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Review

Aneuploidy in human spermatozoa: FISH analysis in men with constitutional chromosomal abnormalities, and in infertile men

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Reproductive difficulties are associated intimately with cytogenetic abnormalities. This article reviews multicolour fluorescence *in situ* hybridization studies on spermatozoa from men with constitutional chromosomal abnormalities and the consequences for spermatozoa, and on chromosomal abnormalities in the spermatozoa of infertile men who have normal somatic karyotypes. In 47,XYY men, the frequencies of 24,XY and 24,YY spermatozoa appear to be ⩽ 1%. Klinefelter (47,XXY) and mosaic Klinefelter patients had sperm aneuploidy frequencies of 2–25% and 1.5–7.0%, respectively. Robertsonian translocation carriers had 3–27% spermatozoa unbalanced for the chromosomes involved in the translocation, with a possible modest interchromosomal effect, but none of the increased frequencies of chromosomal disomy approached 1%. The frequency of chromosomally unbalanced spermatozoa in reciprocal translocations averages 50%, is strongly dependent on the chromosomes involved in the individual translocation, and may be slightly increased as a result of a small interchromosomal effect. Infertile men with a normal karyotype and low sperm concentration or certain types of morphologically abnormal spermatozoa have a significantly increased risk of producing aneuploid spermatozoa, particularly for the sex chromosomes. An increased risk of sperm aneuploidy was not observed in infertile men with poor sperm motility or in those with a normal karyotype and normal semen parameters.

Difficulties with reproduction have been associated with cytogenetic abnormalities since the introduction of human chromosome karyotyping. In recent years, reproductive difficulties have been associated not only with somatic chromosomal abnormalities, but also with cytogenetic abnormalities in the germ cells of infertile individuals with a normal constitutional karyotype. Reproductive difficulties for both types of individuals include an increased risk of pregnancy loss and the birth of children with mental and physical disabilities. Research in this area has become more clinically relevant in the past few years with the advent of intracytoplasmic sperm injection (ICSI). ICSI has been extremely successful for the treatment of male infertility but transmission of cytogenetic defects to offspring is a major concern and in the past few years has been demonstrated to be a reality. Fluorescence *in situ* hybridization (FISH) analysis involves hybridization of chromosome-specific DNA probes labelled with fluorochromes to complementary DNA sequences on target chromosomes, followed by detection of the bound probes under a fluorescence microscope. FISH analysis of decondensed sperm heads allows the study of thousands of spermatozoa (at present 10^4 spermatozoa per individual per probe are analysed in most laboratories) in a relatively short period. This is especially important for the accurate evaluation of the incidence of aneuploidies in human spermatozoa, because the frequency of disomic spermatozoa for most chromosomes is lower than 0.5% in normal men (Shi and Martin, 2000a). As well as analysis of adequate numbers of spermatozoa, it is important to use multicolour FISH for reliable results. Two-colour FISH is required for autosomes and three-colour FISH for sex chromosomes to distinguish diploidy (for example, 46,XY with two probes for the sex chromosomes and one probe with two signals for an autosome) from disomy (for example, 24,XY with two probes for the sex chromosomes and one probe with one signal for an autosome). The effects of donor age and lifestyle factors on the frequency and distribution of chromosomal abnormalities in the spermatozoa of normal men have been reviewed elsewhere (Shi and Martin, 2000a). The present article reviews studies on the consequences of constitutional chromosomal abnormalities observed in spermatozoa and chromosomal abnormalities in the spermatozoa of infertile men who have normal somatic karyotypes.

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Constitutional chromosomal abnormalities

Studies in infertile men have demonstrated that 2–14% have constitutional chromosomal abnormalities. The incidence of chromosomal aberrations is dependent on the definition of 'infertility', and is approximately 2% in males with combined indications of infertility (Meschede *et al.*, 1997), 5% in oligozoospermic men and 14% in azoospermic men (Johnson, 1998). These frequencies are all considerably higher than the population incidence of 0.7% in newborns (Thompson *et al.*, 1986). The most common types of karyotypic abnormality detected include sex chromosomal abnormalities and Robertsonian translocations.

Sex chromosomal abnormalities

47,XYY. In 1988, 75 sperm karyotypes from a 47,XYY male were studied using the hamster oocyte–human sperm fusion technique and no spermatozoa disomic for the sex chromosomes were found (Benet and Martin, 1988). However, when FISH analysis was performed on the same man, a significantly increased frequency of XY disomy (0.6%) was observed in more than 10^4 spermatozoa studied (Martin *et al.*, 1999). Similar experimental protocols revealed increased frequencies for 24,YY and 24,XY spermatozoa in more than 10^4 spermatozoa in another 47,XYY man (Shi and Martin, 2000b). Similar FISH studies on 47,XYY men by other laboratories have demonstrated increased frequencies of spermatozoa with two sex chromosomes ranging from 0.3 (Chevret *et al.*, 1997) to 15% (Mercier *et al.*, 1996) (for a review, see Shi and Martin, 2000b). These findings indicate that XYY cells are able to progress through meiosis and produce 24,YY or 24,XY spermatozoa (Hultén, 1970; Hultén and Pearson, 1971). The birth of 47,XYY children to 47,XYY males also supports this hypothesis (Boucharlat and Jalbert, 1969; Sundequist and Hellstrom, 1969). However, most children of 47,XYY males are normal and only 1% or less of 24,YY and 24,XY spermatozoa were found in 47,XYY men after three-colour FISH (see Shi and Martin, 2000b). These results indicate that the extra Y chromosome is lost during meiosis in most XYY cells since the majority of spermatozoa are chromosomally normal.

In theory, it is possible that abnormalities in the segregation of one chromosome affect the segregation of other chromosomes (termed an interchromosomal effect). For example, aneuploidy of the sex chromosomes might affect the segregation of an autosome. Therefore, a number of studies have investigated the normality of other chromosomes as well as the sex chromosomes. Two-colour FISH analysis of spermatozoa from two 47,XYY men did not show a significant increase in the frequency of disomy 21 (Martin *et al.*, 1999; Shi and Martin, 2000b). Similarly, for all six autosomes assessed in nine 47,XYY men, frequencies of disomic spermatozoa did not show any significant difference from concurrent controls, with the exception of one patient who had an increased frequency of chromosome 13 aneuploidy (Shi and Martin, 2000b). Thus, if an interchromosomal effect occurs during meiosis of 47,XYY males, it would affect specific chromosomes in a minority of men.

