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The importance of aneuploidy screening in reciprocal translocation carriers

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Abstract

The purpose of this study is to investigate the aneuploidy rate and the mosaicism of chromosomes not involved in reciprocal translocations. Aneuploidy screening (AS) (13, 16, 18, 21 and 22) was performed as a re-analysis on fixed blastomeres from 126 embryos already analysed in preimplantation genetic diagnosis (PGD) cycles of eight female and five male reciprocal translocation carriers who had not achieved a pregnancy. A successful diagnosis for AS was achieved in 91.3% of embryos; 30.9% were euploid and 60.3% were aneuploid for the five chromosomes analysed. Of the embryos, 8.7% were euploid for AS and normal-balanced for the translocation and 22.2% were euploid for AS but unbalanced for the translocation; 8% of the embryos were aneuploid for AS but normal-balanced for the translocation and 52.4% were aneuploid for AS and also unbalanced for the translocation. At least 58.7% of the embryos were mosaic regarding mosaicism for the chromosomes involved and not involved in the translocations. Six of the 16 embryos transferred in the PGD cycles were aneuploid for the AS study; four of them were also mosaics. AS should be performed in reciprocal translocation carriers after segregation analysis in PGD.
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Introduction

Reciprocal translocations are produced when there is an exchange of terminal chromosome segments between non-homologous chromosomes. These translocations are a common form of chromosomal abnormality, occurring in about 1 in every 625 newborns (Van Dyke *et al.* 1983) and they are usually phenotypically neutral because there is a balanced complement of genes. Some kinds of abnormalities can appear if the breakpoints disrupt important genes. However, due to segregation modes, a germ cell with a balanced reciprocal translocation can produce 32 types of gametes, only two of which would result in a chromosomally normal child (Scriven *et al.* 1998). For a carrier of a balanced reciprocal translocation, the frequency of the different modes of segregation depends on the specific translocation itself. For male carriers and for alternate segregation, the frequency ranges between 18.6% and 80.7% (Benet *et al.* 2005).

The production of genetically unbalanced gametes in such a high proportion leads translocation carriers to experience difficulty in achieving pregnancy, to frequently suffer spontaneous abortions and to have a high risk of delivering phenotypically abnormal offspring.

Preimplantation genetic diagnosis (PGD) has been used to select chromosomally normal and balanced embryos in carriers of chromosome reorganisations (Sermon *et al.* 2005). Different strategies have been used for this purpose. (a) The use of translocation-specific fluorescence in-situ hybridisation (FISH) probes on individual blastomeres has been consistently successful for the detection of the products of both Robertsonian (Rob) and reciprocal translocations (Conn *et al.* 1998, Munné *et al.* 1998, Pierce *et al.* 1998, Van Assche *et al.* 1999). (b) Blastomere biopsy followed by blastomere fusion with mouse zygotes, which has been described as a method for obtaining metaphase chromosomes from individual human blastomeres (Verlinsky & Evsikov 1999, Willadsen *et al.* 1999).

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This technique has been used to karyotype embryos from patients with translocations (Verlinsky *et al.* 2002). (c) Selection of morphologically normal blastocysts (Ménezo *et al.* 1997) although some studies (Verlinsky & Evsikov 1999, Evsikov *et al.* 2000) suggest that the presence of an unbalanced form of a specific chromosomal translocation does not affect the embryo's ability to reach the blastocyst stage *in vitro*. Consequently, this method can be considered as not applicable for the detection of the unbalanced status of the translocations described. (d) PGD analysing of polar bodies (PBs), which can be used when the female is the carrier of a genetic disease or of a balanced chromosomal rearrangement (Durban *et al.* 2001, Verlinsky *et al.* 2004) or for aneuploidy screening (AS) in cases of advanced maternal age or in vitro fertilization (IVF) failures (Munné *et al.* 2000, Verlinsky *et al.* 2004). This modality of PGD can be used alone or in combination with blastomere biopsy (Magli *et al.* 2004).

PGD analysing of blastomeres for aneuploidy screening (PGD-AS) is being extensively used in patients of advanced maternal age and repeated IVF or implantation failures (Gianaroli *et al.* 1999, Kahraman *et al.* 2000, Munné *et al.* 2002, 2003). In applying PGD-AS to translocation carriers after the analysis of the chromosomes implicated in the translocation, some authors have found a high degree of numerical aberrations and have pointed out the existence of an interchromosomal effect (ICE). ICE, aneuploidies affecting chromosomes not implicated in the translocation, played a role in Rob translocations, and the relevant contribution of aneuploidy exposed the couple to an additional risk of abnormal pregnancy (Gianaroli *et al.* 2002, Pujol *et al.* 2003*a*).

There is some evidence for considering chromosomal translocations as a risk factor for aneuploidy, and for this reason testing for translocations has to be considered in combination with aneuploidy analysis (Kuliev & Verlinsky 2002). Chromosome alterations related to chromosomes different from those involved in the translocation (Malmgren *et al.* 2002) could be the cause for the high incidence of arrest and poor embryo development in translocation carriers (Findikli, 2003).

Until now, only the chromosomes involved in translocations have been routinely analysed although, according to data collected by ESHRE (Harper *et al.* 2006), there is an 18.4% positive heartbeat detection per transfer in PGD in reciprocal translocations and 25.4% positive heartbeat per transfer in Rob translocations.

In the present work the aneuploidy rate of different chromosomes (13, 16, 18, 21 and 22) not involved in reciprocal translocations in female and male carriers with poor PGD outcome, i.e. without pregnancy after a PGD cycle, has been studied. Cytogenetic results have been analysed taking into account the sex of the carrier and the maternal age in order to evaluate their possible effect on the origin of aneuploidy. The degree of mosaicism detected has also been analysed.

Materials and Methods

Patients, fixation and FISH procedure

Aneuploidy screening was performed as a re-analysis of fixed blastomeres of 13 reciprocal translocation carriers (eight females and five males) selected at random (Table 1). These patients had undergone a total of 18 PGD cycles for chromosome segregation analysis in the Centre of Genetics and for Reproductive Medicine of the Dutch-Speaking Brussels Free University in 2002–2003. PGD had been performed to analyse the segregation of the chromosomes involved in each reciprocal translocation. From one to three embryos were transferred in six female carrier and three male carrier cycles, but none of the patients included in this study achieved a pregnancy. Four of the seven semen samples used in the male translocation carriers' cycles (57.1%) were abnormal according to WHO criteria (1999). In female translocation carriers' cycles, 2 out of 11 semen samples were abnormal (18.2%). Sperm morphology had not been evaluated.

