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# Study of the occurrence of interchromosomal effect in spermatozoa of chromosomal rearrangement carriers by fluorescence in-situ hybridization and primed in-situ labelling techniques

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**The possibility that a chromosomal rearrangement might disturb the meiotic behaviour of chromosomes not involved in the rearrangement and favour non-disjunction is a controversial issue in human cytogenetics. Using two-colour fluorescence in-situ hybridization and primed in-situ labelling techniques, we have investigated the segregation pattern of 10 chromosomes (chromosomes 1, 4, 9, 13, 15, 16, 20, 21, X and Y) in spermatozoa from nine carriers of balanced structural rearrangements and three normal men. The patients were divided into two groups according to their semen parameters. In rearrangement carriers and normal subjects, sex chromosomes and chromosome 21 displayed a higher rate of disomy than the other chromosomes. No evidence for the occurrence of interchromosomal effect was found in the spermatozoa of fertile rearrangement carriers, but significant variations were observed for all chromosomes tested in the group of infertile translocation carriers, suggesting a direct correlation between poor quality spermatozoa and increased aneuploidy rate in this group. In fertile carriers of chromosomal rearrangements, the occurrence of non-disjunction of chromosomes not involved in the rearrangement might therefore be considered as fortuitous, whereas in infertile carriers, the risk for interchromosomal effect appears to be real and should be taken into consideration in the genetic counselling of infertile couples with a male partner carrying a chromosomal rearrangement.**

*Key words:* FISH/interchromosomal effect/PRINS/rearrangements/spermatozoa

## Introduction

In humans, the concept of interchromosomal effect was first postulated by Lejeune (Lejeune, 1963). Its occurrence has been claimed for various types of chromosome rearrangements. Thus, cases of trisomy 21 and sex chromosome aneuploidy have been reported in association with chromosomal inversion, reciprocal translocation and Robertsonian translocation (Stoll *et al*., 1978; Canki and Dutrillaux, 1979; Couzin *et al*., 1987; Serra *et al*., 1990). However, most reports have been anecdotal and even results of more comprehensive epidemiological studies have failed to confirm the occurrence of an interchromosomal effect in meiosis of rearrangement carriers (Hecht and Patil, 1977; Lindenbaum *et al*., 1985; Schinzel *et al*., 1992). An alternative approach to investigate this point is to analyse directly the chromosomal constitution of gametes obtained from carriers of structural rearrangements. Using the human–hamster system which allows human sperm karyotyping (Rudak *et al*., 1978), 50 different rearrangements were analysed (Guttenbach *et al*., 1997, review). The frequencies of numerical abnormalities unrelated to the rearrangement were not significantly increased, with the exception of one case of a double translocation heterozygote (Burns *et al*., 1986). The number of sperm karyotypes usually obtained for each rearrangement was low (mean: 92) and consequently might be insufficient to allow the detection of increased disomy resulting from interchromosomal effect. With the advent of molecular cytogenetics, two procedures have proven their efficiency on sperm nuclei, i.e. fluorescence in-situ hybridization (FISH) (Guttenbach and Schmid, 1990; Robbins *et al*., 1993), and primed in-situ labelling (PRINS) (Pellestor *et al*., 1996). Both led to the specific identification of human chromosomes in sperm nuclei using either chromosome-specific probes (for FISH) or chromosome-specific primers (for PRINS). To date, molecular cytogenetic studies of meiotic segregation in spermatozoa from rearrangement carriers have been performed for 25 translocations (Guttenbach *et al*., 1997, review) and one inversion (Colls *et al*., 1997). Among these reports, the question of interchromosomal effect has been studied in 14 cases, by estimating the disomy rates of one or two autosomes and gonosomes. Significant increases of disomy have been

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reported for four translocations: t(14;21), t(2;14), t(6;11) (Rousseaux *et al.*, 1995a,b) and t(7;8) (Mercier *et al.*, 1998), suggesting the occurrence of interchromosomal effect. The existence of an interchromosomal meiotic effect has also been analysed by FISH in spermatozoa of 12 infertile translocation carriers, 10 of whom had abnormal seminal parameters. Compared with spermatozoa from 13 normal fertile subjects, the group of translocation carriers showed higher frequency of aneuploidy and diploidy. Similar results were found in spermatozoa of 15 infertile men with abnormal semen and normal karyotypes. These data suggested a correlation between poor semen quality and non-disjunction events (Vegetti *et al.*, 2000). In addition, two FISH studies on spermatozoa concerning interchromosomal effect have been recently published. One study (Blanco *et al.*, 2000) evaluated the incidence of disomy and diploidy for chromosomes 6 and 21 in spermatozoa of three translocations carriers and one inversion carrier; they reported a significant increase of disomy 21 in the spermatozoa of a t(3;15) carrier. Another group (Estop *et al.*, 2000) studied disomy for chromosomes 13, 18, 21, X and Y in spermatozoa of nine reciprocal translocation carriers and found no evidence for an interchromosomal effect. All these FISH data, representing the analysis of 37 different chromosomal rearrangements, are summarized in Table I.

Because these data are controversial and too scanty to draw any conclusions, the present study was specially designed to investigate the occurrence of interchromosomal effect in male gametes. For this purpose, we analysed sperm samples from nine carriers of structural rearrangements and three normal subjects divided into three groups: six fertile men with normal seminal parameters and rearranged karyotypes, three infertile men with abnormal semen parameters and rearranged karyotypes, and three healthy controls with normal semen analysis and normal karyotypes. In each case, we chose to analyse the diploidy rate and the disomy rate of gonosomes and eight autosomes not involved in the rearrangements and belonging to different chromosome groups in order to address the question of the mode of occurrence of interchromosomal effect, i.e. randomly or restricted to a particular chromosome group. Both FISH and PRINS labelling technique were used in parallel in order to improve the reliability and the statistical significance of the results.

## Materials and methods

### *Donors*

Nine carriers of balanced chromosomal rearrangements were involved in our study and divided into two groups according to their semen quality. All donors gave informed consent for the use of their spermatozoa in the present study. Our research programme was approved by the Research Ethics Committee of our Research Institution.