47,XXY. Patients with Klinefelter syndrome (47,XXY) or mosaic variants of Klinefelter syndrome have greatly impaired spermatogenesis, with severe oligozoospermia or azoospermia. Nevertheless, these men are candidates for ICSI, particularly with the new methods used for recovering testicular spermatozoa. Studies on sperm chromosomes from men with Klinefelter syndrome have also demonstrated that the extra sex chromosome appears to be eliminated during spermatogenesis. There have been multicolour FISH studies on spermatozoa from seven men with mosaic Klinefelter syndrome (Table 1). The frequency of sperm aneuploidy in the patients varied from 1.5 (Lim *et al.*, 1999) to 7% (Kruse *et al.*, 1998), which was significantly higher than in the concurrent controls. There have also been studies of seven men who appear to have a non-mosaic 47,XXY karyotype, with higher sperm aneuploidy frequencies varying from 2 (Rives *et al.*, 2000) to 25% (Estop *et al.*, 1998a) (Table 1).

There have been several attempts to establish pregnancies using spermatozoa from men with Klinefelter syndrome for ICSI. To date, six normal children have been born (Bourne *et al.*, 1997; Reubinoff *et al.*, 1998; Ron-El *et al.*, 1999, 2000a) and three chromosomally abnormal embryos have been reported: one with 47,XXY at amniocentesis (Ron-El *et al.*, 2000b) and two embryos with chaotic chromosome constitutions, including XXY, discovered by preimplantation genetic diagnosis (Reubinoff *et al.*, 1998).

It appears that men with Klinefelter syndrome who undergo ICSI have a significant risk of producing offspring with sex chromosomal aneuploidies and therefore genetic counselling is appropriate.

Translocations

Robertsonian translocations. Robertsonian translocations, with a translocated chromosome composed of the long arms of two acrocentric chromosomes, result in balanced Robertsonian translocation carriers with 45 chromosomes. Robertsonian carriers with fusions between chromosomes 13 and 14 are very common among infertile men. When the chromosomes pair during meiosis, the translocated chromosome and its homologues do so as a trivalent. The resulting gametes can be chromosomally normal or aneuploid with an extra or missing chromosome q arm.

Sperm karyotyping studies (using the hamster oocyte system) have demonstrated that the frequency of chromosomally unbalanced spermatozoa is lower than was predicted, with only 3–27% of spermatozoa unbalanced because of the translocation (Martin, 1995). Three recent FISH studies have also reported the segregation of two Robertsonian translocations in four men: two with t(14q;21q) (Rousseaux *et al.*, 1995a; Honda *et al.*, 2000) and two with t(13q;14q) (Escudero *et al.*, 2000). Similar

Table 1. Summary of normal and disomic sperm frequencies detected by multicolour fluorescence *in situ* hybridization (FISH) in indivdual patients with Klinefelter syndrome

Karyotype of patients	Number of spermatozoa scored	Normal spermatozoa (%)		Disomy frequency (%)							Study
		X-bearing	Y-bearing	1	8	12	18	XX	YY	XY	
47,XXY											
47,XXY	26	20.8	29.2				4.2			25	Estop *et al.,* 1998a
47,XXY	10 000	51.87	24.60[a]					6.92	0.21	14.58[a]	Foresta *et al.,* 1998
47,XXY	10 000	56.00	28.63[a]					3.34	0.09	10.03[a]	Foresta *et al.,* 1998
47,XXY	25	48.0	24.0					0	0	12	Foresta *et al.,* 1999
47,XXY	20	45.0	25.0					0	0	20	Foresta *et al.,* 1999
47,XXY	2206	43.43[b]	48.82[b]	0.50				1.22[c]	0.09	1.36[c]	Guttenbach *et al.,* 1997b
47,XXY	10 123	49.60	48.33			0.42[d]		0.45[d]	0.37[d]	0.54[d]	Rives *et al.*, 2000
Mosaic 47,XXY											
46,XY (1.7%)/47,XXY (98.3%)	358	50.28	44.13					1.12	0.56	2.23	Bielanska *et al.*, 2000
46,XY (90%)/47,XXY (10%)	27 097	52.78[a]	43.88	0.18				0.11[e]	0.003[e]	2.09[e]	Chevret *et al.,* 1996
46,XY (6.1%)/47,XXY (91.8%)/ 48,XXXY (2.1%)	202	50.5	42.1					2.0	0	5.0	Kruse *et al.,* 1998
46,XY (70%)/47,XXY (30%)	1701	46.74	49.62				0.71[d]	0.29[f]	0.06	0.41[e]	Lim *et al.,* 1999
46,XY (30%)/47,XXY (70%)	3581	50.5[b]	42.81[b]		0.32		0.26	0.71[e]	0	1.3[e]	Morel *et al.*, 2000
46,XY (22%)/47,XXY (78%)	1831	47.1	44.88		0.20		0.10	0.86[e]	0.86[eg]	1.73[e]	Morel *et al.*, 2000
46,XY (95%)/47,XXY (5%)	20 814	49.68	48.90			0.49[d]		0.24[e]	0.20	0.62[d]	Rives *et al.*, 2000

[a]$P < 0.05$, Student's *t* test, compared with concurrent controls; also significantly different from the expected 1:1 for the sex ratio.
[b]$P < 0.01$, significantly different from the expected 1:1, chi-squared test.
[c]$P < 0.05$, [d]$P < 0.0001$, [e]$P < 0.001$, [f]$P < 0.01$, compared with concurrent controls; [g]compared with patient immediately above, chi-squared test.

results were found for the patterns and frequencies of the translocation chromosome segregation, with 11–12% chromosomally unbalanced spermatozoa for t(14q;21q) and 19–23% for t(13q;14q). These results corroborate the data from karyotyping analysis of spermatozoa from one t(14q;21q) and three t(13q;14q) carriers, in which most spermatozoa were normal or balanced, and 5–20% were unbalanced for the chromosomes involved in translocation (Balkan and Martin, 1983; Pellestor *et al.*, 1987; Martin, 1988; Ogawa *et al.*, 2000). Similarly, analysis of cells at meiotic prophase in heterozygous carriers of a Robertsonian translocation showed a predominance of the *cis*-configuration of the meiotic trivalent structure hypothesized to lead to alternate segregation and normal or balanced chromosomes (Vidal *et al.*, 1982; Templado *et al.*, 1984).

