

# Cytogenetic determinants of male fertility

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**BACKGROUND:** Cytogenetic abnormalities have been known to be important causes of male infertility for decades. **METHODS:** Research publications from 1978 to 2008, from *PubMed*, have been reviewed. **RESULTS:** These studies have greatly improved our information on somatic chromosomal abnormalities such as translocations, inversions and sex chromosomal anomalies, and their consequences to the cytogenetic make-up of human sperm. Also, we have learned that infertile men with a normal somatic karyotype have an increased risk of chromosomally abnormal sperm and children. New techniques such as single sperm typing and synaptonemal complex analysis have provided valuable insight into the association between meiotic recombination and the production of aneuploid sperm. These meiotic studies have also unveiled errors of chromosome pairing and synapsis, which are more common in infertile men. **CONCLUSIONS:** These studies allow us to provide more precise information to infertile patients, and further our basic knowledge in the causes of male infertility.

*Keywords:* male infertility; meiosis; sperm aneuploidy; sperm chromosome abnormalities

## Introduction

Infertility is a significant problem, affecting up to 15% of couples of reproductive age (de Kretser, 1997). For many years, it was assumed that most reproductive problems could be attributed to the female partner but research in recent years has demonstrated that ~30–50% of infertility is caused by a male factor (Lipshultz and Howards, 1997). Treatment of male-factor infertility by *in vitro* fertilization (IVF) was largely unsuccessful. However, the development of intracytoplasmic sperm injection (ICSI) (Palermo *et al.*, 1992) revolutionized the treatment of male infertility, providing many men the chance to father their own children. ICSI also greatly stimulated research into the causes of male infertility, and our knowledge has increased tremendously in the past decade.

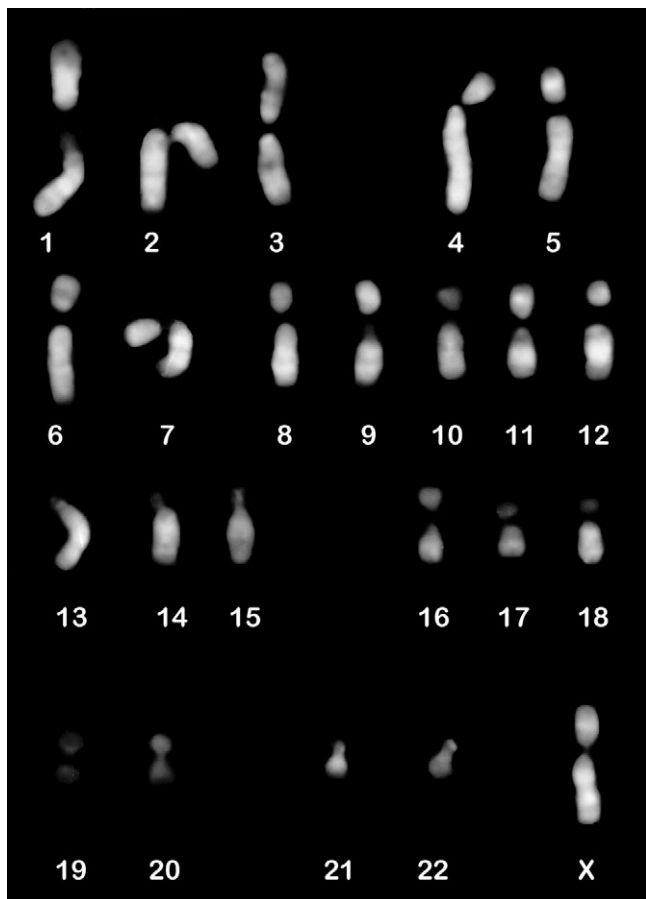
The development of new techniques has provided a wealth of information on the cytogenetics of human sperm. The human sperm–hamster oocyte fusion system allowed the first analysis of human sperm chromosomes (Rudak *et al.*, 1978; Martin *et al.*, 1983; Brandriff *et al.*, 1985; Mikamo *et al.*, 1990; Templado *et al.*, 1996). This technique provides precise karyotypes in which numerical and structural abnormalities can be assessed for each chromosome (Fig. 1). Unfortunately, the technique is very difficult

with only a handful of laboratories achieving success; also the data yield, though of great significance, is low.