### *Group 1 (normal semen and abnormal karyotype)*

Donor A, aged 34 years, carried a t(14;22) (q10;q10) Robertsonian translocation ascertained through systematic sperm donor screening for artificial insemination by donor. He has two normal children. His spermogram was normal with a sperm count of $77\times10^6$/ml and a motility of 70%. Donor B, aged 44 years, was heterozygous for a t(1;14) (p22;q21) ascertained after his wife underwent amniocentesis for advanced maternal age. He was the father of three normal children. His spermogram was normal with a sperm count of $85\times10^6$/ml and a motility of 60%. Donor C, aged 36 years, carried a t(7;9) (q33; p21) inherited through his mother. He was the father of a healthy 7 year old child. His spermogram was normal with a sperm count of $80\times10^6$/ml and a motility of 65%. Donor D, aged 30 years, was heterozygous for a t(7;18) (q35;q11) and was ascertained because of repeated reproductive failure. His wife had three spontaneous abortions. The spermogram of this man was normal with a sperm count of $43\times10^6$/ml and a motility of 55%. The study of meiotic segregation in spermatozoa of subjects C and D has been previously reported (Pellestor *et al.*, 1997). Donor E, aged 35 years, carried a t(17;18) (p11;q11) inherited through his father. His spermogram was normal with a sperm count of $50\times10^6$/ml and a motility of 50%. Donor F, aged 38 years, carried an inv(2) (p11q13). The rearrangement was ascertained after two reproductive failures that ended in termination. The spermogram of this man was normal with a sperm count of $72\times10^6$/ml and a motility of 60%.

### *Group 2 abnormal semen and abnormal karyotype)*

Donor G, aged 39 years, was heterozygous for a t(8;13) (p22;q13) ascertained after 4 years of infertility. Semen analysis revealed an oligoasthenoteratozoospermia (semen count $4.3\times10^6$/ml, motility 13%, nomal morphology 10%). Donor H, 30 years old, carried a t(13;15) (q10;q10) Robertsonian translocation, ascertained through a fertility work-up after 3 years of infertility. Seminal parameters showed oligoasthenoteratozoospermia (semen count $3.2\times10^6$/ml, motility 20%, normal morphology 8%). Donor I, aged 35 years, carried a t(5;9) (p12;p11) inherited through his mother. His sister was also carrier of the same translocation and had two spontaneous abortions. The semen analysis of this subject revealed an oligoteratozoospermia (semen count $15\times10^6$/ml, motility 40%, normal morphology 11%).

### *Controls*

Three healthy normal volunteers, aged 27, 32 and 37 years, provided sperm specimens during the same period as the rearrangement carriers, and constituted the control group (group 3). The three subjects were of proven fertility and had normal seminal parameters (sperm count $>50\times10^6$/ml, motility >50%, normal morphology >20%).

### *Sperm treatment*

All experiments were performed on fresh ejaculates. The processing of sperm samples was identical for carrier and control specimens. The ejaculate was collected in a sterile container and kept at room temperature for 30 min. After liquefaction, an aliquot of the specimen was used for semen analysis. The rest of the sample was washed twice in phosphate-buffered saline (PBS) by centrifugation (8 min at 300 *g*) and fixed for 1 h in fresh fixative (3:1 methanol:glacial acetic acid) at –20°C. The sperm suspension was then dropped onto clean microscope slides and air-dried. Slides were aged 3 days at room temperature before use for in-situ chromosomal labelling. Before PRINS or FISH procedure, the slides were immersed in a 3 mol/l NaOH solution at room temperature for 5 min, passed through ethanol series (70, 90, 100%) and air-dried. The use of 3 mol/l NaOH solution allowed the simultaneous decondensation and denaturation of sperm nuclei, enabling rapid control of the degree of nucleus decondensation under the microscope.

### *In-situ chromosomal labelling procedures*

For each subject, the same combination of probes or primers was used. Details are given in Table II.

**Table I.** Summary of the fluorescence in-situ hybridization studies of interchromosomal effect in spermatozoa from patients carrying chromosomal rearrangements