An interchromosomal effect occurs when the translocated chromosome has an effect on the segregation of chromosomes not involved in the translocation, for example, an increased frequency of trisomy 21 associated with t(13q;14q). No significant interchromosomal effect has been detected in Robertsonian translocation carriers detected by sperm karyotyping (for review, see Guttenbach *et al.*, 1997a). However, because the sample sizes are small (24–149 spermatozoa) when this technique is used, a small interchromosomal effect would be overlooked.

Interchromosomal effects on meiotic segregation of the sex chromosomes in three Robertsonian translocation carriers have been investigated using three-colour FISH (Rousseaux *et al.*, 1995a; Vegetti *et al.*, 2000). Vegetti *et al.* (2000) found a significant increase in the frequency of XY disomy in two carriers with t(14;21)(q10;q10) and t(13;15)(q10;q10) when compared with concurrent control donors, but Rousseaux *et al.* (1995a) did not observe a significant increase in the sex chromosome disomy frequency in a t(14q;21q) carrier.

In addition, interchromosomal effects of a Robertsonian translocation on autosomes have been investigated by multicolour FISH in spermatozoa from four carriers in three studies. Vegetti *et al.* (2000) reported an increased frequency of disomy 18 and 13 in a t(14;21)(q10;q10) carrier, and of disomy 18 and 21 in a t(13;15)(q10;q10) carrier. Rousseaux *et al.* (1995a) demonstrated an increase in the frequency of disomy 1 in a t(14q;21q) carrier (0.67 versus 0.20%, $P < 0.001$). Conversely, Blanco *et al.* (2000) did not detect a significant increase in frequency of disomy 6 or 21 in a t(13;22)(q10;q10) carrier, when compared with concurrent controls. Thus, these preliminary data from FISH analyses indicate some modest interchromosomal effects, but none of the increased frequencies of chromosomal disomy approached 1%.

In summary, the risk of an unbalanced karyotype resulting from a Robertsonian translocation in spermatozoa is between 3 and 27%, depending on the specific translocation. The final outcome can be a spontaneous

abortion or a chromosomally abnormal child, depending on the lethality of the chromosome involved. Many of these unbalanced chromosome constitutions are not viable since only 1–2% of paternally derived Robertsonian translocations are unbalanced at prenatal diagnosis (Boué and Gallano, 1984). Although the risks are low, prospective parents should be informed of them, as the abnormalities can be devastating. In addition, a Robertsonian translocation between two of the same chromosome, for example a t(13q;13q) homozygote, would produce only disomy 13 or nullisomy 13 spermatozoa. ICSI would not be an alternative for such an individual, as all embryos would be trisomy 13 or monosomy 13, with no hope of long-term survival. A case with this particular problem was discovered in the Netherlands after three unsuccessful ICSI attempts (In't Veld *et al.*, 1997) and a male with a t(13q;13q) has also been observed in the authors' cohort of infertile men contemplating ICSI.

Reciprocal translocations. Reciprocal translocations are an exchange of chromosome material between arms of any two chromosomes, and the risks of chromosomally unbalanced offspring from male carriers are higher than they are for Robertsonian translocations. Sperm karyotyping studies of over 30 reciprocal translocation carriers have demonstrated that 19–77% of spermatozoa are chromosomally unbalanced, and an average of about 50% are chromosomally abnormal (Martin and Spriggs, 1995).

During meiosis I, quadrivalents are formed between translocated chromosomes and their normal homologues in reciprocal translocation carriers. In alternate segregation, the translocated chromosomes segregate to one pole and the normal homologues to the other, producing balanced and normal gametes, respectively. Chromosomally unbalanced gametes are produced in adjacent 1 and adjacent 2 segregations (in which homologous centromeres move to opposite poles and the same poles, respectively) and in 3:1 segregation. FISH analysis of interphase spermatozoa using two centromeric probes specific to the involved chromosomes does not allow differentiation of alternate and adjacent 1 segregants; the large number of spermatozoa analysed allows detection of the rarer adjacent 2 and 3:1 segregations. However, it is possible, using centromeric probes combined with locus-specific probes, to detect all types of segregations using FISH analysis. In comparison with the labour-intensive and time-consuming sperm karotyping analysis, FISH analysis of decondensed sperm heads allows the analysis of very large numbers of spermatozoa.

A total of 159 920 spermatozoa from 19 reciprocal translocation carriers has been analysed using the multicolour FISH technique for the segregation of the chromosomes involved in translocations (Table 2). The segregation pattern for an affected chromosome is dependent upon the chromosome involved. The frequencies for each type of segregation are very similar between individuals carrying translocations involving the same chromosomes, for example, two donors with t(3;9) (Honda *et al.*, 1999), two t(6;11) donors (Rousseaux *et al.*, 1995b) and two t(11;22) donors (Estop *et al.*, 1999; Van Assche *et al.*, 1999). Conversely, among pairs of donors in which one of the translocated chromosomes is the same and the other is in the same chromosome group, for example, in t(1;10) versus t(1;11), t(5;7) versus t(5;8), t(7;8) versus t(7;9), and t(7;9) versus t(8;9), the segregation patterns are very different. These results corroborate earlier observations from sperm karyotyping that the risk of producing chromosomally unbalanced gametes depends greatly on the chromosomes involved in the individual translocation (for a review, see Guttenbach *et al.*, 1997a).