Fluorescence *in situ* hybridization (FISH) analysis with chromosome-specific DNA probes was developed in the 1990s, providing a faster, cheaper, easier alternative for detecting aneuploidy in human sperm (Martin *et al.*, 1993; Robbins *et al.*, 1993; Wyrobek *et al.*, 1994; Martin *et al.*, 1996). Also, sperm hampered by abnormalities in motility or other aspects of fertilization can be assessed using FISH analysis (Downie *et al.*, 1997; Egozcue *et al.*, 1997; Robbins *et al.*, 1997; Martin *et al.*, 1999a; Hristova *et al.*, 2002). This is a simple technique that has been embraced by many laboratories, but it must be remembered that it is indirect: fluorescent signals, rather than chromosomes, are counted (Fig. 2).

Single sperm polymerase chain reaction (PCR) or single sperm typing is a difficult, time-consuming technique but it can be used in a very powerful manner for specific studies on recombination in delimited areas of the genome (Shi *et al.*, 2001, 2002; Tiemann-Boege *et al.*, 2006).

The latest development to shed light on the causes of chromosomal abnormalities and infertility is meiotic analysis of the synaptonemal complex (SC). Various important meiotic structures

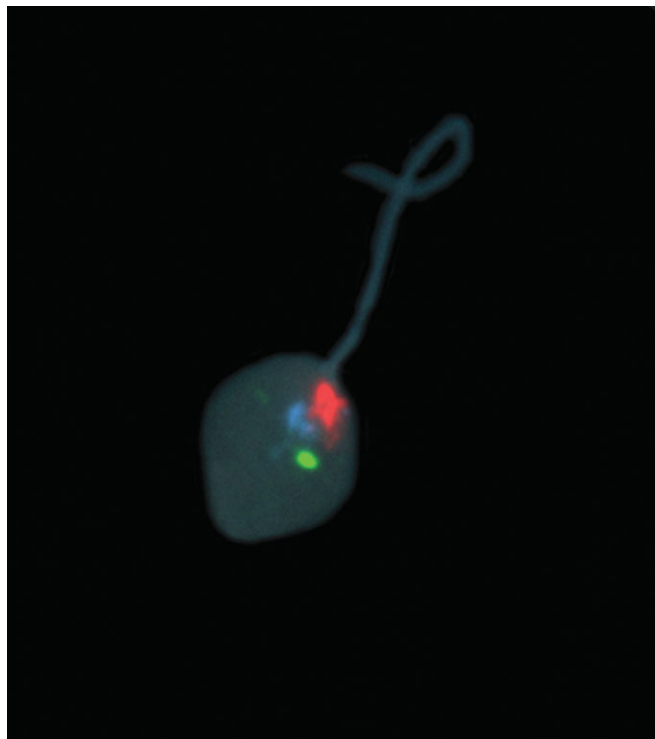


**Figure 1:** Q-banded karyotype of a normal 23,X sperm.

can be identified by the use of immunofluorescence. Antibodies against SCP1 (transverse elements) or SCP3 (lateral elements) can be used to visualize the SCs (the proteinaceous structure linking homologous chromosomes in prophase of meiosis I). The centromere can be localized with CREST antisera. Most importantly, recent studies have demonstrated that antibodies against the DNA mismatch repair protein MLH1 identify the sites of meiotic exchange in SCs in both mouse (Baker *et al.*, 1996; Anderson *et al.*, 1999) and human spermatocytes and oocytes (Barlow and Hultén, 1998; Lynn *et al.*, 2002; Tease *et al.*, 2002).

The use of a multacentromeric FISH technique in conjunction with SC meiotic analysis is a powerful tool to study recombination and the fidelity of pairing in individual identified chromosomes (Sun *et al.*, 2004b, 2006b, 2007a; Oliver-Bonet *et al.*, 2006) (Fig. 3). These studies have provided some important information on the meiotic abnormalities in infertile men and the association between meiotic recombination and aneuploidy (Gonsalves *et al.*, 2004; Sun *et al.*, 2004a, 2006a, 2007b; Codina-Pascual *et al.*, 2005; Martin, 2005).

It is clear that cytogenetic abnormalities (both somatic and meiotic) are a major cause of male infertility. This review will summarize our knowledge of somatic chromosome abnormalities and their effects on sperm, susceptibility to sperm chromosome anomalies in chromosomally normal infertile men, and meiotic errors in translocation carriers and infertile men with a normal chromosome constitution.