| Rearrangement | Age (years) | Semen | 1 | 6 | 12 | 13 | 15 | 17 | 18 | 21 | X | Y | XY | Diploidy | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| t(2;4;8) | 28 | O | | | 0.20 | | | | | | | | | | Lu *et al*. (1994) |
| t(14;21) | 34 | OAT | 0.67[a] | | | | | | | | 0.00 | 0.00 | 0.52 | | Rousseaux *et al*. (1995) |
| t(6;11) | ? | N | 0.30[a] | | | | | | | | 0.04 | 0.02 | 0.30 | 0.21 | Rousseaux *et al*. (1995) |
| t(6;11) | ? | N | 0.49[a] | | | | | | | | 0.00 | 0.00 | 0.65 | 0.21 | Rousseaux *et al*. (1995) |
| t(2;14) | ? | N | 0.39[a] | | | | | | | | 0.01 | 0.01 | 0.43 | 0.27 | Rousseaux *et al*. (1995) |
| t(1;10) | 40 | N | | | | | | | 0.01 | 0.07 | 0.03 | 0.02 | 0.02 | 0.34[a] | Van Hummelen *et al*. (1997) |
| inv(9) | 34 | N | | 0.12 | | | | | | 0.30 | | | | 0.20 | Colls *et al*. 1997 |
| t(3;11) | 33 | N | | | | 0.00 | | | | 0.26 | 0.02 | 0.12 | 0.23 | 0.06 | Martini *et al*. (1998) |
| t(5;8)[c] | 42 | N | | 0.12 | | | | | | 0.23 | | | | 1.18[a] | Blanco *et al*. (1998) |
| t(7;8) | 30 | N | | | | | 0.22 | | 1.21[a] | | | | | 0.44[a] | Mercier *et al*. (1998) |
| t(5;7) | ? | N | | | 0.09 | | | | 0.03 | 0.23 | 0.01b | 0.07[b] | 0.07[b] | 0.09[b] | Cifuentes *et al*. (1999) |
| t(Y;16) | 43 | OAT | | | | | | | ? | | | | | | Giltay *et al*. (1999) |
| t(3;9) | 32 | N | | | 0.12 | | | 0.14 | 0.19 | | 0.11 | 0.12 | 0.12 | 0.15 | Honda *et al*. (1999) |
| t(3;9) | 34 | N | | | 0.13 | | | 0.16 | 0.23 | | 0.13 | 0.14 | 0.12 | 0.14 | Honda *et al*. (1999) |
| t(2;7) | 35 | OAT | | | | 0.29[a] | | | 0.31[a] | 0.38[a] | 0.16[a] | 0.31[a] | 0.31[a] | 0.19[a] | Vegetti *et al*. (2000) |
| t(14;21) | 34 | OAT | | | | 0.37[a] | | | 0.32[a] | | 0.05[a] | 0.09[a] | 0.18[a] | 0.32[a] | Vegetti *et al*. (2000) |
| t(11;22) | 28 | OAT | | | | 0.19[a] | | | 0.14[a] | 0.19[a] | 0.14[a] | 0.19[a] | 0.19[a] | 0.10[a] | Vegetti *et al*. (2000) |
| t(17;22) | 41 | AT | | | | 0.10[a] | | | 0.19[a] | 0.20[a] | 0.09[a] | 0.09[a] | 0.14[a] | 0.00 | Vegetti *et al*. (2000) |
| t(2;5) | 39 | AT | | | | 0.15[a] | | | 0.19[a] | 0.10[a] | 0.05[a] | 0.05[a] | 0.10[a] | 0.10[a] | Vegetti *et al*. (2000) |
| t(3;18) | 31 | AT | | | | 0.24[a] | | | | 0.24[a] | 0.09[a] | 0.22[a] | 0.39[a] | 0.24[a] | Vegetti *et al*. (2000) |
| t(6;7) | 41 | AT | | | | 0.19[a] | | | 0.14[a] | 0.29[a] | 0.09[a] | 0.05[a] | 0.09[a] | 0.29[a] | Vegetti *et al*. (2000) |
| t(13;15) | 38 | AT | | | | | | | 0.23[a] | 0.24[a] | 0.05[a] | 0.05[a] | 0.18[a] | 0.24[a] | Vegetti *et al*. (2000) |
| t(2;10) | 30 | T | | | | 0.15[a] | | | 0.15[a] | 0.10[a] | 0.05[a] | 0.10[a] | 0.05[a] | 0.24[a] | Vegetti *et al*. (2000) |
| t(5;20) | 36 | T | | | | 0.19[a] | | | 0.14[a] | 0.14[a] | 0.10[a] | 0.19[a] | 0.10[a] | 0.10[a] | Vegetti *et al*. (2000) |
| t(6;15) | 42 | N | | | | 0.15[a] | | | 0.14[a] | 0.10[a] | 0.10[a] | 0.10[a] | 0.05[a] | 0.09[a] | Vegetti *et al*. (2000) |
| t(1;2) | 29 | N | | | | 0.28[a] | | | 0.20[a] | 0.46[a] | 0.20[a] | 0.10[a] | 0.20[a] | 0.16[a] | Vegetti *et al*. (2000) |
| t(3;13) | 47 | OAT | | 0.48 | | | | | | 1.90[a] | | | | 5.71[a] | Blanco *et al*. (2000) |
| t(y;7) | 41 | OAT | | | | | | | | 0.43 | | | | 0.17 | Blanco *et al*. (2000) |
| t(13;22) | 28 | OAT | | 0.12 | | | | | | 0.29 | | | | 0.21 | Blanco *et al*. (2000) |
| inv(9) | 37 | N | | 0.02 | | | | | | 0.19 | | | | 0.29 | Blanco *et al*. (2000) |
| t(10;12) | 40 | ? | | | | | | | 0.03 | | 0.01 | 0.02 | 0.04 | | Estop *et al*. (2000) |
| t(2;18) | 32 | ? | | | | 0.13 | | | | 0.21 | 0.03 | 0.02 | 0.09 | | Estop *et al*. (2000) |
| t(3;19) | 35 | ? | | | | 0.10 | | | 0.05 | 0.26 | 0.04 | 0.03 | 0.09 | | Estop *et al*. (2000) |
| t(5;8)[c] | 42 | N | | | | 0.05 | | | 0.12 | 0.13 | 0.03 | | 0.34 | | Estop *et al*. (2000) |
| t(11;22) | 44 | ? | | | | 0.12 | | | 0.05 | 0.27 | 0.03 | | 0.13 | | Estop *et al*. (2000) |
| t(3;4) | 37 | ? | | | | 0.14 | | | 0.03 | 0.24 | 0.03 | | 0.12 | | Estop *et al*. (2000) |
| t(8;9) | 39 | ? | | | | 0.03 | | | 0.00 | 0.11 | 0.12 | | 0.14 | | Estop *et al*. (2000) |
| t(10;18) | 32 | ? | | | | 0.07 | | | | 0.19 | 0.04 | 0.02 | 0.20 | | Estop *et al*. (2000) |
| t(4;10) | 42 | ? | | | | 0.02 | | | 0.01 | 0.16 | 0.03 | 0.01 | 0.09 | | Estop *et al*. (2000) |

N = normal; O = oligozoospermia; T = teratozoospermia; AT = asthenoteratozoospermia; OAT = oligoasthenoteratozoospermia.
[a]Rate of disomy higher than in control.
[b]Rate of disomy lower than in control.
[c]Spermatozoa from the same patient studied twice by two different laboratories.

**Table II.** Combinations of probes and primers* used for in-situ chromosome detection

| | Chromosome | Probe/Primer | Label |
|---|---|---|---|
| FISH | Ch. 1/Ch. 21 | alpha-sat D1Z5/Quint Essential 21 (Oncor) | FITC/Rhodamine |
| | Ch. 4/Ch. 21 | alpha-sat D4Z1/LSI 21 (Vysis) | FITC/SpectrumOrange |
| | Ch. 9/Ch. 15 | alpha-sat D9Z1/alpha-sat D15Z | FITC/Rhodamine |
| | Ch. 13/Ch. 20 | LSI 13/alpha-sat D20Z1 | SpectrumGreen/Rhodamine |
| | Ch. 16/Ch. 1 | alpha-sat D16Z2/alpha-sat D1Z5 | Rhodamine/FITC |
| | Ch.×/Ch.Y/Ch. 9 | alpha-sat CEPX/alpha-sat CEPY/alpha-sat CEP9 | SpectrumAqua/Spectrum Orange/SpectrumGreen |
| PRINS | Ch. 1/Ch. 13 | sat II J52/alpha-sat 13A | FITC/Rhodamine |
| | Ch. 9/Ch. 21 | alpha-sat 9c/alpha-sat 21A | FITC/Rhodamine |
| | Ch. 16/Ch. 1 | alpha-sat 16c/sat II J52 | Rhodamine/FITC |
| | Ch.×/Ch. Y/Ch. 9 | alpha-sat Xc/sat III D600 / alpha-sat 9c | Rhodamine/FITC/Coumarin |

*The sequences of PRINS primers and the optimal technical conditions for use (concentration, annealing temperature) have been detailed elsewhere (Coullin *et al*., 1997)