In most reciprocal translocation carriers, alternate segregants are the most common, occurring at approximately 44–51%; adjacent 1 segregants have a frequency of 16–40%; adjacent 2 segregants are less common with a mean frequency of 9% (range 3–16%); and 3:1 segregants occur at a mean frequency of 11% with a wide range of 2–40% (Table 2). These results are in agreement with those from sperm karyotyping analysis (Martin and Spriggs, 1995; Guttenbach *et al.*, 1997a), although it has been suggested that under certain conditions, this typical segregation pattern does not occur. Jalbert *et al.* (1980) postulated that in the presence of a quadrivalent with unequal sizes of translocated segments and with the participation of an acrocentric chromosome, a 3:1 segregation would preferentially occur. The common t(11; 22)(q23; q11) has a higher proportion of 3:1 segregants among chromosomally unbalanced children. However, in an early study of sperm karyotyping, a t(11;22) heterozygote was shown to have approximately equal frequencies of adjacent 1, adjacent 2 and 3:1 segregations, indicating that the other segregants are formed but do not survive embryonic development (Martin, 1984). Similarly, a recent study of a t(11;22) male by electron microscope analysis of the synaptonemal complexes and dual-colour FISH in metaphase I and II did not demonstrate a preferential 3:1 segregation, again indicating post-zygotic selection as the reason for the predominance of 3:1 segregants at birth (Armstrong *et al.*, 2000). Two other FISH studies (Estop *et al.*, 1999; Van Assche *et al.*, 1999) have shown more frequent 3:1 segregation in spermatozoa but, as pointed out by Armstrong *et al.* (2000), the majority of these spermatozoa were monosomic with only 5–7% carrying the extra chromosome that would be consistent with no preference toward a 3:1 segregation.

A total of 323 931 spermatozoa from 29 reciprocal translocation carriers has been analysed for an interchromosomal effect of the translocation on uninvolved chromosomes using the multicolour FISH approach (Table 3). Comparisons of disomic spermatozoa frequencies for chromosomes not involved in translocations between each translocation carrier and the concurrent controls were carried out using a 2×2 chi-squared test. An increased frequency was observed for at least one type of disomy in 58% of carriers (17/29), while a decreased sex chromosome

Table 2. Segregation of chromosomes in spermatozoa from reciprocal translocation heterozygotes studied by multicolour fluorescence *in situ* hybridization (FISH) or primed *in situ* (PRINS)

Translocation	Number of cells scored	Segregation pattern (%)						Reference
		Normal	Balanced	Adjacent 1	Adjacent 2	3:1	4:0	
t(1;10)(p22.1;q22.3)	4036	90.5[a]			4.9	3.9	–	Van Hummelen *et al.,* 1997
t(1;11)(p36.3;q13.1)	13 071	82.5[a]			8.3	9.2		Spriggs and Martin, 1994
t(2;14)(p23.1;q31)	4610	88[a]			5.5	6.5	–	Rousseaux *et al.,* 1995b
t(2;18)(p21;q11.2)	3139	45[b]		30.8	10.9	13.2	0	Estop *et al.,* 1998b
t(3;9)(q26.2;q32)	10 022	88.4[a]			5.4	5.9		Honda *et al.,* 1999
t(3;9)(q25;q32)	10 278	89.2[a]			6.0	4.5		Honda *et al.,* 1999
t(3;11)(q27.3;q24.3)	4029	44.3[b]		15.9	6.6	28.9	0.8	Martini *et al.,* 1998
t(5;7)(q21;q32)	296	28	21.6	32.5	16.2	1.7	–	Cifuentes *et al.,* 1999
t(5;8)(q33;q13)	10 344	46.5[b]		39.5	7.2	6.8	–	Blanco *et al.,* 1998
t(6;11)(q14;p14)	13 968	86[a]			10.5	3.5	0.1	Rousseaux *et al.,* 1995b
t(6;11)(q14;p14)	13 876	87.4[a]			9.0	3.1	0.5	Rousseaux *et al.,* 1995b
t(7;8)(q11.21;cen)	34 527	30.4	26.3	25.1	11.1	7.1		Mercier *et al.,* 1998
t(7;9)(q33;p21)[c]	10 658	86[a]			10.4	3	~0.5	Pellestor *et al.,* 1997
t(7;18)(q35;q11)[c]	10 462	77.2[a]			15.9	6.6	~0.4	Pellestor *et al.,* 1997
t(8;9)(q24.2;q32)	3118	45.4[b]		41.9	3.17	9.6	0	Estop *et al.,* 1998b
t(10;12)(q26.1;p13.3)	10 049	84.3[a]			11	4.4		Estop *et al.,* 1997
t(11;22)(q23;q11)	1925	27.4[b]		17.6	12.5	40.1	–	Estop *et al.,* 1999
t(11;22)(q25;q22)	1012	29.1[b]		21.2	15.2	34.6		Van Assche *et al.,* 1999
t(Y;16)(q11.21;q24)	500	51[b]		36		12		Giltay *et al.,* 1999
Mean	8417	38.6	24	28.9	9.4	10.8	0.3	

[a]Alternate and adjacent 1 segregants cannot be differentiated.
[b]Normal and balanced segregants cannot be differentiated.
[c]PRINS labelling technique. All other studies were FISH.

disomy was reported in 3% of carriers (1/29) (Cifuentes *et al.*, 1999). In the remaining 11 of 29 carriers (38%), the frequencies of disomy investigated were in the control range (Table 3). No interchromosomal effect has been observed in sperm karyotyping analyses of 4445 spermatozoa from 36 reciprocal translocation carriers (for a review, see Guttenbach *et al.*, 1997a). However, the smaller number of spermatozoa analysed per donor probably accounts for the failure to detect increased hyperhaploidy frequency by karyotype analysis.

The presence of an interchromosomal effect and the increase in the disomy frequency when there is an interchromosomal effect, varied greatly among the chromosomes studied. For example, an interchromosomal effect with an increased disomy frequency of 1.5–2.5 times that of concurrent controls was observed for chromosome 1 in all of three carriers analysed. An interchromosomal effect for chromosome 21 was observed in 9 of 22 carriers (41%), with disomy frequencies 1.4–6.6 times those of control donors. Sex chromosome disomy in 8 of 29 (28%) carriers was 1.7–6.0 times greater than that seen in controls, and no interchromosomal effect at all was detected for chromosomes 6, 12, 15 and 17. These results indicate that during meiosis, segregation of certain chromosomes such as chromosomes 1 and 21 are more frequently affected by the presence of a translocation. Studies of the interchromosomal effect on more chromosomes in reciprocal translocation carriers are needed to confirm or refute these findings.

