observers. The FISH signals were scored according to previous criteria of Hopman *et al*.

Table I Karyotypes of the patients and probes combination used to establish segregation modes.

Patient	Karyotype	FISH probes
Males		
1	46,XY,t(1;7)(q42.1;q32)	Vysis TelVysion 1q (Spectrum Orange), Cytocell CEP 7 (FITC spectrum), Vysis Telvysion 7p (Spectrum Green)
2	46,XY,t(1;12)(p32;p11.1)	Vysis TelVysion 1q (Spectrum Orange), Vysis CEP 1 (Spectrum Aqua), Vysis Telvysion 12p (Spectrum Green)
3	46,XY,t(2;3)(q14.2;q27)	Cytocell LPT 2q (FITC spectrum), Vysis CEP 3 (Spectrum Aqua), Cytocell LPT 3q (Texas Red spectrum)
4	46,XY,t(2;13)(q33;q14)	Cytocell LPT 2q (Texas Red spectrum), Cytocell CEP 2 (Texas Red spectrum), Vysis LSI 13q34 (Spectrum Green)
5	46,XY,t(3;11) (q23;q23)	Cytocell LPT 3p (Texas Red spectrum), Vysis CEP 3 (Spectrum Aqua), Cytocell LPT 11q (FITC spectrum)
6	46,XY,t(5;10)(q33.2;q26)	Cytocell LPT 5p (Texas Red spectrum), Cytocell CEP 10 (FITC Spectrum), Cytocell LPT 5q (FITC spectrum)
7	46,XY,t(7;11)(p15;q22)	Cytocell LPT 7p (Texas Red spectrum), Vysis CEP 11 (Spectrum Aqua), Cytocell LPT 11q (FITC spectrum)
Females		
1	46,XX,t(1;8)(q25;p23.2)	Vysis TelVysion 1q (SpectrumOrange), Oncor CEP 8 (Spectrum FITC), Vysis TelVysion 8p (Spectrum Green)
2	46,XX,t(1;16)(p31.2;q13)	Vysis TelVysion 1p (Spectrum Green), Vysis CEP 16 (Spectrum Orange), Vysis Telvysion 1q (Spectrum Orange)
3	46,XX,t(3;4)(q10;q10)	Cytocell LPT 3p (Texas Red spectrum), Vysis CEP 3 (Spectrum Aqua), Cytocell LPT 4q (FITC spectrum)
4	46,XX,t(3;12)(p25;p11.2)	Cytocell LPT 3p (Texas Red spectrum), Vysis CEP 3 (Spectrum Aqua), Vysis Telvysion 12p (Spectrum Green)
5	46,XX,t(4;16)(q25;q24)	Cytocell LPT 4p (Texas Red spectrum), Vysis CEP 16 (Spectrum Orange), Cytocell LPT 4q (FITC spectrum)
6	46,XX,t(5;7)(p13.3;q11.2)	Cytocell LPT 5p (Texas Red spectrum), Cytocell CEP 7 (FITC spectrum), Cytocell LPT 5q (FITC spectrum)
7	46,XX,t(5;21)(q10;q10)	Cytocell LPT 5p (Texas Red spectrum), Cytocell LPT 21q (FITC spectrum), Cytocell LPT 5q (FITC spectrum)

(1988). All slides were evaluated and interpreted by two investigators. Meiotic segregation type of gametes was estimated according to the criteria of Scriven *et al.* (1998). In order to estimate the error (4%) and mosaicism/discordant rate (12%) the abnormal embryos were reanalysed. The error rate fell within the limits set in the ESHRE PGD Consortium's guidelines (PGDIS, 2008) <10%.

Statistical analysis

Statistical analysis was performed using the Student's test and χ^2 analyses. $P < 0.05$ was considered statistically significant.

Results

Analysis of meiotic segregation mode

The meiotic segregation mode was analyzed in 118 blastomeres. Table III shows, for each translocation, the number of embryos in each segregation mode and the overall percentage allocated to each mode. Data for male and female carriers are shown separately. The allocation of embryos to alternate and adjacent-1 modes assumes either no meiotic crossing-over in the interstitial segment, or an even number or crossover events. Overall, 2:2 segregation was observed in 78 embryos (66.1%), 3:1 segregation was observed in 28 embryos (23.6%) and 4:0 and chaotic patterns were observed in 12 embryos (10.2%). The segregation mode most frequently found was alternate. In the 2:2 segregations, incidence of the alternate mode (31.4%) was higher than that of adjacent-1 (23.6%) or adjacent-2 (8.5%) modes.