During the PGD cycle, embryo biopsy was performed on day 3 using laser (1.48 μm non-contact infrared laser; Fertilase, Octax, Herbron, Germany) (De Vos *et al.* 2003). Two blastomeres were aspirated from each embryo and fixed according to Coonen's method (Coonen *et al.* 1994). The analysis had been carried out using telomeric (Tel), centromeric (CEP) and locus-specific (LSI) probes for the chromosomes involved in each specific reciprocal translocation. FISH had been performed using a procedure published elsewhere (Staessen *et al.* 1999) based on a standard protocol with an overnight incubation and separate denaturation of blastomere DNA and probes. After the PGD cycle, the slides with the hybridised blastomeres were stored at −20 °C.

Our group reported a FISH efficiency of 90–100% in the second round of FISH and between 63.6% and 91.6% in the third round of FISH in PGD cycles for the analysis of chromosome segregation and AS (Pujol *et al.* 2003*a*).

A total of 126 embryos, 76 of female and 50 of male carriers that had been analysed for the meiotic segregation have been re-analysed. An average of 7 embryos (2–16) has been analysed per cycle, 6.9 and 7.1 in the female and the male carrier groups respectively. Two blastomeres have been analysed per embryo. The mean maternal age was 33.8 years (24–41), 33.1 years (female carriers) and 34.6 years (male carriers) (Table 1).

The AS re-analysis was performed using CEP and LSI probes for the chromosomes implicated in the most frequent autosomal abnormalities (13, 16, 18, 21 and 22) (MultiVysionPB, Vysis, NY, USA). In four female and three male translocation carriers the rearrangement involved one of the chromosomes checked in the AS. The FISH procedure used was the same as in the PGD cases for translocations (Staessen *et al.* 1999).

Visualisation and analysis was made with a Zeiss microscope equipped with a high-sensitivity camera

Table 1 Female and male translocation carriers' details.

Case	Rearrangement	Female's age	Cycle	Embryos	Embryos transferred	Semenogram's results
Female carriers						
1	46, XX t(2;18) (q31;q23)	24	1	9	–	Normozoospermia
2	46, XX, t(9;21) (q11;q22)	26	1	6	1	Normozoospermia
3	46, XX,t(12;15) (q24.1;q24)	26	1	8	1	Asthenozoospermia
4	46, XX t(2;6) (p21;q25.1)	31	1	4	1	Normozoospermia
		32	2	6	2	Normozoospermia
5	46, XX t(7;14) (q11.23;q22)	35	1	11	1	Normozoospermia
6	46, XX t(3;10) (q26.2;q21.21)	35	1	4	–	Normozoospermia
		36	2	3	–	Normozoospermia
7	46, XX t(12;21) (q15;q22)	38	1	5	–	Oligoasthenozoospermia
8	46, XX, t(3;16) (p25;q22)	40	1	16	3	Normozoospermia
		41	2	4	–	
Subtotal		x=33.1	*n*=11	*n*=76, x=6.9	*n*=9, x=1.5	
Male carriers						
9	46, XY t(4;18) (q31.1;p11.2)	28	1	9	2	Oligozoospermia
10	46, XY t(10;17) (q22.1;q21.3)	33	1	2	–	Oligoasthenozoospermia
		33	2	4	–	Normozoospermia
11	46, XY t(2;13) (p16;q31)	34	1	13	–	Normozoospermia
12	46, XY t(11;12) (q12;p11.1)	35	1	3	–	Azoospermia
13	46, XY t(4;22) (q21;q13.3)	39	1	11	3	Oligoasthenozoospermia*
		40	2	8	2	Normozoospermia*
Subtotal		x=34.6	*n*=7	*n*=50, x=7.1	*n*=7, x=2.3	
Total		x=33.8	*n*=18	*n*=126	*n*=16	

Cycle 1: 1st PGD cycle; Cycle 2: 2nd PGD cycle; x= average; *n*=total number; *These semen samples were frozen.

(Roger Scientific, Photometrics, Tucson, AZ, USA), with filters for the fluorochromes used and connected to a Power Macintosh G3 computer with software for Smartcapture (Digital Scientific, Cambridge, UK).

Scoring criteria and statistical analysis

Two chromosomes were scored when the FISH signals were separated by more than the distance that the diameter of two additional signals would allow. When no diagnosis was achieved in either of the two blastomeres analysed, the corresponding embryo was diagnosed according to the results obtained in the other single blastomere. When the two analysed blastomeres of one embryo showed different FISH results, the corresponding embryo was classified as mosaic and considered to be an abnormal embryo. The results of the AS only included the abnormalities of the analysed chromosomes not involved in each translocation. The aneuploid events observed per embryo (Table 2) include all the aneuploidies identified in each one of the blastomeres. Cytogenetic results were analysed taking into account the gender of the translocation carrier and the maternal age ($\leq$37 years and $>$ 37 years) in each couple.

Either media or data distribution were compared using the Mann–Whitney U test, Fisher's exact test or Chi-squared test.

Results

PGD for chromosome segregation analysis had been performed for the mentioned 13 different reciprocal translocation carriers (eight females and five males) in order to select and transfer normal or balanced embryos. Two blastomeres of transferable and non-transferable embryos were re-analysed (Table 3) with the AS probe panel.

Embryos with chromosome complements derived from alternate segregation (tranferable embryos) were detected in all cases except for the following four translocation carriers: 46, XX, t(3;10)(q26.2;q21.21), 46, XX t(12;21)(q15;q22), 46, XY, t(10;17)(q22.1;q21.3) and 46, XY t(11;12)(q12;p11.1); the frequency of alternate

Table 2 Aneuploid events.