**Figure 2:** Disomic XY sperm, with green X signal, red Y signal and a single blue chromosome 1 signal (an internal control to distinguish disomy from diploidy).

## Materials and Methods

Research publications from 1978 to 2008, from PubMed and the author's personal library, were reviewed. Methods for selecting and synthesizing the data were based on personal experience.

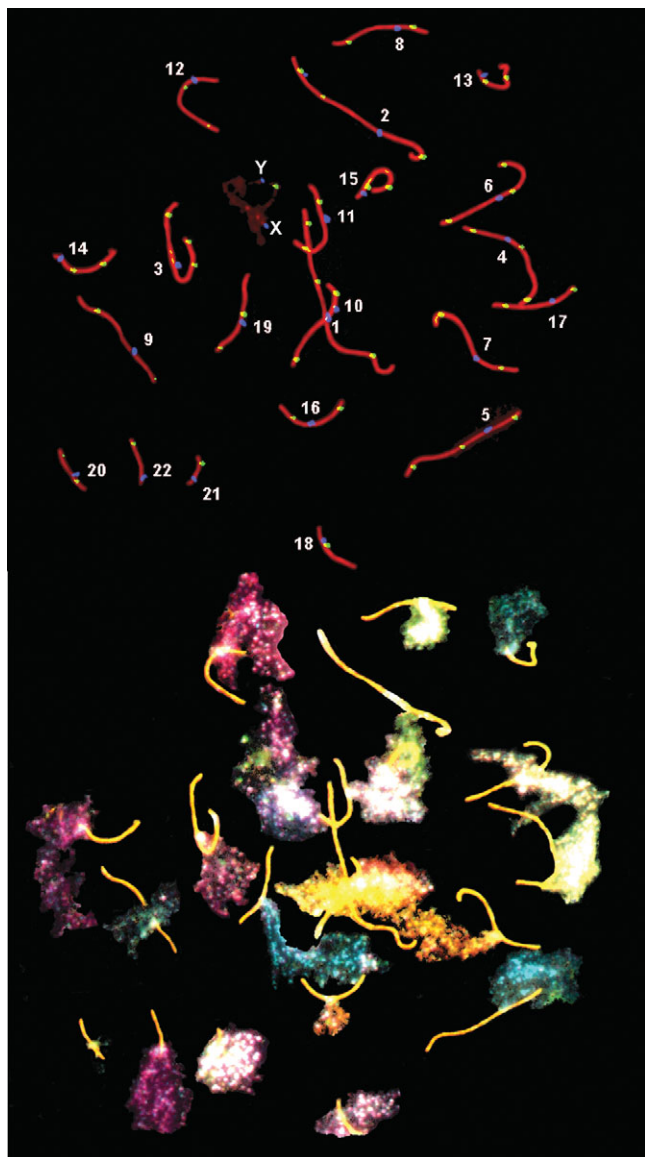
## Somatic chromosomal abnormalities

Somatic chromosomal abnormalities are relatively common in humans. These can be numerical chromosomal abnormalities, such as an extra chromosome, or structural abnormalities such as a translocation. It has long been recognized that somatic chromosomal abnormalities are associated with infertility, an increased probability of pregnancy loss and the birth of handicapped children. The frequency of somatic chromosomal abnormalities in infertile men varies from 3% to 19%: 3% in the cases of mild infertility and 19% in men with non-obstructive azoospermia (NOA) (Yoshida *et al.*, 1997). Thus, it is imperative that chromosome karyotyping be performed in all infertile men so that they can be apprised of their risks. Somatic chromosomal abnormalities have definite consequences on the cytogenetic abnormalities observed in sperm and consequently those observed in newborns.

### Sex chromosomal abnormalities

#### 47,XYY

Men with a 47,XYY karyotype are generally fertile, but they are seen more frequently in infertile populations. Many 47,XYY men produce normal children, but there has been no systematic analysis of children born to these men. Theoretically, 50% of the sperm cells should be abnormal. In a study of 75 sperm



**Figure 3:** (upper) Human pachytene spermatocyte with SCs shown in red, centromeres in blue and MLH1 foci in yellow. (lower) Subsequent cenM-FISH analysis permits identification of individual chromosomes so that recombination (MLH1) foci can be analyzed for each SC.

karyotypes from a 47,XXY male, we found no sperm disomic for a sex chromosome (Benet and Martin, 1988). Our results supported the hypothesis that the extra sex chromosome is eliminated during spermatogenesis. FISH analysis on the same male with 10 000 sperm studied demonstrated a small but significant increase for XY disomy to 0.6% (Martin *et al.*, 1999b). Other laboratories have demonstrated increased frequencies of sperm aneuploidy for the sex chromosomes ranging from 0.3% to 15% (Mercier *et al.*, 1996; Chevret *et al.*, 1997; Shi and Martin, 2000b).