Meiotic segregation modes were analyzed according to the gender of translocation carriers (Table III). The proportion of alternate segregation for normal or balanced chromosome contents in reciprocal male and female carriers was not significant (35.5 versus 23.8%, $P = 0.12$). However, the frequencies of adjacent-1 and adjacent-2 segregation were lower in embryos from female reciprocal translocation carriers PGD cycles than male carriers PGD cycles ($P < 0.05$). For male translocations, alternate segregation (35.5%) was apparently the most frequent mode. The proportion of 3:1 segregation was the most frequent mode (31.0%) in female translocations carriers.

Clinical outcomes of PGD for reciprocal translocations

In this study, outcomes of 20 cycles of PGD carries out for 14 couples carrying different reciprocal translocations were analysed. Clinical

Table II Clinical outcomes of PGD- FISH for carriers of reciprocal translocations.

	Total	Male carriers	Female carries
PGD results of translocation carriers			
Patients	14	7	7
Started cycles	20	11	9
Female age (years)	32.2 ± 3	32.1 ± 2	31.7 ± 4
Male age	34.2 ± 4.7	34.8 ± 5.2	32.9 ± 4.2
Retrieved oocytes	319	187	132
Injected oocytes	260	160	100
2-pronuclei zygotes	183	115	68
Biopsied embryos	127	84	43
Diagnosed embryos	118	76	42
Transferable embryos	37	27	10
Embryo transfer cycles	17	11	6
No. of embryos transferred (mean ± SD)	30	21(1.8 ± 1)	9(1 ± 0.9)
Positive β-hCG (per embryo transfer)	8	5	3
Biochemical pregnancies	2	1	1
Miscarriages	0	0	0
Deliveries	6	4	2

outcomes of PGD cycles are shown in Table II. A total of 319 oocyte–cumulus were retrieved and ICSI was performed on 260 metaphase II. There were 183 (70.38%) fertilized oocytes. Single blastomere biopsy was performed on 127 embryos. The embryos were successfully evaluated in 118 blastomeres (92.9%). Of these, 37 embryos (31.4% of total diagnosed embryos) were identified as normal or balanced embryos and 30 embryos were transferred in 17 cycles (85% of started cycles). Unfortunately, embryo transfer was cancelled in three cycles due to the lack of chromosomally balanced embryos. Positive β-hCG was shown in eight cycles (41.2%) and six deliveries (35.2%) were achieved.

Discussion

We describe the investigation of segregation modes in male and female translocation carriers by FISH on preimplantation embryos. As the fetal genome is not thought to become active until Day 2/3 (Braude *et al.*, 1988), it is unlikely that any selection would be operating on the cleavage stage embryos. Therefore, it can be assumed that for female carriers, the distribution of segregation patterns found at cleavage stage embryos reflects the frequency at female meiosis.

The overall incidence of unbalanced embryos was 68.6% (all patterns other than alternate segregation) and there was no difference between female and male carriers. Our present data are in accordance with previous studies, reporting similar frequencies of unbalanced embryos with respect to the gender of carrier of reciprocal translocation (Ogilvie and Scriven, 2002). According to the IX European Society of Human Reproduction and Embryology (ESHRE) PGD consortium data collection (Goossens *et al.*, 2009), about 80% of embryos from reciprocal translocation carriers are diagnosed as non-transferable and similar frequencies of balanced embryos were reported with respect to gender of carriers.

Previous studies have reported that reciprocal translocations produce similar frequencies of alternate segregation for transferable embryos without respect to the gender (Munné, 2005). However, the frequency of adjacent-2 and 3:1 segregation may be different between the genders of the carriers (Ogilvie and Scriven, 2002; Lim *et al.*, 2008). Although, the general picture of the differences between male and female meiotic processes has been known for a long time (Ogilvie and Scriven, 2002), the impact of these differences on chromosome segregation remains unclear. Analysis of the meiotic behaviour of chromosomes and the differences between the meiotic products of female and male for a chromosomal rearrangement could provide clues as to the respective roles of the mechanism in operation (Pinton *et al.*, 2005). Numerous experimental meiotic segregation studies have been carried out in males using sperm-FISH. In contrast, the limited accessibility of human oocytes has restricted the study of female meiosis. The development of PGD cycles for translocation carriers has brought new data for chromosomal segregation studies. This report contributes further information about meiotic segregation in order to understand the behaviour of meiosis in translocation carriers.

The production of unbalanced gametes was very different between the two sexes, with a higher proportion of products derived from adjacent-1 segregation in males than in females (27.6 versus 23.8%, respectively). These results match previous studies in mammals (Tease, 1998). These data may be explained by differences in the frequency and localization of crossing-over between male and female, because both parameters determine the meiotic configurations which form during prophase I, as well as the orientation of multivalents, and consequently affect the proportion of segregation patterns.