Both sperm karyotype and FISH data indicate that the frequency of chromosomally unbalanced spermatozoa from reciprocal translocation heterozygotes is, on average, 50% with possibly a small additional risk owing to an interchromosomal effect. Many of these imbalances are not compatible with survival, and the average frequency of paternally derived translocation imbalances at prenatal diagnosis is 12% (Boué and Gallano, 1984) (the same frequency as maternally derived translocation imbalances). However, some translocations have higher risks of imbalance and survival, and all surviving fetuses have serious consequences including mental and physical disabilities. A number of fetuses with unbalanced segregations of reciprocal translocations have been reported after ICSI (Meschede *et al.*, 1997; Belin *et al.*, 1999). These significant risks indicate that chromosome karyotyping is important for all men contemplating ICSI, and this should be followed by genetic counselling if an abnormality is discovered.

Table 3. Sperm disomy frequencies detected by multicolour fluorescence *in situ* hybridization (FISH) in reciprocal translocation carriers and concurrent normal controls

Carrier/Control	Age of donors (years)	Number of cells scored	Disomy frequency (%)												Reference
			1	6	12	13	15	17	18	21	XX	YY	XY	X + Y	
t(5;8)(5q33;8q13)	42	10059		0.12						0.23					Blanco *et al.*, 1998
Control	23–37	20077		0.14[a]						0.37					
t(5;7)(q21;q32)	–	20118		0.09					0.03	0.23	0.01	0.07	0.07	0.15[b]	Cifuentes *et al.*, 1999
Control	–	71445		0.14[a]					0.10	0.37	0.10	0.16	0.11	0.37	
t(2;18)(p21;q11.2)	32	20030				0.13[b]				0.21	0.03	0.02	0.09	0.14	Estop *et al.*, 2000
t(3;4)(p25;p16)	37	20282				0.14[c]			0.03	0.24[d]	0.03	0	0.12	0.15	
t(3;19)(p25;q12)	35	20587				0.10			0.05	0.26[b]	0.04	0.03	0.09	0.16	
t(4;10)(q33;p12.2)	42	20393				0.02			0.01	0.16	0.03	0.01	0.09	0.13	
t(5;8)(q33;q13)	42	20171				0.05			0.12	0.13	0.03	0	0.34[e]	0.37[b]	
t(8;9)(q24.2;q32)	39	15163				0.03			0	0.11	0.12[d]	0	0.14	0.28	
t(10;12)(p26.1;p13.3)	40	9219							0.03		0.01	0.02	0.04	0.07	
t(10;18)(q24.1;p11.2)	32	20403				0.07				0.19	0.04	0.02	0.20	0.26	
t(11;22)(q23;q11)	44	20572				0.12[b]			0.05	0.22[d]	0.03	0	0.13	0.16	
Control	23–42	175038				0.05			0.07	0.14[a]	0.05	0.02	0.15	0.22[a]	
t(3;9)(q26.2;q32)	32	21393			0.12			0.14	0.19		0.11	0.12	0.12	0.35	Honda *et al.*, 1999
t(3;9)(q25;q32)	34	20156			0.13			0.16	0.23		0.13	0.14	0.12	0.39	
Control	25,29,33	62572			0.17			0.16	0.23		0.11	0.11	0.15	0.37	
t(3;11)(q27.3;q24.3)	33	10718				0				0.26	0.02	0.12	0.23	0.37	Martini *et al.*, 1998
Control	–	12644				0.07				0.37	0.28	0.09	0.40	0.77	
t(7;8)(q11.21;cen)	30	2416					0.22		1.21[c]		0.07	0.13	0.20	0.40	Mercier *et al.*, 1998
Control	–	56116					0.06		0.21		0.04	0.05	0.16	0.25	
t(6;11)(q14;p14)	–	10328	0.30[d]								0.04	0.02	0.30	0.36	Rousseaux *et al.*, 1995b
t(6;11)(q14;p14)	–	4088	0.49[c]								0	0	0.65	0.65[b]	
t(2;14)(p23.1;q31)	–	8459	0.39[c]								0.01	0.01	0.43	0.45	
Control	–	94575	0.20								0.04	0.01	0.34	0.39	
t(1;10)(p22.1;q22.3)	40	10017							0.01	0.07				0.05	Van Hummelen *et al.*, 1997
Control	43, 47	20054							0.02	0.05				0.09	
t(1;2)(p36.1;p11.2)	29	4198				0.28[f]			0.20[d]	0.46[e]	0.20[e]	0.10	0.20[f]	0.50[e]	Vegetti *et al.*, 2000
t(2;5)(p25;p12)	39	4123				0.15			0.19[d]	0.10	0.05	0.05	0.10	0.20	
t(2;7)(p23;p22)	33	1684				0.29[d]			0.31[d]	0.38[c]	0.16	0.31[b]	0.31[b]	0.78[e]	
t(2;10)(q23;q11.2)	30	4079				0.15			0.15	0.10	0.05	0.10	0.05	0.20	
t(3;18)(p21.3;q21.1)	31	4408				0.24[d]			–	0.24[f]	0.09	0.22[c]	0.39[e]	0.70[e]	
t(5;20)(p22;p13)	36	4158				0.19			0.14	0.14	0.10	0.19[f]	0.10	0.39[b]	
t(6;7)(q21;p21)	41	4199				0.19			0.14	0.29[c]	0.09	0.05	0.09	0.23	
t(6;15)(p22;q26.3)	42	4129				0.15			0.14	0.10	0.10	0.10	0.05	0.25	
t(11;12)(q24.3;q12)	28	4214				0.19			0.14	0.19[d]	0.14[f]	0.19[f]	0.19[f]	0.52[e]	
t(17;22)(q11;q11)	41	4167				0.10			0.19[d]	0.20[d]	0.09	0.09	0.14	0.32[d]	
Control	36.3 (28–46)	112918				0.08			0.06	0.07	0.03	0.05	0.05	0.13	

Only studies using two-colour FISH for autosomes and three-colour FISH for X and Y, and scoring at least 10^3 spermatozoa per donor for each probe are included in this table.
Comparisons of disomy frequencies reported in the literature were carried out between each translocation carrier and concurrent controls using the 2 × 2 chi-squared test: [a]Significant interdonor heterogeneity was detected (no *P* value was reported); [b]$P < 0.005$; [c]$P < 0.001$; [d]$P < 0.05$; [e]$P < 0.0001$; [f]$P < 0.01$.