Rearrangement	Embryos	Aneuploid embryos	Nullisomies	Monosomies	Trisomies	Tetrasomies	Pentasomies
Female carriers							
46, XX t(2;18)(q31;q23)	9	7	5	9	4	–	–
46, XX, t(9;21)(q11;q22)	6	3	–	4	2	–	–
46, XX,t(12;15)(q24.1;q24)	8	5	4	6	1	–	–
46, XX t(2;6)(p21;q25.1)	10	5	1	8	2	–	–
46, XX t(7;14)(q11.23;q22)	11	2	1	3	1	–	–
46, XX t(3;10)(q26.2;q21.21)	7	5	10	3	–	2	–
46, XX t(12;21)(q15;q22)	5	5	9	2	1	–	–
46, XX, t(3;16)(p25;q22)	20	10	3	12	6	–	–
Subtotal	76	42	33	47	17	2	–
Male carriers							
46, XY t(4;18)(q31.1;p11.2)	9	4	–	1	5	–	–
46, XY t(10;17)(q22.1;q21.3)	6	6	5	9	2	1	–
46, XY t(2;13)(p16;q31)	13	11	5	11	6	2	1
46, XY t(11;12)(q12;p11.1)	3	3	–	1	1	1	–
46, XY t(4;22)(q21;q13.3)	19	10	12	9	1	3	–
Subtotal	50	34	22	31	15	7	1
Total	126	76	55	78	32	9	1

All aneuploidies found were counted, i.e. if an embryo had two different alterations in the two analysed blastomeres, both alterations were counted.

chromosome segregation was 16.6% (21/126) (Table 3). The 2:2 Adjacent 1 (Adj 1) and 3:1 chromosome segregations were observed with frequencies of 15% (19/126) and 16.6% (21/126) respectively being as frequent as the alternate chromosome segregation. The 2:2 Adjacent 2 (Adj 2) chromosome segregation was also observed but was less frequent (8.7%; 11/126). Only one embryo resulting from a 4:0 chromosome segregation was detected in translocation 46, XX, t(2;6)(p21;q25.1). Mosaic embryos affecting chromosomes involved in the translocation were similar to the unbalanced embryos due to chromosome segregation; 37.3% (47/126) and 41.1% (52/126) respectively. Moreover, embryos with other abnormal chromosome complements with a difficult segregation interpretation were observed in four of the carriers (Table 3). No significant differences were observed when cytogenetic results were compared between genders or between maternal age groups ($\leq$37 years old and $>$37 years old).

Euploid or aneuploid embryos were successfully identified throughout the AS analysis of the blastomeres analysed (Table 4) in a total of 91.3% (115/126) of the embryos, while in 8.7% (11/126) cell material losses or FISH negative results did not allow for a final embryo diagnosis. Of the embryos, 30.9% (39/126) were euploid for the AS-analysed chromosomes and 60.4% (76/126) were aneuploid; 8.7% (11/126) were euploid for AS and normal/balanced for the translocation shown and 22.2% (28/126) were euploid for AS but unbalanced for the translocation. Eight percent (10/126) of the embryos were aneuploid for AS but normal/balanced for the translocation and 52.4% (66/126) of the embryos were aneuploid for AS and also unbalanced for the translocation. No significant differences were observed when the cytogenetic results obtained between genders or between maternal age groups ($\leq$37 years old and $>$37 years old) were compared.

In all of the cases included in the present work, aneuploid events, detected as extra or missing chromosomes and affecting one or more of the chromosomes analysed, were observed in at least two embryos of each cycle (Table 5). The AS analysis showed aneuploid events for chromosomes 13, 16, 18, 21 and 22 (when they were not involved in translocations) a total of 35 (20.2%), 41 (23.7%), 21 (12.1%), 39 (22.5%) and 37 (21.4%) times respectively. No significant differences were observed when aneuploid events observed between genders or maternal age groups were compared. When the aneuploidies observed in normal/balanced and in unbalanced embryos were compared, significant differences were not found.

The aneuploidy rate for chromosomes 13, 16, 18, 21 and 22 (calculated for each chromosome, dividing the number of altered chromosomes into the total number of

Table 3 Chromosome segregations obtained after PGD cycles.

	Embryos (*n*)	Transferable Alternate	Non-transferable				Mosaics	Segregation not identifiable[a]
			Adj1	Adj2	3:1	4:0		
46, XX, t(2;18)(q31;q23)	9	1	2	–	–	–	4	2
46, XX, (9;21)(q11;q22)	6	1	1	–	3	–	1	–
46, XX, (12;15)(q24.1;q22)	8	1	1	1	2	–	3	–
46, XX,(2;6)(p21;q25.1)	10	3	4	–	1	1	1	–
46, XX, (7;14)(q11.23;q22)	11	1	1	3	2	–	3	1
46, XX, (3;10)(q26.2;q21.21)	7	–	–	2	1	–	4	–
46, XX, (12;21)(q15;q22)	5	–	–	1	2	–	2	–
46, XX, (3;16)(p25;q22)	20	5	–	–	2	–	11	2
Subtotal	76	12	9	7	13	1	29	5
%		15.7	11.8	9.2	17.1	1.3	38.0	6.5
46, XY, (4;18)(q31.1;p11.2)	9	2	–	–	–	–	6	1[b]
46, XY, (10;17)(q22.1;q21.3)	6	–	1	–	1	–	4	–
46, XY, (2;13)(p26;q31)	13	2	2	2	4	–	3	–
46, XY, (11;12)(q12;p11.1)	3	–	1	1	1	–	–	–
46, XY, (4;22)(q21;q13.3)	19	5	6	1	2	–	5	–
Subtotal	50	9	10	4	8	–	18	1
%		18	20	8	16	0	36	2
Total	126	21	19	11	21	1	47	6
%		16.6	15.0	8.7	16.6	0.7	37.3	4.7

[a]Embryos considered abnormal for the translocation analysis but in which the segregation could not be diagnosed. [b]Alternative segregation.

diagnosed chromosomes and multiplied by 100) was 7.48%, 8.76%, 4.49%, 8.76% and 7.91% respectively with an average of 7.48%.

Embryo mosaicism for the two chromosomes involved in the translocation and also for those included in the AS analysis (Table 6) was evaluated in 67.5% (85/126) of embryos. In the remaining 32.5% (41/126), FISH results which could be interpreted were available only for one single blastomere, thus mosaicism could not be evaluated.

We observed that at least in 34.9% (44/126) of the embryos, corresponding to 51.8% (44/85) of the embryos in which mosaicism was evaluated, a mosaicism for the chromosomes studied for AS was detected. Summarising mosaicism results, mosaicism for both translocation chromosomes and AS chromosomes was present in at least 13.5% (17/126) of embryos, corresponding to 20% (17/85) of the embryos in which mosaicism was evaluated; mosaicism for only AS chromosomes was found in at least 21.4% (27/126) of embryos, corresponding to 31.8% (27/85), mosaicism for only the translocation chromosomes was found in 7.1% (9/126) corresponding to 10.6% (9/85) and there were 16.7% (21/126) embryos mosaic for translocation and non-diagnosed for AS. Considering only the results of those embryos in which a mosaicism diagnosis for translocation and for the AS analysis was achieved, no significant differences were observed when the results referring to mosaicism were compared between genders and maternal age groups.