The majority of the more-stringent three-color FISH studies have demonstrated a low risk of 24,YY or 24,XY sperm of ~1%. However, a recent study of two 47,XXY men with severe oligozoospermia demonstrated a higher frequency of aneuploid sperm of 37–38%, with approximately one-half of the abnormalities caused by sex chromosomal aneuploidy (Gonzalez-Merino

*et al.*, 2007). This group found that the frequency of sperm aneuploidy was concordant with the frequency of aneuploidy in preimplantation embryos (32%).

Since many 47,XXY men have normal semen parameters, the severe oligozoospermia observed in these men may indicate more perturbations during meiotic pairing, subsequent loss of germ cells and the production of aneuploid sperm.

#### 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. FISH analysis has demonstrated that the frequency of aneuploidy for the sex chromosomes varies from 1.5% (Lim *et al.*, 1999) to 7% (Kruse *et al.*, 1998) in sperm from Klinefelter mosaics, and 2% (Rives *et al.*, 2000) to 45% (Estop *et al.*, 1998) in the sperm of men who appear to have a non-mosaic 47,XXY karyotype. The majority of babies born to 47,XXY men have been normal although chromosomally abnormal fetuses have been reported (Ron-el *et al.*, 2000; Friedler *et al.*, 2001). Staessen *et al.* (2003) studied 113 embryos by preimplantation genetic diagnosis (PGD) and found a significantly increased frequency of autosomal and sex chromosomal abnormalities. Thus, there appears to be a small increased risk for these men.

#### Structural aberrations

##### Translocations

- (i) Robertsonian translocations: Robertsonian translocation carriers have a fusion of the long arms of two acrocentric chromosomes. The fused short arms (containing redundant DNA) are generally lost so that the carrier has a balanced chromosomal constitution with 45 chromosomes. When the chromosomes pair during meiosis, they do so as a trivalent, and the resulting gametes can be chromosomally normal or aneuploid with an extra or missing chromosome q arm. This can cause translocation Down's syndrome or Patau's syndrome, e.g. sperm karyotyping studies in our laboratory, and others have demonstrated that the actual frequency of unbalanced sperm in seven men is lower than theoretically expected, with 3–27% of sperm being unbalanced because of the translocation (Martin, 1995; Ogawa *et al.*, 2000). Similarly, FISH studies in 71 Robertsonian translocation heterozygotes have demonstrated that 7% to 40% are unbalanced, with a mean of 15% unbalanced (Rousseaux *et al.*, 1995; Blanco *et al.*, 2000; Escudero *et al.*, 2000; Honda *et al.*, 2000; Vegetti *et al.*, 2000; Frydman *et al.*, 2001; Morel *et al.*, 2001; Pellestor *et al.*, 2001; Acar *et al.*, 2002; Baccetti *et al.*, 2002; Anton *et al.*, 2004; Anahory *et al.*, 2005; Douet-Guilbert *et al.*, 2005; Machev *et al.*, 2005; Brugnion *et al.*, 2006; Hatakeyama *et al.*, 2006; Moradkhani *et al.*, 2006a,b; Ogur *et al.*, 2006; Chen *et al.*, 2007; Nishikawa *et al.*, 2008). Thus, all

Robertsonian translocations have relatively similar segregation behaviors despite the participation of different acrocentric chromosomes. The risks of chromosomal imbalance at prenatal diagnosis are even lower, with generally only 1–2% of paternally derived Robertsonian translocations being unbalanced (Boué and Gallano, 1984). Even though the risks are low, prospective parents deserve to be informed of them as the abnormalities can be devastating, and trisomy 13 fetuses have been detected after ICSI using sperm from a father with a Robertsonian translocation (In't Veld *et al.*, 1997a). Also, a special category of Robertsonian translocation, if found, would nullify the usefulness of ICSI: a Robertsonian translocation between the same two chromosomes, e.g. two chromosomes 13, would produce only abnormal embryos, 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.*, 1997b).