***FISH labelling***

Two types of probe were used. Detection of chromosomes 1, 4, 9, 15, 16, 20, X and Y was performed using centromeric satellite probes obtained from Oncor (Oncor, Gaithersburg, MD, USA) or Vysis (Vysis, Downers Grove, IL, USA). Locus-specific probes were used for the identification of chromosomes 13 and 21. The specific labelling of chromosome 13 was carried out with the Vysis probe LSI 13, spanning the 13q14 region. This probe was directly labelled with SpectrumGreen. For chromosome 21, we used both the Vysis specific probes LSI 21, spanning the 21q22.13-21q22.2 region and directly labelled with SpectrumOrange, and the Oncor probe Quint-Essential 21 spanning the 21q22.2 region. The FISH reactions were carried out according to the manufacturer's instructions. Probes were applied to the denatured slides in commercially available hybridization solution and the slides were incubated overnight at 37°C. Post-hybridization washes were performed in 50% formamide, 2×standard saline citrate (SSC), pH 7.0, followed by 2 min washes in 2×SSC at 37°C. Biotinylated and digoxigenin-labelled probes were detected with avidin-fluorescein isothiocyanate and antidigoxigenin-rhodamine (Roche Diagnostics, Meylan, France) diluted 1:300 and 1:100 in 4×SSC, 5% skimmed milk respectively. Slides were counterstained with DAPI (0.5 μg/ml) and propidium iodide (0.02 μg/ml), and mountained with Vectashield antifade solution (Vector laboratories, Burlingame, CA, USA).

***PRINS labelling***

Specific oligonucleotide primers for satellite DNA of chromosomes 1, 9, 13, 16, 21, X and Y were used in double or triple PRINS reactions. The specificity and the efficiency of these primers were previously tested in both metaphase and interphase nuclei. Their sequences and optimal technical conditions have been given elsewhere (Coullin *et al*., 1997). PRINS reactions were performed according to our sequential PRINS protocol (Pellestor *et al*., 1996). For each primer, a mix was prepared in a final volume of 50 μl containing the oligonucleotide (50-250 pmol/l), the nucleotide mixture including a labelled dUTP (fluorescein-12-dUTP, rhodamine-4-dUTP or coumarin-6-dUTP), the Taq polymerase buffer and 2 units of Taq DNA polymerase (Roche Diagnostics). The mix was preheated on a waterbath at the annealing temperature of the used primer. The denatured preparation slides were put on the plate block of the thermocycler. The reaction consisted of two programmed steps: 10 min at the specific annealing temperature of the primer and 10–30 min at 72°C in order to allow the nucleotide chain elongation. The slides were not sealed. Both the volume of the mix and the short incubation time prevented the slides from drying during the reaction. The PRINS reaction was stopped by immersing the slides in a stop buffer (500 mmol/l NaCl, 50 mmol/l EDTA, pH 8) at 72°C for 1 min, and the slides were transferred to 2×SSC, 0.5% Tween 20 at room temperature. The slides were then treated for 15 min at 37°C with a dideoxynucleotide mix and 2 units of Klenow enzyme in order to block the free 3′-ends of the elongation fragments generated by the first PRINS reaction. This intermediate step prevented mixing of labelling. The slides were washed and again placed on the plate block of the thermocycler. A second and then a third PRINS reaction mix, involving a primer specific for another chromosome and another labelled dUTP, was applied to the slides and a new reaction was run. Finally, the preparations were washed twice in 2×SSC, 0.05% Tween 20 at 37°C and counterstained with DAPI (0.5 μg/ml) and propidium iodide (0.02 μg/ml) in antifade solution (Vectashield).

***Microscopy***

Slides were viewed under a Leitz fluorescence microscope DRMB (Leica SA, Rueil-Malmaison, France), equipped with a DAPI single band-pass filter, a fluorescein single band-pass filter, a rhodamine single band-pass filter, a fluorescein/rhodamine double band-pass filter, and a triple filter set for simultaneous observation of fluorescein, rhodamine and coumarin signals.

***Sperm scoring***

Slides were analysed by two independent observers, each scoring ~5000 nuclei per slide. Scorers were blinded with respect to the identity of the specimen. The scored sperm nuclei were intact, not overlapping and with a well-defined border. Nuclei that were decondensed to greater than thrice the size of an undecondensed spermatozoa were not scored because of the diffusion of fluorescent signals. The haploid nuclei displayed two spots of different colour corresponding to the two labelled chromosomes. Nuclei were scored as disomic when they displayed a total of three signals. Two of these signals had to be similar in size, colour and intensity, and separated from each other by at least one signal domain diameter. Nuclei were classified as diploid when they contained four distinct fluorescent spots, two for each tested chromosome. We scored for disomy and diploidy but not for nullisomy since failure to detect a signal could be the result of technical artefact as well as non-disjunction.

***Statistical analyses***

Student's *t*-test was used to verify the homogeneity of mean ages in the three groups of subjects. The $\chi^2$-test was used: (i) to check the homogeneity of disomy rates for the 10 chromosomes tested in each sperm sample (interchromosomal variation); (ii) to compare the frequencies of disomy for each chromosome between the 12 subjects (interindividual variation); (iii) to test the variability of the diploidy frequencies. A value of $P < 0.05$ was considered to be significant. The homogeneity of the mean autosomal disomy rates among groups was also tested by the non-parametric Mann-Whitney *U*-test. $P < 0.05$ was considered to be significant.

## Results

A total of 1 193 744 spermatozoa was analysed for this study: 598 543 in group 1, 306 876 in group 2 and 288 325 in the control group. Donor ages ranged from 27 to 44 years with a total mean age of 34 ± 4.6 years. The mean age in the two groups of patients (36.1 ± 4.5 years in group 1; 34 ± 4.6 years in group 2) was not significantly different ($P > 0.05$) when compared to the control mean age (32 ± 5 years).

The labelling efficiency of each probe and primer was determined by scoring the proportion of labelled nuclei on samples of 1000 sperm nuclei. The labelling efficiency was similar in FISH and PRINS assays (from 98.4 to 99.9% of labelled nuclei according to both the sperm samples and the chromosomes analysed), but we noted a slightly higher efficiency in samples from normal men compared to re-arrangement carriers (mean values 99.5 versus 98.7%). For all chromosomes tested, no significant difference ($P > 0.05$) was found between the results of FISH and PRINS assays. The details of disomy rates per subject and chromosome are given in Table III.

In group 1, the incidence of disomy ranged from 0.08 to 0.45% for autosomes and from 0.05 to 0.25% for gonosomes. In group 2, values varied from 0.13 to 0.55% in autosomes and from 0.09 to 0.31% in gonosomes. In the control group, the frequency of disomy ranged from 0.09 to 0.37% for autosomes and from 0.07 to 0.14% for sex chromosomes.