Analysing aneuploid events in the 76 aneuploid embryos, either mosaic or not, a total of 133 missing chromosomes (55 nullisomies and 78 monosomies) and 42 extra chromosomes (32 trisomies, 9 tetrasomies and 1 pentasomy) were found (Table 2).

In general, there were more missing chromosomes than extra chromosomes. Only in two translocations, t(4;18)(q31.1;p11.2) and t(11;12)(q12;p11.1), were there more extra chromosomes. Non-significant differences were found in the distributions of the aneuploid events among the different translocations.

From the 16 embryos which had been transferred in the PGD cycles (diagnosed as normal/balanced for the corresponding translocation), 10 were euploid for the AS study; 8 of them were non-mosaic and in 2 mosaicism could not be diagnosed. In the remaining six embryos transferred, aneuploidies for one to three of the chromosomes analysed in the AS were observed (Table 5). Four of them were also mosaic, one was non-mosaic and in the other mosaicism could not be diagnosed. This means that during the PGD cycles carried out, six aneuploid embryos had been transferred, and in at least four of them mosaicism was also present.

Discussion

In the present study, aneuploidy screening cytogenetic re-analysis of the embryos already diagnosed for PGD chromosome segregation analysis has been performed. A total of 13 different reciprocal translocation carriers are included in this work. Chromosomes 13, 16, 18, 21 and 22 were analysed by FISH in the AS study, since 82% of all abnormal embryos, including most mosaic ones, involve these chromosomes (Abdelhadi *et al.* 2003). None of the patients included in the AS study had achieved a pregnancy after the PGD cycle for translocation analysis despite a one-, two- or three-embryo transfer having been performed in some of them (Table 1).

Table 4 Summary of the PGD and AS results obtained for female and male carriers. Normal and abnormal embryos for each translocation are detailed.

Rearrangement	Cycles	Embryos	Normal	Abnormal			
			NB for t & E for AS	NB for t & A for AS	UB for t & E for AS	UB for t & A for AS	UB for t & ND for AS
46, XX t(2;18) (q31;q23)	1	9	–	1	2	6	–
46, XX, t(9;21) (q11;q22)	1	6	1	–	2	3	–
46, XX,t(12;15) (q24.1;q24)	1	8	–	1	3	4	–
46, XX t(2;6) (p21;q25.1)	2	10	1	2	3	3	1
46, XX t(7;14) (q11.23;q22)	1	11	1	–	6	2	2
46, XX t(3;10) (q26.2;q21.21)	2	7	–	–	1	5	1
46, XX t(12;21) (q15;q22)	1	5	–	–	–	5	–
46, XX, t(3;16) (p25;q22)	2	20	3	2	3	8	4
Total for female carriers	11	76	6	6	20	36	8
%			7.9	7.9	26.3	47.4	10.5
46, XY t(4;18) (q31.1;p11.2)	1	9	1	1	3	3	1
46, XY t(10;17) (q22.1;q21.3)	2	6	–	–	–	6	–
46, XY t(2;13) (p16;q31)	1	13	–	2	2	9	–
46, XY t(11;12) (q12;p11.1)	1	3	–	–	–	3	–
46, XY t(4;22) (q21;q13.3)	2	19	4	1	3	9	2
Total for male carriers	7	50	5	4	8	30	3
%			10	8	16	60	6
Total for females and males	18	126	11	10	28	66	11
%			8.7	8.0	22.2	52.4	8.7

NB: normal-balanced; UB: unbalanced; E: euploid; A: aneuploid; t: translocation-related chromosomes; AS: chromosomes analysed in aneuploidy screening, i.e. chromosomes not related to the translocation; ND: non-diagnosed chromosomes.

Chromosome segregation results, normal, balanced or unbalanced embryos, which had been identified in the PGD cycles in female and male carriers of different chromosome translocation have been summarised in the present study. Most of the embryos analysed, 105/126 (83.3% average: 84.2% for female carriers and 82% for male carriers; Table 4) had, including mosaics, unbalanced chromosome complements, due to the segregation of the chromosomes involved in the translocation that had been detected during the PGD cycles. Similar frequencies of unbalanced embryos in reciprocal translocation carriers have been published. Verlinsky *et al.* (2002) reported 72.3% of unbalanced embryos, 76.4% being in female carriers and 68.2% in male carriers. Findikli (2003) found 78.3% unbalanced embryos in female carriers and 63.2% in male carriers. According to data collected by ESHRE between 1997 and 2001 (Harper *et al.* 2006), 615 PGD cycles of reciprocal translocation carriers were made (308 in female carriers and 307 in male carriers) and 78.4% of embryos were diagnosed as non-transferable (78.8% in female carriers and 78% in male carriers).

In the present study, 84.2% of embryos from female carriers were unbalanced. A review on maternally derived reciprocal translocations (Durban *et al.* 2001) showed that 56% of the oocytes diagnosed through first polar body (1PB) were unbalanced. On the other hand, Findikli (2003) also found a high percentage (69.2%) of unbalanced oocytes in female reciprocal translocation carriers.

In the present study, 82% of embryos from male carriers were unbalanced. The frequencies of unbalanced spermatozoa found in reciprocal translocation carriers using the hamster-test or FISH in decondensed sperm heads, vary between 23% and 81% (Benet *et al.* 2005). The differences in the frequencies could be a consequence of the size of the samples and the characteristics of each translocation.

Some authors have pointed out the existence of ICE when studying translocation carriers using FISH in decondensed sperm heads (Estop *et al.* 2000, Pellestor

Table 5 Aneuploidy screening results: aneuploid and mosaic embryos and aneuploid events detected.