- (ii) Reciprocal translocations: reciprocal translocations occur when there are exchanges of chromosome material between any chromosomes. During meiosis, four chromosomes must pair in reciprocal translocation heterozygotes and the resulting segregations have a higher frequency of unbalanced chromosomes than Robertsonian translocations. Sperm karyotyping studies of 37 reciprocal translocation heterozygotes have shown that 19–77% of spermatozoa are unbalanced (Estop *et al.*, 1995; Martin and Spriggs, 1995; Cifuentes *et al.*, 1999). FISH analyses of chromosome segregations in 99 reciprocal translocation heterozygotes have also shown a large range in the frequency of unbalanced sperm, from 37% to 91% (Goldman and Hultén, 1993; Spriggs and Martin, 1994; Rousseaux and Chevret, 1995; Estop *et al.*, 1997, 1998, 1999, 2000; Van Hummelen *et al.*, 1997; Blanco *et al.*, 1998a, 2000; Martini *et al.*, 1998; Mercier *et al.*, 1998; Cifuentes *et al.*, 1999; Giltay *et al.*, 1999; Honda *et al.*, 1999; Vegetti *et al.*, 2000; Oliver-Bonet *et al.*, 2001, 2002, 2004; Pellestor *et al.*, 2001; Cora *et al.*, 2002; Geneix *et al.*, 2002; Trappe *et al.*, 2002; Baccetti *et al.*, 2003; Escudero *et al.*, 2003; Lim *et al.*, 2003; Anton *et al.*, 2004; Morel *et al.*, 2004; Douet-Guilbert *et al.*, 2005; Machev *et al.*, 2005; Brugnion *et al.*, 2006; Midro *et al.*, 2006; Yakut *et al.*, 2006; Perrin *et al.*, 2007; Wiland *et al.*, 2007; Nishikawa *et al.*, 2008; Vozdova *et al.*, 2008). In one study, four male family members of a kindred segregating a chromosome 15;17 translocation were studied by FISH analysis (Cora *et al.*, 2002; Vozdova *et al.*, 2008). The segregation patterns were very similar in all four men, with ~50% of sperm chromosomally unbalanced. Also, Morel *et al.* (2004) found similar frequencies of imbalance of 37% and 43% in two brothers heterozygous for a chromosome 7;8 translocation. These studies demonstrate that the risk of meiotic imbalance is primarily determined by the characteristics of the chromosomes involved, and the break-point positions. They also demonstrate the reproducibility of the method. In both karyotyping and FISH studies, the mean frequency of sperm with unbalanced chromosomes is

~50% in reciprocal translocation carriers. 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). However, some translocations have higher risks of imbalance and survival, and all have serious consequences of mental and physical handicaps. A number of fetuses with unbalanced segregations of reciprocal translocations have been reported after ICSI (Baschat *et al.*, 1996; Meschede *et al.*, 1997). Because the frequency of chromosome abnormality is very high, some men carrying reciprocal translocations have undergone PGD to implant only chromosomally normal or balanced embryos. Studies comparing the frequency of chromosome abnormalities in sperm and embryos from reciprocal translocation carriers show a close agreement in the abnormality frequencies (Escudero *et al.*, 2003).

#### Inversions

Inversions occur when two chromosome breaks occur in the same chromosome and the breaks heal in an inverted order. All the genes are present in the correct number but difficulties arise during pairing of homologous chromosomes during meiosis. If a single crossover occurs in the inverted region of the paired chromosomes, offspring with chromosomal duplications and deficiencies can result from recombinant chromosomes.