Infertile men with normal karyotypes

Sperm chromosome complements in infertile men with normal 46,XY karyotypes have been studied to determine whether meiosis in these men is prone to errors of nondisjunction, leading to aneuploidy. More than 20 sperm FISH studies from 364 patients have reported chromosome disomy frequencies (Table 4).

Infertile men with abnormal semen parameters

Five men with oligo-, aestheno- or teratozoospermia and a normal somatic 46,XY karyotype were studied by both sperm chromosome and FISH analysis (Moosani *et al.*, 1995). Approximately 100 sperm karyotypes were analysed for each infertile patient. The results demonstrated a significant increase in the frequency of total sperm chromosome abnormalities in infertile patients compared with control donors. For each patient, multi-colour FISH analysis was performed on a minimum of 10^4 sperm nuclei per chromosome probe for chromosomes 1, 12, X and Y. There was a significant increase in the frequency of disomy for chromosome 1, and particularly for XY disomy, in infertile patients. This study was expanded to analyse sperm chromosomal abnormalities in additional infertile men by FISH analysis, and the original results were corroborated with significantly increased frequencies of disomy for chromosome 1 and XY disomy (Martin, 1996). If a spermatozoon with disomy 1 fertilized a normal oocyte, a trisomy 1 embryo would be produced, which would probably be lost before implantation because of the lethality of this condition. However, a 24,XY spermatozoon would produce a 47,XXY embryo with Klinefelter syndrome, which has a high probability of surviving to term.

Along with the sex chromosomes, aneuploidies for chromosomes 13 and 21 are clinically significant because these trisomies can sometimes survive to term (resulting in Patau syndrome and Down syndrome, respectively). The same infertile men were studied to determine whether they have an increased risk of aneuploidy for chromosomes 13 and 21 in their spermatozoa. More than 9×10^4 spermatozoa were studied by FISH analysis, and there was a statistically significant increase in the frequency of disomy for both chromosomes 13 and 21, although not as marked an increase as that for the sex chromosomes (McInnes *et al.*, 1998). Similar results were obtained and extended to other chromosomes by most other studies using two- or three-colour FISH analysis. For example, Pang *et al.* (1999) detected a higher incidence of disomy in 10 oligoaesthenoteratozoospermia (OAT) patients for autosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18 and 21 and for the sex chromosomes than in fertile donors, and the frequency of sex chromosome disomy increased the most markedly. Aran *et al.* (1999) and Nishikawa *et al.* (2000) found a significantly increased XY disomy frequency in OAT patients. In addition, a significantly increased disomy frequency for chromosomes 13, 21, X and Y in OAT patients was observed by Pfeffer *et al.* (1999), Ushijima *et al.* (2000) and Vegetti *et al.* (2000). These results clearly indicate that infertile men with abnormal semen parameters have an increased risk of aneuploid spermatozoa, particularly for the sex chromosomes. Reports based on prenatal diagnosis in ICSI pregnancies have indicated an increased risk of sex chromosomal abnormalities in approximately 1% of cases (Liebaers *et al.*, 1995), and these abnormalities have been shown to be of paternal origin (Van Opstal *et al.*, 1997). One of the men in our original study had a very high frequency of XY disomy in his spermatozoa, 14-fold higher than in controls. He subsequently underwent ICSI and produced a 47,XXY fetus (Moosani *et al.*, 1999). Thus, the frequency of abnormalities in spermatozoa may predict a real risk of abnormal offspring.

These first studies of sperm chromosomal abnormalities in infertile men have tended to group all types of infertility together. The next avenue of study will be to separate out various types of infertility and determine which subsets are associated with an increased risk. Some studies have started to address this question.

Disomy frequency and sperm morphology. Studies carried out to investigate the correlation between chromosome aneuploidy and morphologically abnormal spermatozoa by FISH have produced no consistent results (Table 4). No statistical correlations between the frequency of disomy for all the chromosomes analysed and sperm morphology were found in two studies (Rives *et al.*, 1999; Vegetti *et al.*, 2000) of 50 and 19 infertile men, respectively. These results are in agreement with the data obtained by sperm karyotyping using the human spermatozoa–hamster egg fusion technique (Martin and Rademaker, 1988), and after sperm injection into mouse oocytes (Lee *et al.*, 1996). In addition, Carrell *et al.* (1999) reported the frequencies of disomy for chromosomes 13, 18, 21, X and Y in two siblings with round-headed sperm syndrome and similar semen parameters. Compared with healthy controls, sibling 1 had significantly increased disomy for chromosomes 13, 21 and the sex chromosomes, while sibling 2 with the exception of disomy 21 did not. However, Ushijima *et al.* (2000) reported that the incidence of sex chromosome abnormalities increased significantly with an increase in the percentage of morphologically abnormal spermatozoa in eight OAT patients. Bernardini *et al.* (1998) indicated that regardless of the type of semen analysed, a high percentage of certain types of morphologically abnormal spermatozoa (that is, macrocephalic, and one head with two tails) were found to be disomic in 13 infertile men including seven cases with normal semen parameters. Thus, there is conflicting evidence for a relationship between sperm morphology and the frequency of sperm aneuploidy. It is possible that only certain types of morphologically abnormal spermatozoa are associated with an increased frequency of aneuploidy.

Disomy frequency and sperm motility. In contrast to the contradictory results of studies on the relationship between

Table 4. Sperm disomy frequencies in infertile men with a normal 46,XY karyotype detected using two-colour fluorescence *in situ* hybridization (FISH) for autosomes and three-colour FISH for X and Y (with the exceptions of *, where two-colour FISH for X and Y was used)