**Table III.** Results of both PRINS and FISH estimates of disomy frequencies for 8 autosomes and gonosomes in sperm from 9 chromosomal rearrangement carriers (groups 1 and 2) and three normal men (Group 3). For chromosome 21, FISH labelling was performed using in paralllel the probes from Oncor (FISHo) and vysis (FISHv).

| Subject | Age | Semen | Chromosome no. | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | | 4 | 9 | | 13 | | 15 | 16 | | 20 | 21 | | | X | | Y | | XY | |
| | | | FISH | PRINS | FISH | FISH | PRINS | FISH | PRINS | FISH | FISH | PRINS | FISH | FISHo | FISHv | PRINS | FISH | PRINS | FISH | PRINS | FISH | PRINS |
| Group 1 | | | | | | | | | | | | | | | | | | | | | | |
| t(14;22) | 34 | N | 0.18 | 0.24 | 0.27 | 0.23 | 0.24 | 0.26 | 0.31 | 0.28 | 0.18 | 0.22 | 0.27 | 0.40 | 0.37 | 0.40 | 0.05 | 0.07 | 0.07 | 0.08 | 0.21 | 0.18 |
| t(1;14) | 44 | N | — | — | 0.29 | 0.14 | 0.20 | 0.20 | 0.20 | 0.17 | 0.21 | 0.24 | 0.26 | 0.26 | 0.29 | 0.24 | 0.09 | 0.10 | 0.11 | 0.09 | 0.10 | 0.12 |
| t(7;9) | 36 | N | 0.12 | 0.10 | 0.25 | — | — | 0.30 | 0.33 | 0.22 | 0.17 | 0.17 | 0.21 | 0.42 | 0.39 | 0.45 | 0.10 | 0.08 | 0.07 | 0.08 | 0.22 | 0.25 |
| t(7;18) | 30 | N | 0.22 | 0.22 | 0.19 | 0.37 | 0.34 | 0.32 | 0.33 | 0.18 | 0.19 | 0.22 | 0.16 | 0.30 | 0.31 | 0.28 | 0.20 | 0.18 | 0.17 | 0.18 | 0.23 | 0.20 |
| t(17;18) | 35 | N | 0.19 | 0.23 | 0.17 | 0.25 | 0.29 | 0.24 | 0.25 | 0.19 | 0.25 | 0.31 | 0.15 | 0.33 | 0.35 | 0.33 | 0.07 | 0.06 | 0.10 | 0.10 | 0.14 | 0.15 |
| inv(2) | 38 | N | 0.15 | 0.13 | 0.20 | 0.22 | 0.22 | 0.18 | 0.20 | 0.08 | 0.14 | 0.17 | 0.20 | 0.35 | 0.38 | 0.37 | 0.12 | 0.10 | 0.08 | 0.11 | 0.20 | 0.17 |
| Group 2 | | | | | | | | | | | | | | | | | | | | | | |
| t(8;13) | 39 | OT | 0.20 | 0.18 | 0.18 | 0.35 | 0.38 | — | — | 0.13 | 0.27 | 0.30 | 0.28 | 0.39 | 0.37 | 0.42 | 0.20 | 0.18 | 0.26 | 0.25 | 0.30 | 0.31 |
| t(13;15) | 30 | OAT | 0.32 | 0.30 | 0.27 | 0.44 | 0.48 | — | — | — | 0.32 | 0.34 | 0.41 | 0.55 | 0.49 | 0.53 | 0.25 | 0.22 | 0.32 | 0.29 | 0.22 | 0.20 |
| t(5;9) | 35 | OAT | 0.24 | 0.28 | 0.30 | — | — | 0.50 | 0.47 | 0.30 | 0.18 | 0.20 | 0.36 | 0.47 | 0.50 | 0.44 | 0.13 | 0.10 | 0.10 | 0.09 | 0.17 | 0.20 |
| Group 3 | | | | | | | | | | | | | | | | | | | | | | |
| C1 | 27 | N | 0.17 | 0.18 | 0.20 | 0.25 | 0.29 | 0.28 | 0.31 | 0.20 | 0.15 | 0.16 | 0.15 | 0.29 | 0.34 | 0.31 | 0.09 | 0.10 | 0.10 | 0.12 | 0.11 | 0.14 |
| C2 | 32 | N | 0.14 | 0.11 | 0.09 | 0.18 | 0.15 | 0.11 | 0.16 | 0.22 | 0.17 | 0.15 | 0.10 | 0.22 | 0.25 | 0.25 | 0.07 | 0.07 | 0.07 | 0.10 | 0.13 | 0.12 |
| C3 | 37 | N | 0.25 | 0.23 | 0.14 | 0.16 | 0.17 | 0.30 | 0.32 | 0.11 | 0.24 | 0.22 | 0.19 | 0.35 | 0.37 | 0.37 | 0.08 | 0.09 | 0.07 | 0.07 | 0.09 | 0.08 |

**Table IV.** Estimate of interchromosomal variations in the frequencies of disomy found in each subject for autosomes and gonosomes, and levels of significance

| | Groups | Autosomes | Gonosomes | Autosomes + gonosomes |
|---|---|---|---|---|
| G1 | t(14;22) | $\chi^2 = 8.88$<br>$P > 0.20$<br>NS | $\chi^2 = 0.33$<br>$P > 0.50$<br>NS | $\chi^2 = 33.35$<br>$P < 0.01$<br>S |
| | t(1;14) | $\chi^2 = 6.20$<br>$P > 0.30$<br>NS | $\chi^2 = 0.19$<br>$P > 0.50$<br>NS | $\chi^2 = 25.17$<br>$P < 0.001$<br>S |
| | t(7;9) | $\chi^2 = 22.83$<br>$P < 0.001$<br>S | $\chi^2 = 0.52$<br>$P > 0.30$<br>NS | $\chi^2 = 36.39$<br>$P < 0.001$<br>S |
| | t(7;18) | $\chi^2 = 14.05$<br>$P > 0.05$<br>NS | $\chi^2 = 0.10$<br>$P > 0.50$<br>NS | $\chi^2 = 17.31$<br>$P < 0.05$<br>S |
| | t(17;18) | $\chi^2 = 11.39$<br>$P > 0.10$<br>NS | $\chi^2 = 0.53$<br>$P > 0.30$<br>NS | $\chi^2 = 31.48$<br>$P < 0.001$<br>S |
| | inv(2) | $\chi^2 = 24.23$<br>$P < 0.01$<br>S | $\chi^2 = 0.18$<br>$P > 0.50$<br>NS | $\chi^2 = 31.27$<br>$P < 0.001$<br>S |
| G2 | t(8;13) | $\chi^2 = 23.20$<br>$P < 0.001$<br>S | $\chi^2 = 0.78$<br>$P > 0.30$<br>NS | $\chi^2 = 23.53$<br>$P < 0.01$<br>S |
| | t(13;15) | $\chi^2 = 11.44$<br>$P < 0.05$<br>S | $\chi^2 = 0.64$<br>$P > 0.30$<br>NS | $\chi^2 = 28.95$<br>$P < 0.001$<br>S |
| | t(5;9) | $\chi^2 = 32.13$<br>$P < 0.001$<br>S | $\chi^2 = 0.39$<br>$P > 0.50$<br>NS | $\chi^2 = 67.41$<br>$P < 0.001$<br>S |
| G3 | C1 | $\chi^2 = 17.39$<br>$P < 0.02$<br>S | $\chi^2 = 0.18$<br>$P > 0.50$<br>NS | $\chi^2 = 34.81$<br>$P < 0.001$<br>S |
| | C2 | $\chi^2 = 12.93$<br>$P > 0.05$<br>NS | $\chi^2 = 0.24$<br>$P > 0.50$<br>NS | $\chi^2 = 20.04$<br>$P < 0.01$<br>S |
| | C3 | $\chi^2 = 22.81$<br>$P < 0.01$<br>S | $\chi^2 = 0.06$<br>$P > 0.50$<br>NS | $\chi^2 = 43.78$<br>$P < 0.001$<br>S |