- (i) Paracentric inversions: paracentric inversions occur when both break points are in one chromosome arm. If a single crossover occurs within the inversion pairing loop, one-half of the gametes are normal, one-quarter are acentric (and would be lost) and one-quarter are dicentric (which could lead to a break between the centromeres and chromosome imbalance). Paracentric inversions are rarely reported, since they can only be detected by the use of banding procedures (Pettenati *et al.*, 1995). Some investigators have suggested that paracentric inversions in man are generally harmless (Madan, 1995); however, recombinant chromosomes have been observed in newborns, and the risk of viable recombinants has been estimated to be 3.8% (Pettenati *et al.*, 1995). Only two men with paracentric inversions have been studied by sperm karyotyping (Martin, 1986, 1999). Neither showed any recombinant chromosomes in sperm, suggesting that either an inversion loop was not formed or that crossing over was suppressed within the loop. Similarly, one case has been studied by FISH analysis and 1% of sperm were recombinant, with both dicentric and acentric chromosomes observed (Devine *et al.*, 2000). Thus, the risk for paracentric inversions appears to be small.
- (ii) Pericentric inversions: pericentric inversions occur when the chromosome breaks occur in both chromosome arms and include the centromere in the inversion. Sperm karyotyping studies have been performed in seven men heterozygous for pericentric inversions (Martin, 1999). Four men had no recombinant chromosomes and three men had frequencies of imbalance varying from 11% to 31%. FISH studies have been performed in 24 pericentric inversion carriers with the frequency of recombinant

chromosomes varying from 0% to 54% (Anton *et al.*, 2007; Chantot-Bastarud *et al.*, 2007; Morel *et al.*, 2007). The inversions that produce recombinant chromosomes are, in general, large inversions encompassing more than half of the chromosome length. An overall risk at prenatal diagnosis has been estimated to be 10–15% (Daniel *et al.*, 1989), but it is clear that the risks are dependent on the individual inversion.

#### *Interchromosomal effects*

A number of researchers have suggested that there is an increased frequency of chromosomal abnormalities unrelated to the structural abnormality and have termed this an 'interchromosomal effect' (Aurias *et al.*, 1978), e.g. an increased frequency of trisomy 21 children born to Robertsonian translocation carriers. Sperm karyotype studies have not shown any support for an interchromosomal effect in translocation (Martin and Spriggs, 1995) or inversion carriers (Martin, 1999), despite the fact that all chromosomes can be analyzed. However, the number of sperm karyotypes is limited with a maximum of 548 sperm cells analyzed in one translocation carrier (Spriggs *et al.*, 1992). Thus, a small interchromosomal effect would be missed by these studies.

Studies employing FISH analysis have the advantage of much larger sample sizes, with a few hundred to several thousand sperm assessed. There is some suggestion for an interchromosomal effect in 58% (21/36 studied for an interchromosomal effect) of Robertsonian translocations and 64% (35/55) of reciprocal translocations studied for the segregation of other chromosomes by FISH analysis, since an increased frequency of numerical abnormalities was observed in at least one of the chromosomes evaluated. For inversions, only one of seven cases demonstrated a possible interchromosomal effect (Amiel *et al.*, 2001). However, it is doubtful whether this is a true interchromosomal effect, since carriers of translocations and inversions are often infertile, with altered sperm profiles. In fact, almost all the Robertsonian translocation carriers studied for an interchromosomal effect had abnormal semen profiles, with only three normospermic men (none of whom demonstrated an interchromosomal effect). In reciprocal translocation heterozygotes, abnormal semen profiles were much more common among the cases demonstrating an interchromosomal effect (67%) compared with those that did not (11%). Infertile men with oligozoospermia, asthenozoospermia or teratozoospermia are known to have an increased frequency of chromosome abnormalities in their sperm (Moosani *et al.*, 1995; Martin, 1996; Aran *et al.*, 1999; Pang *et al.*, 1999; Nishikawa *et al.*, 2000). Therefore, it is possible that the increased frequency of numerical chromosomal abnormalities in some carriers of structural rearrangements may be related to infertility factors rather than the rearrangement. On the other hand, clear examples of translocations with asynaptic segments pairing with sex chromosomes or autosomes suggest that this type of interchromosomal effect may be causing the infertility (Oliver-Bonet *et al.*, 2005a).

PGD studies have demonstrated that embryos of translocation carriers have a high frequency of genetic imbalance. Some studies have suggested an interchromosomal effect for Robertsonian translocation carriers, since there appears to be a higher frequency of aneuploidy (Conn *et al.*, 1998, 1999; Gianaroli *et al.*,

2002), whereas others have not found a significant increase (Scriven *et al.*, 2001; Munné *et al.*, 2005). In summary, an interchromosomal effect may be a reality for some translocations, especially in infertile patients, and sperm chromosome studies may be useful to determine the level of risk.