Cases	NB t & A AS embryos (mosaics)	UB t & A AS embryos (mosaics)	Missing/extra chr in NB t & A AS embryos					Missing/extra chr in UB t & A AS embryos					UBt & ND AS embryos
			13	16	18	21	22	13	16	18	21	22	
46, XX, t(2;18) (q31;q23)	1(1)	6(5)	−/−	1/−	−/−	1/−	−/−	3/−	2/2	2*/1*	5/−	2/2	–
46, XX, (9;21) (q11;q22)	–	3(1)	−/−	−/−	−/−	−/−	−/−	1/−	−/−	−/−	2*/−	1/1	–
46, XX, (12;15) (q24.1;q22)	1(1) 1T	4(4)	1/−	1/−	−/−	1/−	−/−	1/−	2/1	2/−	1/−	1/−	–
46, XX,(2;6) (p21;q25.1)	2(2) 2T	3(2)	1/−	1/−	−/−	2/−	−/−	2/−	1/2	−/−	1/−	1/−	1
46, XX, (7;14) (q11.23;q22	–	2(2)	−/−	−/−	−/−	−/−	−/−	2/−	−/−	2/1	2*/−	−/1	2
46, XX, (3;10) (q26.2;q21.21)	–	5(2)	−/−	−/−	−/−	−/−	−/−	4/−	1/2	−/−	5/−	2/1	1
46, XX, (12;21) (q15;q22)	–	5(4)	−/−	−/−	−/−	−/−	−/−	4/−	1/1	2/−	4*/−	4/−	–
46, XX, (3;16) (p25;q22)	2(1) 1T	8(6)	−/1	−/−	−/−	1/−	1/−	3/1	2*/2*	3/1	2/1	5/2	4
46, XY, (4;18) (q31.1;p11.2)	1 1T	3(3)	−/−	−/−	−/−	−/−	1/−	−/3	−/−	−/−	−/1	−/1	1
46, XY, (10;17) (q22.1;q21.3)	–	6(5)	−/−	−/−	−/−	−/−	−/−	1/−	3/1	3/1	3/−	4/1	–
46, XY, (2;13) (p26;q31)	2(1)	9(5)	−/−	−/2	−/−	−/−	−/−	4*/−	5/4	−/3	6/−	5/−	–
46, XY, (11;12) (q12;p11.1)	–	3(1)	−/−	−/−	−/−	−/−	−/−	−/−	−/1	−/−	1/−	−/1	–
46, XY, (4;22) (q21;q13.3)	1(1) 1T	9(9)	−/−	1/−	1/−	1/−	−/−	7/−	3/3	2/−	6/1	6*/1*	2
Total without*	10(7)	66(49)	2/1	4/2	1/−	6/−	2/−	28/4	18/17	14/6	30/3	25/10	11
Total aneuploidies	76(56)		3	6	1	6	2	32	35	20	33	35	

Abnormalities affecting the chromosomes involved in the translocation are not counted as AS alterations and are indicated in grey.
chr: chromosome; NB: normal-balanced; UB: unbalanced; t: translocation analysis; A: aneuploid; AS: aneuploidy screening; ND: non diagnosed; *chromosome abnormality due to translocation; nT: number of embryos transferred which were NB for t and A for AS.

et al. 2001, Oliver-Bonet *et al.* 2002, Anton *et al.* 2004). In general, it seems that this phenomenon is basically observed in translocation carriers with abnormal semenograms, as in 57.1% of the male translocation carriers' cycles included in the present work (Table 1). When the genetic risk is evaluated the importance of the existence of an ICE is the undesirable consequences of non-disjunction more than its frequency, because non-disjunction can lead to the production of spermatozoa with viable aneuploidies which could generate viable abnormal embryos (Oliver-Bonet *et al.* 2002).

In the present study, 115 (91.3%) abnormal embryos, 28 of them (24.3%) altered only for chromosomes involved in translocations, 66 (57.4%) altered for chromosomes involved and not involved in translocations and 10 (8.7%) altered only for chromosomes not involved in translocations were found (Table 7). Performing PGD in female and male reciprocal translocation carriers and analysing the chromosomes involved and also others not involved in translocations, Gianaroli *et al.* (2002) found 54 (89%) abnormal embryos, 35 of them (65%) altered only for translocation-chromosomes, 9 (16%) altered for chromosomes involved and not involved in translocations and 3 (6%) altered only in chromosomes not involved in translocations. The rate of abnormalities for the chromosomes not involved in translocations was quite high and, when also studying Rob translocation carriers, that kind of chromosome abnormality was observed much more frequently than in reciprocal translocation carriers (67% vs 22%) and was considered a consequence of ICE. Other authors also found a high rate of aneuploidies in the chromosomes not involved in translocations (90.5% of the abnormal embryos) when performing PGD by analysing 1PB in two Rob t(13;14) female carriers; on the other hand, for a female carrier of a rec t(8;13), the frequency was lower (12.5%) (Pujol *et al.* 2003*a*).

The high number of chromosome abnormalities involving abnormalities related and unrelated to the chromosome translocation may explain why, in the group of patients included in the present work, none of them ended in a pregnancy.

In males, some studies indicate that there is a strict checkpoint in gametogenesis which stops meiosis when unbalanced chromosome complements are detected, thus reducing the generation of altered spermatozoa

Table 6 Summary of the results obtained for female and male carriers. Mosaicism detected in each translocation for the chromosomes involved in translocations, for those analysed for AS and for both.

		Mosaic embryos detected					Non-mosaic embryos detected	
Cases	**Analysed embryos**	Only for t	Only for AS	For t & for AS	For t & ND for AS	For t & for AS	For t & ND for AS	ND for t & ND for AS
46, XX t(2;18) (q31;q23)	9	1	2	2	1	–	3	–
46, XX, t(9;21) (q11;q22)	6	–	1	–	1	3	–	1
46, XX,t(12;15) (q24.1;q24)	8	2	4	–	1	1	–	–
46, XX t(2;6) (p21;q25.1)	10	1	4	–	–	2	3	–
46, XX t(7;14) (q11.23;q22)	11	3	2	–	–	2	3	1
46, XX t(3;10) (q26.2;q21.21)	7	–	–	1	3	1	2	–
46, XX t(12;21) (q15;q22)	5	–	2	0	2	1	–	–
46, XX, t(3;16) (p25;q22)	20	1	1	4	6	5	2	1
Subtotal	76	8	16	7	14	15	13	3
%		10.5	21.0	9.2	18.5	19.7	17.1	4.0
46, XY t(4;18) (q31.1;p11.2)	9	1	–	1	4	3	–	–
46, XY t(10;17) (q22.1;q21.3)	6	–	1	3	1	1	–	–
46, XY t(2;13) (p16;q31)	13	–	3	3	–	5	1	1
46, XY t(11;12) (q12;p11.1)	3	–	1	–	–	2	–	–
46, XY t(4;22) (q21;q13.3)	19	–	6	3	2	6	1	1
Subtotal	50	1	11	10	7	17	2	2
%		2.0	22.0	20.0	14.0	34.0	4.0	4.0
Total	126	9/126	27/126	17/126	21/126	32/126	15/126	5/126
%		7.1	21.4	13.5	16.7	25.4	11.9	4.0

t: translocation-related chromosomes; AS: chromosomes analysed in aneuploidy screening, i.e. chromosomes not related to the translocation; ND: non-diagnosed chromosomes.