S = significant.
NS = non-significant.

### *Interchromosomal variation*

Variations of disomy rates were first estimated separately in autosomes and gonosomes. In the pooled analysis, we considered only the total gonosome disomy rate (XX+XY+YY) for statistical analysis. Results are given in Table IV.

In group 1, the interchromosomal variation in autosomes reached a significant level in two cases, the translocation t(7;9) ($\chi^2 = 22.83$; $P < 0.001$) and the inversion inv(2) ($\chi^2 = 24.23$; $P < 0.01$). No significant variation ($P > 0.05$) was observed for disomy rate in gonosomes. Nevertheless, when autosome and gonosome data were pooled, significant variations ($P < 0.05$) were found in all cases.

In group 2, the three rearrangements displayed significant interchromosomal variations ($P < 0.05$) in autosomes as well as in pooled data (autosomes + gonosomes) ($P < 0.01$). No significant variation ($P > 0.05$) was found for disomy in gonosomes.

In the control group, only donor C1 showed significant interchromosomal variations in autosomes ($\chi^2 = 17.39$; $P < 0.02$). Variations were significant ($P < 0.01$) in the three donors when autosome and gonosome data were pooled.

Details of the calculation indicated that in both the translocation t(7;9) and the inversion inv(2), the significance was reached because of the high incidence of disomy scored for chromosome 21. In fact, chromosome 21 consistently displayed a higher disomy frequency (from 0.22 to 0.55%) than the other autosomes tested in both rearrangement carriers and control donors (Table III).

### *Interindividual variation*

In group 1, the disomy values found for each chromosome were homogeneous ($P > 0.10$), except for chromosome 15 ($\chi^2 = 11.37$; $P < 0.05$) and chromosome X ($\chi^2 = 12.93$; $P < 0.05$) (Table V). In group 2, there were significant interdonor differences in the disomy frequency for chromosome 1 ($\chi^2 = 8.41$; $P < 0.02$), chromosome 15 ($\chi^2 = 6.70$; $P < 0.01$) and chromosome Y ($\chi^2 = 10.75$; $P < 0.01$). The control group displayed interindividual variations only for chromosome 1 ($\chi^2 = 6.29$; $P < 0.05$).

When pooling data from groups 1 and 3, significant variation was found only for chromosome 4 ($\chi^2 = 16.54$; $P < 0.05$), whereas consistent interindividual variations ($P < 0.05$) were observed between groups 1 and 2 (for chromosomes 1, 9, 13, 15, 20, X and Y) and between groups 2 and 3 (for all

**Table V.** Estimate of interindividual variations in the frequencies of disomy for autosomes and gonosomes and levels of significance found in the 3 groups of subjects and between each group.

| Chromosome | G1 | G2 | G3 | G1–G3 | G1–G2 | G2–G3 |
|---|---|---|---|---|---|---|
| Ch. 1 | $\chi^2 = 3.99$<br>$P > 0.30$<br>NS | $\chi^2 = 8.41$<br>$P < 0.02$<br>S | $\chi^2 = 6.29$<br>$P < 0.05$<br>S | $\chi^2 = 10.29$<br>$P > 0.10$<br>NS | $\chi^2 = 14.20$<br>$P < 0.05$<br>S | $\chi^2 = 16.72$<br>$P < 0.01$<br>S |
| Ch. 4 | $\chi^2 = 5.47$<br>$P > 0.30$<br>NS | $\chi^2 = 3.54$<br>$P > 0.10$<br>NS | $\chi^2 = 4.22$<br>$P > 0.05$<br>NS | $\chi^2 = 16.54$<br>$P < 0.05$<br>S | $\chi2 = 9.71$<br>$P > 0.30$<br>NS | $\chi^2 = 17.10$<br>$P < 0.05$<br>S |
| Ch. 9 | $\chi^2 = 7.39$<br>$P > 0.10$<br>NS | $\chi^2 = 0.93$<br>$P > 0.30$<br>NS | $\chi^2 = 3.35$<br>$P > 0.10$<br>NS | $\chi^2 = 12.57$<br>$P > 0.05$<br>NS | $\chi^2 = 20.72$<br>$P < 0.01$<br>S | $\chi^2 = 22.26$<br>$P < 0.001$<br>S |
| Ch. 13 | $\chi^2 = 6.14$<br>$P > 0.20$<br>NS | —<br>—<br>— | $\chi^2 = 5.68$<br>$P > 0.05$<br>NS | $\chi^2 = 11.83$<br>$P > 0.10$<br>NS | $\chi^2 = 20.25$<br>$P < 0.01$<br>S | $\chi^2 = 17.94$<br>$P < 0.001$<br>S |
| Ch. 15 | $\chi^2 = 11.37$<br>$P < 0.05$<br>S | $\chi^2 = 6.70$<br>$P < 0.01$<br>S | $\chi^2 = 3.91$<br>$P > 0.10$<br>NS | $\chi^2 = 15.45$<br>$P > 0.05$<br>NS | $\chi^2 = 19.08$<br>$P < 0.01$<br>S | $\chi^2 = 12.05$<br>$P < 0.02$<br>S |
| Ch. 16 | $\chi^2 = 4.53$<br>$P > 0.30$<br>NS | $\chi^2 = 3.24$<br>$P > 0.10$<br>NS | $\chi^2 = 1.74$<br>$P > 0.30$<br>NS | $\chi^2 = 6.97$<br>$P > 0.30$<br>NS | $\chi^2 = 11.73$<br>$P > 0.10$<br>NS | $\chi^2 = 10.76$<br>$P > 0.05$<br>NS |
| Ch. 20 | $\chi^2 = 5.85$<br>$P > 0.30$<br>NS | $\chi^2 = 2.47$<br>$P > 0.20$<br>NS | $\chi^2 = 3.25$<br>$P > 0.10$<br>NS | $\chi^2 = 14.44$<br>$P > 0.05$<br>NS | $\chi^2 = 24.68$<br>$P < 0.01$<br>S | $\chi^2 = 33.91$<br>$P < 0.001$<br>S |
| Ch. 21 | $\chi^2 = 3.88$<br>$P > 0.50$<br>NS | $\chi^2 = 1.59$<br>$P > 0.30$<br>NS | $\chi^2 = 2.45$<br>$P > 0.20$<br>NS | $\chi^2 = 7.33$<br>$P > 0.30$<br>NS | $\chi^2 = 13.54$<br>$P > 0.05$<br>NS | $\chi^2 = 14.67$<br>$P < 0.02$<br>S |
| Ch. X | $\chi^2 = 12.93$<br>$P < 0.05$<br>S | $\chi^2 = 3.76$<br>$P > 0.10$<br>NS | $\chi^2 = 0.55$<br>$P > 0.50$<br>NS | $\chi^2 = 15.30$<br>$P > 0.05$<br>NS | $\chi^2 = 27.12$<br>$P < 0.001$<br>S | $\chi^2 = 18.67$<br>$P < 0.01$<br>S |
| Ch. Y | $\chi^2 = 7.71$<br>$P > 0.10$<br>NS | $\chi^2 = 10.75$<br>$P < 0.01$<br>S | $\chi^2 = 1.33$<br>$P > 0.50$<br>NS | $\chi^2 = 9.52$<br>$P > 0.30$<br>NS | $\chi^2 = 41.58$<br>$P < 0.001$<br>S | $\chi^2 = 32.03$<br>$P < 0.001$<br>S |
| Ch. XY | $\chi^2 = 8.33$<br>$P > 0.10$<br>NS | $\chi^2 = 3.74$<br>$P > 0.10$<br>NS | $\chi^2 = 2.18$<br>$P > 0.20$<br>NS | $\chi^2 = 15.22$<br>$P > 0.05$<br>NS | $\chi^2 = 14.19$<br>$P > 0.05$<br>NS | $\chi^2 = 23.93$<br>$P < 0.001$<br>S |