Group	Number of patients	Age of patients (years) (range)	Mean number of cells scored	Mean semen parameters (range)			Disomy frequency (%)																			Reference
				Concentration (10^6 ml^{-1})	Percentage motility	Percentage normal morphology	1	4	6	7	8	9	10	11	12	13	14	15	17	18	21	22	XX	YY	XY	X + Y	
UI	6	34 (29–44)	> 3000	TNMS 50 (13–122)		> 20 (45–70), WHO	0.38												0.37						0.45	Bernardini *et al.*, 1997*
OAT	9	37 (30–44)	> 3000	TNMS 4.5 (0.01–20)		<20 (0–20), WHO	0.54												0.74[c]						0.82[d]	Bernardini *et al.*, 1997*
UI	7	37 (27–46)	> 3000	Normal	Normal	Normal	0.36												0.37							Bernardini *et al.*, 1998
OAT	6	49 (41–60)	> 3000	TNMS < 5		<20, WHO	0.60[e]												0.60[e]							Bernardini *et al.*, 1998
RHSS	2	–	> 5000	(10–92)	(5–45)	0										0.5				0.1	1.8		0.5	0	6.05		Carrell *et al.*, 1999
OAT	24	38.2	1725	<20	<40	<14														0.6[e]	1.2[e]					0.64[e]	Colombero *et al.*, 1999*
UI	23	38.5		> 20	> 40	> 14														0.31	0.32					0.46	Colombero *et al.*, 1999*
OAT	1	31	1000	15	20	0																			13.8		In't Veld *et al.*, 1997
Group A	8	28.5 (25–35)	10 000	121 (55–203)	61 (55–65)	31 (18–46)	0.08			0.07																	Lahdetie *et al.*, 1997
Group B (OAT)	4	31.3 (24–37)	10 000	12 (8.5–16)	29 (5–45)	8 (6–14)	0.22[f]			0.13[f]																	Lahdetie *et al.*, 1997
Group B	6	–	> 3000	24 (9–34)	31 (15–50)														0.12						0.39[g]	Li and Hoshiai, 1998
Group A	6	–	> 3000	171 (98–380)	72 (52–83)														0.15						0.26	Li and Hoshiai, 1998
Infertile	9	32.8 (28–46)	10 000	10.9 (2.5–21.5)	35.6 (11–69)	29.3 (14–54) WHO	0.14[h]								0.18	0.28[i]					0.48[i]		0.08	0.10	0.42[i]		McInnes *et al.*, 1998
O	5	–	> 4000	–	–	–	0.14																0.16	0.11		0.17	Miharu *et al.*, 1994
Infertile	7	–	> 4000	–	–	–	0.13																0.16	0.11		0.27	Miharu *et al.*, 1994
A	1	30	10 000	37	30	41, WHO	0.08								0.16								0.05	0.08	0.47[d]		Moosani *et al.*,1995
O	2	34, 35	10 000	13, 16	40, 51	40, 51, WHO	0.24[d]								0.24[a]								0.08	0.09	0.33[d]		Moosani *et al.*,1995
T	2	29, 30	10 000	26, 50	35, 40	24, 29, WHO	0.17[e]								0.17								0.10	0.10	0.27[e]		Moosani *et al.*,1995
Infertile	10	–	10 000	11.6 (3–20)	22.2 (0–40)	80 (0–96) WHO														0.15			0.10	0.13	0.36[a]	0.59	Nishikawa *et al.*, 2000
OAT	9	34.3 (25–39)	1000 (autosome); 2000 (XY18)	6.4 (2–15)	27.8 (18–41)	2.4 (1–4.4)		1.58[g]	1.38[g]	1.83[g]	1.74[g]	1.68[g]	1.78[g]	1.68[g]	0.69[g]	1.86[g]			1.62[g]	1.67[g]	1.94[g]		0.57[g]	0.76[g]	1.05[g]	2.38[g]	Pang *et al.*, 1999
OAT	10	37.2 (32–47)	1300	5.8 (1.2–14.5)	30.6 (17–50)	6 (2–10)	0.61[g]									0.91[g]				0.82[g]	0.48[g]					1.09[g]	Pfeffer *et al.*, 1999
Infertile	50	32 (24–63)	> 5000	10.8 (0.1–74)	28.4 (5–45)	44.1 (16–85)	0.34[c]									0.32[c]	0.43[c]			0.51[c]	0.50[c]	0.42[c]	0.20	0.24[g]	0.54[j]		Rives *et al.*, 1999
OAT	8	(26–39)	> 5000	12.1 (2–20)	12.6 (3–30)	2.4 (0–7)										0.13[e]				0.12	0.24[g]		0.16	0.21[e]	0.23[e]	0.59[e]	Ushijima *et al.*, 2000
OAT	9	34.9 (29–41)	1671	3.02 (0.11–11.5)	25.2 (5–48)	3.6 (0–9)										0.30[d]				0.28[d]	0.37[d]		0.17[d]	0.31[d]	0.33[d]		Vegetti *et al.*, 2000
AT	7	37.6 (31–43)	3446	52.6 (20.2–108)	33.1 (12–43)	5.3 (1–10)										0.14[g]				0.12[a]	0.22[d]		0.14[d]	0.10[g]	0.14[d]		Vegetti *et al.*, 2000
T	2	29.5 (29–30)	4019	57.8 (37–78.5)	53 (51–53)	9.5 (9–10)										0.06				0.07	0.11		0.04	0.05	0.04		Vegetti *et al.*, 2000
A	1	31	7087	56	31	18										0.12				0.07	0.10		0.06	0.10	0.03		Vegetti *et al.*, 2000

For some studies, patients were regrouped according to their semen parameters if the corresponding data were given individually in the original reports. Only studies scoring at least 10^3 spermatozoa per donor for each probe are included.
Normal semen parameters were according to the World Health Organization (1993) criteria for sperm concentration ($\geq 20 \times 10^6$ ml^{-1}) and motility (progressive sperm > 40%) and Kruger's strict criteria (Kruger, 1998) for sperm shape (> 14% morphologically normal spermatozoa) unless otherwise stated.
*Disomy frequencies for individual autosomes are obtained by dividing the total autosome disomy frequency by 2.
A: aesthenozoospermia; AT: aesthenoteratozoospermia; OA: oligoaesthenozoospermia; OAT: oligoaesthenoteratozoospermia; RHSS: round-headed sperm syndrome; T: teratozoospermia; TNMS: total number of motile sperm with normal morphology per ejaculate; UI: unexplained infertility.
Values with superscripts were significantly different from concurrent controls: [a]$P < 0.01$ (chi-squared test); [b]$P = 0.01$ (Mann–Whitney U test); [c]$P < 0.002$, [d]$P < 0.0001$, [e]$P < 0.001$ (chi-squared test); [f]$P = 0.0001$ (Poisson regression); [g]$P < 0.05$ (chi-squared test); [h]$P < 0.005$, [i]$P < 0.0001$ (two-tailed Z test); [j]$P = 0.0002$ (chi-squared test).