Without taking into account the gender, the adjacent-2 frequency was the lower segregation mode in 2:2 segregations (9.2% for male carriers versus7.1% for female carriers). This result matches previous studies (Lim *et al.*, 2008). The presence of an interstitial chiasma has an effect on the proportion of adjacent-2 segregation (Faraut *et al.*, 2000), because it favours the co-orientation of homologous centromeres to the opposite poles, thus limiting the probability of adjacent-2 segregation. Thus, the frequency of adjacent-2 segregation is inversely proportional to the frequency of crossing-over on interstitial segments. Localization of chiasma by immunohistochemistry in humans (Barlow and Hultén, 1998) has shown that the frequency in crossing-over is higher and their distribution is more interstitial in females than in males. Finally, the percentage of 3:1 segregation was higher on embryos from female translocation carriers (19.7% for male carriers versus 31.0% for female carriers). This proportion should be higher in females than males because of the apparent predisposition of female meiosis to produce III + I configurations in prophase I.

Understanding the mode of unbalanced segregation in male and female translocation carriers, allows the design of the best probes sets to detect balanced/normal embryos. In some cases, commercially probes are not available. Thus, knowing the segregation mode likely to produce viable unbalanced products is important to predict the fertility risk for couples carrying a reciprocal translocation rearrangement and to design tailor-made strategies.

Table III Meiotic segregation mode by gender of reciprocal translocation carrier.

Karyotype	Alternate	Adjacent-1	Adjacent-2	3:1	4:0/Others*	No. of transferred embryos /embryo transfer (mean ± SD)	Pregnancy outcome	Cycles
Males								
46,XY,t(1;7)(q42.1;q32)	9	5	2	3	1	2.3 ± 1.2	Biochemical pregnancy	3
46,XY,t(1;12)(p32;p11.1)	2	1	1	0	0	2	1 baby	1
46,XY,t(2;3)(q14.2;q27)	1	1	1	2	0	1	No pregnancy	1
46,XY,t(2;13)(q33;q14)	7	4	1	2	1	3	1 baby	1
46,XY,t(3;11) (q23;q23)	2	2	0	3	1	1 ± 0	No pregnancy	2
46,XY,t(5;10)(q33.2;q26)	4	4	0	3	2	2 ± 1.4	1 baby	2
46,XY,t(7;11)(p15;q22)	2	4	2	2	1	2	1 baby	1
Total embryos 76	27	21	7	15	6			
	35.5%	27.6%	9.2%	19.7%	7.9%	1.8 ± 1		
Females								
46,XX,t(1;8)(q25;p23.2)	1	1	1	1	0	0	–	1
46,XX,t(1;16)(p31.2;q13)	3	1	1	2	1	1.5 ± 0.7	1 baby	2
46,XX,t(3;4)(q10;q10)	0	1	0	2	1	0	–	1
46,XX,t(3;12)(p25;p11.2)	4	5	1	4	0	2 ± 0	Biochemical pregnancy	2
46,XX,t(4;16)(q25;q24)	0	0	0	0	1	0	–	1
46,XX,t(5;7)(p13.3;q11.2)	1	1	0	2	2	1	No pregnancy	1
46,XX,t(5;21)(q10;q10)	1	1	0	2	1	1	1 baby	1
Total embryos 42	10	10	3	13	6			
	23.8%	23.8%	7.1%	31.0%	14.3%	1 ± 0.9		
Total embryos 118	37	31	10	28	12			
	31.4%	26.3%	8.5%	23.6%	10.2%			

*Includes 4:0 and chaotic patterns.

In summary, the results presented here show that reciprocal translocations in male and female carriers produce similar frequencies of alternate products. For male translocations, alternate segregation was the most frequent mode. However, there is an indication that the frequency of adjacent-1, adjacent-2 and 3:1 may be different from that of females. The proportions of adjacent-1 and adjacent-2 segregation were significantly lower in embryos from female than male reciprocal translocation carriers PGD cycles. The proportion of 3:1 segregation was the most frequent mode in female translocations carriers.

In conclusion, this study is a new contribution comparing the segregation profiles of male and female translocation carriers, a topic that has only been investigated twice previously. As more PGD cycles for reciprocal translocation carriers are performed, further valuable data will emerge on the behaviour of these common chromosome abnormalities at male and female meiosis, allowing to the clinicians a more adequate prognosis. Analysis of the meiotic behaviour of chromosomes and the differences between the meiotic products of female and male for a chromosomal rearrangement could provide clues as to the respective roles of the mechanism in meiosis operation, in order to predict the outcome of PGD for translocation carriers.