(Roeder & Bailis 2000) although the arrest may be overcome and result in the production of diploid or aneuploid sperm. In female meiosis the anaphase checkpoint is not so stringent and altered oocytes can be produced (LeMarie-Adkins *et al.* 1997). According to this, it would be expected that the frequency of chromosome abnormalities observed in embryos could be different depending on the sex of the rearrangement carrier. In the present work, no statistically significant differences were obtained in the cytogenetic results of the translocation chromosomes when comparing female and male carriers, and all types of segregations were found, even a 4:0 in one case. This could indicate that a sex-linked selective process against the unbalanced

Table 7 Segregation analysis and AS in embryos of translocation carriers.

				Abnormalities			
Translocation carriers	**Diagnosed cells**	**Normal** Total (%)	**Abnormal** Total (%)	NB for t & A for AS (%)	UB for t & E for AS (%)	UB for t & A for AS (%)	Others (%)
rec ($n=13$)[a]	61 embryos	7(11.0)	54(89.0)	3(6.0)	35(65.0)	9(16.0)	7(13.0) haploid/ polyploid
Rob ($n=15$)[a]	111 embryos	26(23.0)	85(77.0)	26(31.0)	18(21.0)	31(36.0)	10(12.0)
rec ($n=1$)[b]	9 oocytes	1(11.1)	8(88.9)	–	7(87.5)	1(12.5)	–
Rob ($n=2$)[b]	24 oocytes/embryos	3(12.5)	21(87.5)	7(33.3)	2(9.5)	12(57.2)	–
rec ($n=13$)[c]	126 embryos analysed	11(8.7)	115(91.3)	10(8.7)	28(24.3)	66(57.4)	11(9.6) UB for t & ND for AS

[a]Gianaroli *et al.* (2002); [b]Pujol *et al.* (2003*a*); [c]Present study.
NB: normal-balanced; UB: unbalanced; E: euploid; A: aneuploid; t: translocation-related chromosomes; AS: chromosomes analysed in aneuploidy screening, i.e. translocation-non related chromosomes; ND: non-diagnosed chromosomes.

chromosome complements generated due to the presence of a translocation does not exist. This is in agreement with Armstrong *et al.* (2000) and also with Oliver-Bonet *et al.* (2001, 2002) who found spermatozoa with all the theoretical unbalanced segregations.

An increase of the aneuploidy rate in embryos affecting chromosomes not involved in a translocation could depend, as already mentioned, on the influence of the quadrivalent on the segregation of chromosomes not involved in the translocation (ICE), but it could also be affected by maternal age. In order to evaluate both mechanisms, a comparison was made between AS results of the chromosomes not implicated in the translocation of patients with a maternal age of $\leq$37 years and $>$37 years. In female carriers, the embryos with aneuploidies in chromosomes not implicated in translocation were 52.8% ($\leq$37 years) and 60% ($>$37 years). These percentages were not statistically different. The presence of a quadrivalent could affect the segregation of the other chromosomes increasing the aneuploidy rate, especially in young female carriers where a high aneuploidy rate would not be expected.

In male carriers with a partner $\leq$37 years, although aneuploidies related to maternal age were not expected, 77.4% of embryos had aneuploidies of chromosomes not implicated in translocation. Nevertheless, it is not possible to know if this high aneuploidy rate is caused by abnormal segregation that took place during meiotic division or during the first few mitotic divisions. Male carriers with a partner $>$37 years had fewer aneuploid embryos (52.7%), but it should be taken into account that there was only one patient in that group.

No statistically significant differences were obtained in cytogenetic results when comparing the two established maternal age groups, neither considering female and male carriers separately nor considering both female and male carriers as a single group. The generation of aneuploidies by the studied patients in the chromosomes not involved in translocations is not increased by maternal age.

All chromosomes studied in the AS had high rates of aneuploidy and this result confirms the importance of including them in the AS analysis performed alone or in combination with the translocation analysis.

In female carriers, a high frequency of aneuploidies for the five chromosomes analysed in the AS was found (61.8%, 42/68) (Table 4). The number of embryos altered for one, two, three, four or five chromosomes fits a Poisson with parameter 1.26 with a significance of $P=0.079$. Then, each embryo would have a probability of 71.6% of being aneuploid and 35.7%, 22.5%, 9.5%, 3% and 0.8% would be aneuploid for one, two, three, four and five chromosomes respectively. In a study made in patients of normal karyotype, using metaphase II (MII) oocytes and 1PBs from IVF cycles, the aneuploidy rate found by FISH analysing chromosomes 1, 13, 15, 16, 17, 18, 21, 22 and X (Pujol *et al.* 2003*b*) was 47.5% and an estimation for the 23 chromosomes reported that 57.2% of oocytes would be aneuploid, and 36.3%, 15.4%, 4.4%, 0.9% and 0.2% would be aneuploid for one, two, three, four and five chromosomes respectively. Recently, in analysing the whole chromosome complement of MII oocytes and 1PBs by comparative genomic hybridisation (CGH) (Gutiérrez-Mateo *et al.* 2004), a very similar frequency of aneuploidy was found (57.1%). Comparing the frequencies of aneuploidy for each of the analysed chromosomes (13, 16, 18, 21 and 22) obtained in the present work with those obtained for oocytes from normal karyotype females (Pujol *et al.* 2003*b*), a slightly significant difference ($P=0.046$) using the Chi-squared test was found.

For the AS analysis made in the female carriers of the present study, each of the five analysed chromosome pairs of the embryos had a 7.48% (4.49–8.76%) risk of being involved in aneuploidy. In oocytes from females with normal karyotype, each of the nine analysed chromosomes of the oocytes had a 0.89% (0.52–1.70%) risk of being involved in aneuploidy. Significant differences ($P<0.0005$, Chi-squared test) are observed for chromosomes 13, 16, 18, 21 and 22. The high increase in the aneuploidy rate found in embryos from female carriers compared with the rate observed in oocytes from normal karyotype females could be, in part, due to the presence of the translocation during the meiotic segregation process (ICE). Errors in the first few mitotic divisions and in male meiosis could also be responsible.

Chromosome 16 showed an equivalent incidence of extra and missing chromosomes (Table 5). For chromosomes 13, 18, 21 and 22, missing chromosomes were more frequent than extra chromosomes, as also happened in global (133 vs 42). This is in accordance with other studies made in embryos (Munné *et al.* 2004), in which one or two rounds of FISH were performed.