S = significant; NS = not significant.

**Table VI.** Analysis of the homogeneity of autosomal disomy rates between the three groups of subjects and the levels of significance

| Group | G1 | G2 | G3 |
|---|---|---|---|
| **G3** | U = 1.57<br>$P > 0.10$<br>NS | U = 2.83<br>$P > 0.001$<br>S | – |
| **G2** | U = 0.84<br>$P < 0.05$<br>S | – | – |

S = significant; NS = not significant.

chromosomes tested except for chromosome 16) (Table V). The homogeneity of the mean frequencies of autosomal disomy among groups was also tested by the non-parametric Mann-Whitney *U*-test (Table VI). These data confirmed the significant difference in disomy rates between groups 1 and 2 ($U = 0.84$; $P < 0.05$) as well as between groups 2 and 3 ($U = 2.83$; $P < 0.001$).

### *Frequency of diploidy*

The mean incidences of diploidy for each subject are given in Table VII. The values ranged from 0.08 to 0.87%. The statistical analysis of these data revealed a highly significant difference ($\chi^2 = 40.63$; $P < 0.001$). The frequency of diploidy was significantly higher in patients from groups 1 and 2 compared to controls ($P < 0.05$) but was not significantly different ($P > 0.05$) between groups 1 and 2.

## Discussion

Whereas the interchromosomal effect is a well-described mechanism in *Drosophila* (Grell, 1962; Portin and Rantanen, 1990), the occurrence of this phenomenon is still an open question in mammals because of the difficulty of obtaining experimental and direct data on gametes. Our approach combined FISH and PRINS labelling techniques. This gave us an internal control on the efficiency of each labelling used as well as on the accuracy of the data obtained. To improve the relevance of our study, both autosomes and gonosomes were considered in our experimental design. Also, we chose to investigate two distinct groups of chromosomal rearrangement carriers according to the semen quality in order to consider the relationship between meiotic disturbance and semen parameters in the occurrence of the interchromosomal effect.

All the control values are within the limits of the disomy rates usually reported for normal subjects (Guttenbach *et al.*, 1997). Both interchromosomal and interindividual variations displayed different patterns according to the semen quality. In

**Table VII.** Frequencies of diploidy in sperm of the 9 carriers of chromosomal rearrangements (groups G1 and G2) and the 3 control donors (Group G3)

| Group | G1 | | | | | | G2 | | | G3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | t(14;22) | t(1;14) | t(7;9) | t(7;18) | t(17;18) | inv(2) | t(8;13) | t(13;15) | t(5;9) | C1 | C2 | C3 |
| % diploidy | 0.33 | 0.19 | 0.36 | 0.11 | 0.87 | 0.42 | 0.15 | 0.44 | 0.37 | 0.21 | 0.08 | 0.35 |
| Mean % diploidy | 0.38 ± 0.24 | | | | | | 0.31 ± 0.14 | | | 0.21 ± 0.13 | | |

the control group and patients with normal semen parameters (group 1), interchromosomal variations were limited in autosomes and non-existant in gonosomes. Variations were highly significant in patients with abnormal sperm (group 2), or globally when autosome and gonosome data were pooled. Chromosome analysis of spermatozoa from infertile males has shown that these patients present a high variability in the behaviour of chromosome segregation during meiosis (Miharu *et al*., 1994; Moosani *et al*., 1995). This variability may be linked to the multifactorial nature of male infertility, but in cases of subjects combining poor semen quality and chromosomal rearrangement, interchromosomal variations in disomy rates may directly reflect meiotic impairment. It might be suggested that both fluctuation in disomy and degree of semen parameter abnormalities are conditioned by the chromosomes involved in the rearrangement.

The analysis of our results indicates that the sex chromosomes and chromosome 21 have a higher frequency of disomy than the other chromosomes tested. This finding confirms previous reported data (Spriggs *et al*., 1995; Blanco *et al*., 1996; Pellestor *et al*., 1996). Increase of disomy for chromosome 21 and sex chromosomes was not specific to rearrangement carriers but observed in all subjects tested. This observation disagrees with the hypothesis of a relationship between the increase of non-disjunction for these chromosomes and the presence of a chromosomal rearrangement, but rather is consistent with the suggestion that sex chromosomes and chromosome 21 might be more susceptible to non-disjunction than other chromosomes. Data from spontaneous abortions and livebirths support this idea (Jacobs, 1992).