References

Barlow AL, Hultén MA. Combined immunocytogenetic and molecular cytogenetic analysis of meiosis I oocytes from normal human females. *Zygote* 1998;**6**:27–38.

Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* 1988;**332**:459–461.

Escudero T, Abdelhadi I, Sandalinas M, Munné S. Predictive value of sperm fluorescence in situ hybridization analysis on the outcome of preimplantation genetic diagnosis for translocations. *Fertil Steril* 2003; **79**:1528–1534.

Estop AM, Van Kirk V, Cieply K. Segregation analysis of four translocations, t(2;18), t(3;15), t(5;7), and t(10;12), by sperm chromosome studies and a review of the literature. *Cytogenet Cell Genet* 1995;**70**:80–87.

Faraut T, Mermet MA, Demongeot J, Cohen O. Cooperation of selection and meiotic mechanisms in the production of imbalances in reciprocal translocations. *Cytogenet Cell Genet* 2000;**88**:15–21.

Goossens V, Harton G, Moutou C, Traeger-Synodinos J, Van Rij M, Harper JC. ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007. *Hum Reprod* 2009;**24**:1786–1810.

Hartshorne GM, Barlow AL, Child TJ, Barlow DH, Hultén MA. Immunocytogenetic detection of normal and abnormal oocytes in human fetal ovarian tissue in culture. *Hum Reprod* 1999;**14**:172–182.

Hopman AH, Ramaekers FC, Raap AK, Beck JL, Devilee P, van der Ploeg M, Vooijs GP. In situ hybridization as a tool to study numerical chromosome aberrations in solid bladder tumors. *Histochemistry* 1988; **89**:307–316.

Lim CK, Cho JW, Song IO, Kang IS, Yoon YD, Jun JH. Estimation of chromosomal imbalances in preimplantation embryos from preimplantation genetic diagnosis cycles of reciprocal translocations with or without acrocentric chromosomes. *Fertil Steril* 2008;**90**:2144–2151.

Munné S. Analysis of chromosome segregation during preimplantation genetic diagnosis in both male and female translocation heterozygotes. *Cytogenet Genome Res* 2005;**111**:305–309.

Ogilvie CM, Scriven PN. Meiotic outcomes in reciprocal translocation carriers ascertained in 3-day human embryos. *Eur J Hum Genet* 2002; **10**:801–806.

Otani T, Roche M, Mizuike M, Colls P, Escudero T, Munné S. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. *Reprod Biomed Online* 2006;**13**:869–874.

Pinton A, Faraut T, Yerle M, Gruand J, Pellestor F, Ducos A. Comparison of male and female meiotic segregation patterns in translocation heterozygotes: a case study in an animal model (*Sus scrofa domestica* L.). *Hum Reprod* 2005;**20**:2476–2482.

Pinton A, Raymond Letron I, Berland HM, Bonnet N, Calgaro A, Garnier-Bonnet A, Yerle M, Ducos A. Meiotic studies in an azoospermic boar carrying a Y;14 translocation. *Cytogenet Genome Res* 2008; **12**:106–111.

Preimplantation Genetic Diagnosis International Society (PGDIS). Guidelines for good practice in PGD: programme requirements and laboratory quality assurance. *RBM online* 2008;**16**:134–147.

Scriven PN, Handyside AH, Ogilvie CM. Chromosome translocations: segregation modes and strategies for preimplantation genetic diagnosis. *Prenat Diagn* 1998;**18**:1437–1449.

Smith A, Gaha TJ. Data on families of chromosome translocation carriers ascertained because of habitual spontaneous abortion. *Aust N Z J Obstet Gynaecol* 1990;**31**:57–62.

Stern C, Pertile M, Norris H, Hale L, Baker HW. Chromosome translocations in couples with in-vitro fertilization implantation failure. *Hum Reprod* 1999;**14**:2097–2101.

Tease C. Chiasma distributions and chromosome segregation in male and female translocation heterozygote mice analysed using FISH. *Chromosoma* 1998;**107**:549–558.

Van Steirteghem A, Joris H, Liu J. Protocol for intracytoplasmic sperm injection. *Hum Reprod Update* 1995;**1**: CD-ROM, item 3.

Verlinsky Y, Kuliev A. Current status of preimplantation genetic diagnosis for single gene disorders. *Reprod Biomed Online* 2003; **7**:145–150.

Vozdova M, Oracova E, Horinova V, Rubes J. Sperm fluorescence in situ hybridization study of meiotic segregation and an interchromosomal effect in carriers of t(11;18). *Hum Reprod* 2008;**23**:581–588.