disomy frequency and sperm morphology, most studies comparing disomy frequency and sperm motility showed very consistent results. Six out of seven studies (86%) did not find any significant association between disomy frequency and sperm motility. Rives *et al*. (1999) reported no correlation between total disomy frequency for the chromosomes analysed and global sperm motility in 50 infertile patients. Aran *et al*. (1999) did not find a significant increase in the frequency of disomy for chromosomes 18, X and Y in five aesthenozoospermic patients. Moreover, normal men and infertile patients with or without normal semen parameters showed no significant differences in the frequency of disomy between unselected spermatozoa and motile spermatozoa selected by the swim-up method (Martinez-Pasarell *et al*., 1997), between morphologically normal, immotile but viable spermatozoa and normal motile spermatozoa (Zeyneloglu *et al*., 2000), or between spermatozoa in swim-up motile fractions and in the pellet (Pfeffer *et al*., 1999; Dyk *et al*., 2000). The only exception was a study by Li and Hoshiai (1998), in which abnormal semen had a significantly higher frequency of disomy for the sex chromosomes in unselected spermatozoa than in spermatozoa selected by the swim-up method.

Disomy frequency and sperm concentration. The association between sperm concentration and sperm aneuploidy has probably been the most extensively studied (Table 4). Finkelstein *et al*. (1998) found significantly higher frequencies of spermatozoa disomic for all the chromosomes analysed in 12 men with a sperm concentration $\leqslant 30 \times 10^6$ ml^{-1} than in eight donors with a sperm concentration $> 60 \times 10^6$ ml^{-1}. Rives *et al*. (1999) reported that the mean frequency of disomy for autosomes 1, 13, 14, 18, 21 and 22 ($P < 0.001$) and gonosomes ($P = 0.002$) in 50 infertile men increased significantly as sperm concentration decreased. Such a correlation has been confirmed by Vegetti *et al*. (2000) in a study of chromosomes 13, 18, 21, X and Y in 15 infertile men. Nishikawa *et al*. (2000) observed a significantly increased frequency of XY disomy in eight of ten OAT patients, and in all of five men with a sperm concentration $< 13 \times 10^6$ ml^{-1}. Aran *et al*. (1999) found a significantly higher frequency of XY disomy in 14 OAT patients when compared with normal donors. Overall, in patients with low sperm concentrations, a significantly increased frequency of disomy for chromosome 21 and the sex chromosomes, particularly XY disomy, was found consistently by most groups (Table 4).

It has been argued that a generalized pairing abnormality in the meiotic chromosomes may predispose to both oligozoospermia and nondisjunction (Martin, 1996). Oligozoospermia has been associated with pairing problems in both autosomes and gonosomes. Egozcue *et al*. (1983) indicated that there is an increased frequency of pairing disruptions resulting in meiotic arrest in infertile men. Martin (1996) hypothesized that it is possible that a pairing abnormality in these infertile men leads to meiotic arrest in some cells, resulting in oligozoospermia, and nondisjunction in other cells capable of completing spermatogenesis, resulting in aneuploid spermatozoa. As sex chromosomes and small chromosomes (which generally have a single crossover) are particularly susceptible to pairing abnormalities, if recombination is absent or reduced, nondisjunction could result. Hassold *et al*. (1991) have determined that 47,XXY of paternal origin is associated with a reduced recombination frequency. Shi *et al*. (2001) have demonstrated by single sperm PCR that XY spermatozoa have a reduced frequency of recombination in the pseudoautosomal region compared with normal X or Y spermatozoa. Savage *et al*. (1998) have observed altered recombination in cases of trisomy 21 of paternal origin. Thus, it is plausible that infertile men have decreased recombination and pairing, leading to both meiotic arrest (oligozoospermia) and nondisjunction (disomic spermatozoa).

In summary, our studies and those of others have demonstrated an increased frequency of sex chromosomal aneuploidy in the spermatozoa of infertile men (particularly oligozoospermia and OAT patients). The relative risk of sex chromosome aneuploidy is 2–3 times that in control donors, which is in agreement with the frequency of 1% sex chromosomal abnormalities observed from prenatal diagnoses after ICSI. Recent data for chromosomes 13 and 21, also clinically significant trisomies, show more modest relative risks. However, the most recent surveys of ICSI results demonstrate an increased frequency of autosomal aberrations, as well as sex chromosomal abnormalities in ICSI children compared with the general neonatal population (Bonduelle *et al*., 1998).

Infertile men with normal semen parameters

There have been several studies of infertile men with normal semen parameters (Table 4). Bernardini *et al*. (1997, 1998) did not find a significant increase in the frequency of disomy for autosomes and gonosomes, when compared with normal donors. These results are consistent with an earlier observation by Miharu *et al*. (1994) in seven patients with normal semen parameters and confirmed by Lahdetie *et al*. (1997). Thus, preliminary studies indicate that there is no increased risk of sperm aneuploidy in infertile patients with normal semen parameters (Table 4).

Testicular spermatozoa

New developments in infertility treatment include surgical retrieval of spermatozoa from testicular tissue for ICSI when there are no spermatozoa in the ejaculate. These testicular spermatozoa may have very different risks for aneuploidy from those of ejaculated spermatozoa. Aneuploidy frequencies for chromosomes 13, 21, X and Y have been studied using FISH analysis in testicular spermatozoa extracted from three 46,XY men with non-obstructive azoospermia (Martin *et al*., 2000). The infertile patients had an increased frequency of disomy for chromosomes 13, 21 and XY disomy compared with controls, but none of these increases reached statistical

significance. There were no significant differences in the sex ratio or the frequency of diploidy in azoospermic patients compared with normal control donors. This first report on chromosomal aneuploidy in spermatozoa extracted from testes of patients with non-obstructive azoospermia indicates that some azoospermic men do not have a substantially increased risk of chromosomally abnormal spermatozoa. However, this study was based on only 3324 spermatozoa since testicular spermatozoa are available only in small numbers. Zech *et al.* (2000) reported congenital malformations in two out of four pregnancies initiated by intracytoplasmic injection of spermatids; one of the fetuses had trisomy 9. Further studies on other patients are urgently required.

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