In the present work at least 58.7% (74/126) of the embryos were mosaic, 37.3% (47/126) were mosaic for the chromosomes involved in translocations and at least 34.9% (44/126) were mosaic for the chromosomes not involved. No statistically significant difference was found between female and male carriers when analysing mosaicism. A high degree of mosaicism has previously been detected in human embryos of patients with balanced structural chromosome aberrations; the frequencies are as high as 73%, 98% and even 100% (Iwarsson *et al.* 2000, Malmgren *et al.* 2002, Emiliani *et al.* 2003).

Until recently, and according to the results published by the ESHRE PGD Consortium (Harper *et al.* 2006), PGD in translocation carriers is carried out to study only the segregation pattern of the chromosomes involved in the rearrangement and AS is only performed in other groups of patients (patients of advanced maternal age, recurrent miscarriages or IVF failures). It has been found that the couples with a translocation carrier have an increased risk of having embryos with slow development,

arrest and, consequently, IVF failures; this is probably due to the production of gametes with alterations not only in translocation chromosomes, but also in others (Findikli, 2003).

In the present work, AS analysis allowed for the detection of, in at least 60.4% (76/126) of the analysed embryos, aneuploid events affecting chromosomes not involved in translocations (Table 4). Ten (8%) of those embryos had been detected as being normal/balanced for the chromosomes involved in translocation and six of those ten embryos had been transferred in the PGD cycles (Table 5).

As expected, a low frequency of the embryos were normal for all analysed chromosomes (8.7% average: 7.9% in females and 10% in males), while a high frequency had chromosome abnormalities (91.3% average: 92.1% in females and 90% in males) (Table 7).

Although there are also other studies which, in translocation carriers, recommend the analysis of chromosomes not involved in translocation (Gianaroli *et al.* 2002, Kuliev & Verlinsky 2002, Pujol *et al.* 2003*a*), this is not yet extensively used.

The number of transferable embryos would diminish if an AS is performed during PGD cycles in translocation carriers, but aneuploidies would be detected in some of the embryos which are normal or balanced for the translocation. The present study allows for the corroboration of the importance of routinely including AS in the PGD cycles carried out in translocation carriers. There is enough time to perform AS after the analysis of the chromosomes involved in a translocation, although a blastocyst transfer could be made and a more strict selection of embryos would benefit the outcome of chromosome reorganisation carriers.

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References

Abdelhadi I, Colls P, Sandalinas M, Escudero T & Munné S 2003 Preimplantation genetic diagnosis of numerical abnormalities for 13 chromosomes. *RBM Online* **6** 226–231.

Anton E, Vidal F, Egozcue J & Blanco J 2004 Preferential alternate segregation in the common t(11;22)(q23;q11) reciprocal translocation: sperm FISH analysis in two brothers. *Reproductive BioMedicine Online* **9** 637–644.

Armstrong S, Goldman A, Speed R & Hultén M 2000 Meiotic studies of a human male carrier of a common translocation, t(11;22), suggest postzygotic selection rather than preferential 3:1 MI segregation as the cause of liveborn offspring with an unbalanced translocation. *American Journal of Human Genetics* **67** 601–609.

Benet J, Oliver-Bonet M, Cifuentes P, Templado C & Navarro J 2005 Segregation of chromosomes in sperm of reciprocal translocation carriers. *Cytogenetic and Genome Research* **111** 281–290.

Conn C, Harper J, Winston R & Delhanty J 1998 Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. *Human Genetics* **102** 117–123.

Coonen E, Dumoulin J, Ramaekers F & Hopman A 1994 Optimal preparation of preimplantation embryo interphase nuclei for analysis by fluorescence in-situ hybridization. *Human Reproduction* **9** 533–537.

De Vos A, Sermon K, De Rijcke M, Grossens V, Henderix P, Van Ranst N, Platteau P, Lissens W, Devroey P, Van Steirteghem A *et al.* 2003 Preimplantation genetic diagnosis for Charcot-Marie-Tooth disease type 1A. *Molecular Human Reproduction* **9** 429–435.

Durban M, Benet J, Boada M, Fernández E, Calafell JM, Lailla JM, Sánchez-García JF, Pujol A, Egozcue J & Navarro J 2001 PGD in female carriers of balanced Robertsonian and reciprocal translocations by first polar body analysis. *Human Reproduction Update* **7** 591–602.

Emiliani S, González-Merino E, Van den Bergh M, Abramowicz M & Englert Y 2003 Higher degree of chromosome mosaicism in preimplantation embryos from carriers of robertsonian translocation t/13;14) in comparison with embryos from karyotypically normal IVF patients. *Journal of Assisted Reproduction and Genetics* **20** 95–100.

Estop AM, Cieply K, Munné S, Surti U, Wakim A & Feingold E 2000 Is there an interchromosomal effect in reciprocal translocation carriers? Sperm FISH studies. *Human Genetics* **106** 517–524.

Evsikov S, Cieslak J & Verlinsky Y 2000 The effect of chromosomal translocations on the development of preimplantation human embryos in vitro. *Fertility and Sterility* **7** 672–677.

Findikli N 2003 Embryo development characteristics in Robertsonian and reciprocal translocations: a comparison of results with non-translocation cases. *RBM Online* **7** 563–571.

Gianaroli L, Magli MC, Ferraretti A & Munné S 1999 Preimplantation diagnosis for aneuploidies in patients undergoing *in vitro* fertilization with a poor prognosis: identification of the categories for wich it should be proposed. *Fertility and Sterility* **72** 837–844.

Gianaroli L, Magli M, Ferraretti A, Munné S, Balicchia B, Escudero T & Crippa A 2002 Possible interchromosomal effect in embryos generated by gametes from translocation carriers. *Human Reproduction* **17** 3201–3207.

Gutiérrez-Mateo C, Benet J, Wells D, Colls P, Bermúdez M, Sánchez-García JF, Egozcue J, Navarro J, Munné S, Sánchez-García JF, Egozcue J, Navarro J & Munné S 2004 Aneuploidy study of human oocytes first polar body comparative genomic hybridization and metaphase II fluorescence in situ hybridization analysis. *Human Reproduction* **19** 2859–2868.

Harper JC, Boelaert K, Geraedts JP, Harton G, Kearns W, Moutou C, Muntjewerff N, Repping S, SenGupta S & Scriven P 2006 ESHRE PGD Consortium data collection V: Cycles from January to December 2002 with pregnancy follow-up to October 2003. *Human Reproduction* **21** 3–21.

Iwarsson E, Malmgren H, Inzunza J, Ährlund-Richter L, Sjöblom P, Rosenlund B, Fridström M, Hovatta O, Nordenskjöld M & Blennow E 2000 Highly abnormal cleavage divisions in preimplantation embryos from translocation carriers. *Prenatal Diagnosis* **20** 1038–1047.