Interindividual variations in disomy rate are restricted to a few chromosomes in each group of subjects (Table V). The significant variations concern chromosomes 1, 15 and sex chromosomes. Interdonor variations for the same chromosome have been reported (Spriggs *et al*., 1995, 1996). These findings are too limited to drawn conclusions on interindividual fluctuations of non-disjunction events. However, molecular approaches offer the possibility to investigate this question, in particular with regard to the recent data on the importance of interindividual difference in the rate of total genomic recombination and genetic control of crossover frequency (Brown *et al*., 2000).

According to our finding, rearrangement carriers with normal semen parameters displayed no significant difference in disomy rates with the normal subjects, except for chromosome 4. The homogeneity of disomy rate between the two groups was confirmed by two statistical tests (Tables V and VI). This result provided no direct evidence for the occurrence of an interchromosomal effect in sperm of fertile rearrangement carriers. On the contrary, infertile men carrying a translocation (group 2) showed significant interindividual variation when compared to control donors. All chromosomes tested, except chromosome 16, displayed a significant difference in disomy rate, and significant variations were also found for several chromosomes between groups 1 and 2. These results support the hypothesis of an interchromosomal effect restricted to the cases of translocation carriers with abnormal semen parameters. This is consistent with a study (Vegetti *et al*., 2000) which found a higher incidence of diploidy and aneuploidy in sperm of infertile men with abnormal karyotypes compared to normal controls. The analysis of several chromosomes belonging to different chromosomal groups does not support the hypothesis of an effect restricted to a particular chromosome group. However, this result needs to be confirmed by similar analysis on other chromosomes. Several studies have reported a correlation between poor semen quality and increased frequency of aneuploidy in spermatozoa (Moosani *et al*., 1995; Rives *et al*., 1999). In cases of rearrangement carriers, such a correlation may depend on the chromosomes involved in the rearrangement and their breakpoints. Pachytene configurations of some structural rearrangements may more directly affect the progression of meiosis and the production of gametes when they are closely associated with the sex vesicle or other autosomal bivalent (Guichaoua *et al*., 1991; Navarro *et al*., 1991). The length of the translocated segments could be an essential factor in the occurrence of an association with another bivalent, and facilitate the malsegregation of other chromosome pairs (Blanco *et al*., 2000). These associations may lead to synaptic anomaly in various chromosome pairs and spermatogenesis impairment (Luciani *et al*., 1984; Batanian and Hultén, 1987). The influence of defective pairing of some chromosomes on the segregation of other chromosomes has been evidenced directly in human trisomic oocytes (Cheng *et al*., 1998) and XO mice oocytes (Hunt *et al*., 1995). However, it seems that the female meiotic checkpoint process was less efficient than the male mechanism (LeMaire-Adkins *et al*., 1997). Disturbances in the pairing process can adversely block the male meiotic progression, which can limit the occurrence of an interchromosomal effect in mature spermatozoa. An interesting observation is also the higher frequency of diploidy found in spermatozoa from infertile rearrangement carriers, which can also be related to synaptic abnormalities. Elevated frequency of diploidy in spermatozoa of rearrangement carriers has been reported in several studies (Table I) and could also reflect a gross disruption of meiosis with the absence of cytokinesis. If both timing accuracy of chromosome pairing

and recombination act as checkpoints in meiosis, the presence of abnormal pairing configurations could upset the progression through meiosis and lead to the increased production of diploid cells (Goldman and Hultén, 1993; Kleckner, 1996).

This interchromosomal effect may be restricted to males since the sterilizing effect of translocations appears to be limited to male heterozygotes. No effects on germ-cell development have been found in women carrying translocations.When compared to the high maternal contribution to chromosomal abnormalities of the human conceptus (Pellestor, 1991; Nakaoka *et al*., 1998), the increase of disomy in spermatozoa of infertile carriers of rearrangements may be of limited clinical releveance, except for sex chromosomes where male non-disjunction is in the same order of magnitude as in female meiosis. However, these data need to be taken into account for counselling of couples with a male partner carrying a translocation before treatment of infertility by intracytoplasmic sperm injection (ICSI), since a higher incidence of sex chromosomal aneuploidy of paternal origin has been reported in children conceived by ICSI (In't Veld *et al*., 1995).

Most cases of interchromosomal effect reported concerned fortuitous observations of trisomy 21 in the lineage of balanced rearrangement carriers. As pointed out elsewhere (Lindenbaum *et al*., 1985), a significant prevalence of rearrangements in parents of trisomy 21 offspring does not necessarily imply that these rearrangements increase the likehood of parental meiotic non-disjunction. In the case of fertile male rearrangement carriers, it has been demonstrated (Schinzel *et al*., 1992) that such associations are fortuitous, since the concomittant non-disjunctions originated in the mother. The potential high incidence of balanced rearrangements in the parents of trisomic 21 offspring could largely result from the higher age of parents due to the difficulty which carriers of chromosomal rearrangements and their partners have to conceive and to carry a pregnancy to term. In fact, the risk of association between a balanced chromosomal rearrangement and trisomy 21 in fertile couples may be assumed to be very low and to correspond to the risk for trisomy 21 in a normal population.

Various lines of evidence do not support the concept of a paternal interchromosomal effect for carriers of structural chromosomal aberrations. Our data indicate that the occurrence of interchromosomal effect may be restricted to the infertile rearrangement carriers. We can assume that the choice of the rearrangements investigated, the use of two different labelling techniques and the number of chromosomes analysed confers a good reliability to the present results. The only restrictive parameter which was not possible to control was the large diversity of structural rearrangements in humans. This is particularly true in reciprocal translocations since almost all observed translocations are unique in terms of configuration and meiotic behaviour. To date, sperm analysis has revealed that the frequecy of chromosomally unbalanced sperm related to translocation varied dramatically from 0 to 77% (Martin and Spriggs, 1995; Pellestor *et al*., 1997). The production of unbalanced gametes depends on various criteria involving the position of breakpoints, the size of the translocated segments and the genetic background. All these factors may affect the occurrence of an interchromosomal effect. Consequently, the present findings need to be confirmed by the sperm analysis of other rearrangements. Nevertheless, the association of poor semen parameters and increased aneuploidy in conjunction with a structural rearrangement probably reflects important meiotic disturbances and should consequently be considered when counselling males heterozygous for a chromosomal rearrangement.

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