Kahraman S, Bahce M, Samli H, Imirzalioglu N, Yakisn K, Cengiz G & Dönmez E 2000 Healthy births and ongoing pregnancies obtained by preimplantation genetic diagnosis in patients with advanced maternal age and recurrent implantation failure. *Human Reproduction* **15** 2003–2007.

Kuliev A & Verlinsky Y 2002 Current features of preimplantation genetic diagnosis. *RBM Online* **5** 294–299.

LeMarie-Adkins R, Radke K & Hunt P 1997 Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. *Journal of Cell Biology* **139** 1611–1619.

Magli M, Gianaroli L, Ferraretti A, Toschi M, Esposito F & Fasolino M 2004 The combination of polar body and embryo biopsy does not affect embryo viability. *Human Reproduction* **19** 1163–1169.

Malmgren H, Sahlén S, Inzunza J, Aho M, Rosenlund B, Fridström M, Hovatta O, Ährlund-Richter L, Nordenskjöld M & Blennow E 2002 Single cell CGH analysis reveals a high degree of mosaicism in human embryos from patients with balanced structural chromosome aberrations. *Molecular Human Repoduction* **8** 502–510.

Ménezo Y, Bellec V, Zaroukian A & Benkhalifa M 1997 Embryo selection by IVF, co-culture and transfer at the blastocyst stage in case of translocation. *Human Reproduction* **12** 2802–2803.

Munné S, Cohen J & Sable D 2002 Preimplantation genetic diagnosis for advanced maternal age and other indications. *Fertility and Sterility* **78** 234–236.

Munné S, Fung J, Cassel M, Márquez C & Weier H 1998 Preimplantation Genetic analysis of translocations:case-specific probes for interphase cell analysis. *Human Genetics* **102** 663–674.

Munné S, Sandalinas M, Escudero T, Velilla E, Walmsley R, Sadowy S, Cohen J & Sable D 2003 Improved implantation after preimplantation genetic diagnosis of aneuploidy. *RBM Online* **7** 91–97.

Munné S, Sapulveda S, Balmaceda J, Fernández E, Fabres C, Mackenna A, López T, Crosby J & Zegers-Hochschild F 2000 Selection of the most common chromosome abnormalities in oocytes prior to ICSI. *Prenatal Diagnosis* **20** 582–586.

Munné S, Bahce M, Sandalinas M, Escudero T, Márquez C, Velilla E, Colls P, Oter M, Alikani M & Cohen J 2004 Differences in chromosome susceptibility to aneuploidy and survival to first trimester. *Reproductive BioMedicine Online* **8** 81–90.

Oliver-Bonet M, Navarro J, Carrera M, Egozcue J & Benet J 2002 Aneuploid and unbalanced sperm in two translocation carriers: evaluation of the genetic risk. *Molecular Human Repoduction* **8** 958–963.

Oliver-Bonet M, Navarro J, Codina-Pascual M, Carrera M, Egozcue J & Benet J 2001 Meiotic segregation analysis in a t(4;8) carrier: comparison of FISH methods on sperm chromosome metaphases and interphase sperm nuclei. *European Journal of Human Genetics* **9** 395–403.

Pellestor F, Imbert I, Andreo 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.

Pierce K, Fitzgerald LM, Seibel M & Zilberstein M 1998 Preimplantation genetic diagnosis of chromosome balance in embryos from a patient with a balanced reciprocal translocation. *Molecular Human Reproduction* **4** 167–172.

Pujol A, Durban M, Benet J, Boiso I, Calafell JM, Egozcue J & Navarro J 2003*a* Multiple aneuploidies in the oocytes of balanced translocation carriers: a PGD study using 1PB. *Reproduction* **126** 701–711.

Pujol A, Boiso I, Benet J, Veiga A, Durban M, Campillo M, Egozcue J & Navarro J 2003*b* Analysis of nine chromosome probes in 1st Polar Bodies and metaphase II oocytes for the detection of aneuploidies. *European Journal of Human Genetics* **11** 325–336.

Roeder G & Bailis J 2000 The pachytene checkpoint. *Trends in Genetics* **16** 395–403.

Scriven P, Handyside A & Ogilvie M 1998 Chromosome translocations:Segregation Modes and strategies for preimplantation genetic diagnosis. *Prenatal Diagnosis* **18** 1437–1449.

Sermon K, Moutou C, Harper JC, Geraedts JP, Scriven P, Wilton L, Magli C, Michiels A, Viville S & DeDie C 2005 ESHRE PGD Consortium data collection IV: May-December 2001. *Human Reproduction* **20** 19–34.

Staessen C, Van Assche E, Joris H, Bonduelle M, Vandervorst M, Liebaers I & Van Steirteghem A 1999 Clinical experience of sex determination by fluorescent in-situ hybridization for preimplantation genetic diagnosis. *Molecular Human Reproduction* **5** 382–389.

Van Assche E, Staessen C, Vegetti W, Bonduelle M, Vandervorst M, VanSteirteghem A & Liebaers I 1999 Preimplantation genetic diagnosis and sperm analysis by fluorescence *in situ* hybridization for the most common reciprocal translocation t(11;22). *Molecular Human Repoduction* **5** 682–690.

Van Dyke D, Weiss L, Roberson J & Babu V 1983 The frequency and mutation rate of balanced autosomal rearrangements in man estimated from prenatal genetic studies for advanced maternal age. *American Journal of Human Genetics* **35** 301–308.

Verlinsky Y & Evsikov S 1999 A simplified and efficient method for obtaining metaphase chromosomes from individual human blastomeres. *Fertility and Sterility* **72** 1127–1133.

Verlinsky Y, Cieslak J, Evsikov S, Galat V & Kuliev A 2002 Nuclear transfer for full karyotyping and preimplantation diagnosis for translocations. *RBM Online* **5** 300–305.

Verlinsky Y, Cohen J, Munne S, Gianaroli L, Simpson J, Ferraretti A & Kuliev A 2004 Over a decade of experience with preimplantation genetic diagnosis. *Fertility and Sterility* **82** 292–294.

Willadsen S, Levron J, Munné S, Schimmel T, Márquez C, Scott R & Cohen J 1999 Rapid visualization of methafase chromosomes in single human blastomeres after fusion with in-vitro matured bovine eggs. *Human Reproduction* **14** 470–475.

World Health Organization 1999 WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction. Cambridge: Cambridge University Press.

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