#### *Y chromosome microdeletions*

Tiepolo and Zuffardi (1976) first recognized that six azoospermic men had deletions of the long arm of the Y chromosome, large enough to be recognized by light microscopy. Since that time, it has been determined that the majority are Yq microdeletions and therefore require analysis by molecular means. Most studies demonstrate that 4–14% of azoospermic or severely oligozoospermic men have an Yq microdeletion, making this a major contribution to male infertility (Foresta *et al.*, 2001). Of significance is the fact that all sons are expected to be infertile.

Three regions have been delineated as azoospermic factor (AZF) a, b and c with AZFc holding the best prognosis for viable testicular sperm retrieval (Foresta *et al.*, 2001; Vogt, 2004). An interesting aspect of Y chromosome microdeletions is the instability of the chromosome suggested by recent studies. Patsalis *et al.* (2002) studied 12 mosaic 46,X/46,XY patients with Turner syndrome traits or sexual ambiguities and 4/12 had Y chromosome microdeletions. Ferlin *et al.* (2007) performed sperm FISH studies in 11 men with AZFc deletions and found only 33% Y-bearing sperm compared with 49% in controls, and a significant increase in disomic XY sperm and sperm nullisomic for the sex chromosomes. More research is clearly indicated, but this suggests that men with a Y chromosome microdeletion may have an increased risk of 45,X and 47,XXY offspring as well as mosaic offspring because of loss of the Y chromosome.

#### *Germinal mosaics*

Men with a normal somatic karyotype may still have an abnormal cell line in their testes. These men are called 'germinal mosaics', and it is difficult to discover them without a testicular biopsy. Studies have discovered that 1–17% of infertile men are germinal mosaics (Chandley *et al.*, 1976; Hendry *et al.*, 1976), so this is still a risk after a normal karyotype result, but the risks for abnormal offspring would be lower than those for non-mosaic individuals.

#### **Infertile men with a normal karyotype**

With the advent of ICSI, it has become clear that infertile men with a normal somatic karyotype also have an increased risk of chromosomally abnormal sperm. Moosani *et al.* (1995) were the first to report that infertile men with a normal 46,XY karyotype have an increased risk for autosomal and sex chromosomal abnormalities in their sperm. More than 30 FISH studies have confirmed this association of increased sperm aneuploidy frequencies in 46,XY infertile men (Aran *et al.*, 1999; Pang *et al.*, 1999; Nishikawa *et al.*, 2000). Most studies have reported the increase of sperm chromosome abnormalities in infertile men to be about three times higher than in control donors (Moosani *et al.*, 1995; Lahdetie *et al.*, 1999; Acar *et al.*, 2000). Reports based on prenatal diagnosis of ICSI pregnancies (Van Steirteghem *et al.*, 2002) and newborns (Aboulghar *et al.*, 2001) have indicated the risk of *de novo* chromosome abnormalities to be ~2% to 3%, which is 3-fold

higher than that in normal pregnancies. Thus, the increased frequency of chromosome abnormalities in ICSI pregnancies and newborns mirrors the increased frequency observed in the sperm of infertile ICSI patients. Furthermore, studies have indicated that these chromosome abnormalities are of paternal origin (Van Opstal *et al.*, 1997), underscoring the fact that chromosomally abnormal sperm in ICSI patients become chromosomally abnormal fetuses and children.

The first studies of sperm chromosomal abnormalities in infertile men tended to lump all types in infertility together. However, it is possible that some subsets of infertility have an elevated risk of sperm chromosomal abnormalities whereas others do not. We have studied men with asthenozoospermia (motility defects; Hristova *et al.*, 2002), teratozoospermia (abnormalities of form; Templado *et al.*, 2002), various degrees of oligozoospermia (low concentration; Martin *et al.*, 2003b) and azoospermia (no sperm in the ejaculate; Martin *et al.*, 2003a). To our surprise, we found that men with any type of infertility had an increased frequency of sperm chromosomal abnormalities, varying from 2 to 10 times higher than that of control donors. Thus, it seems that any perturbation of spermatogenesis confers an increased risk of aneuploid sperm.

One rare type of teratozoospermia appears to confer a very high risk is men with a high percentage of macrocephalic, multinucleated, multiflagellate sperm. A number of studies have reported very high frequencies of aneuploidy and polyploidy in these men (50–100%) (Benzacken *et al.*, 2001; Devillard *et al.*, 2002; Lewis-Jones *et al.*, 2003). It has also been suggested that abnormalities of the centrosome may exist in surgically retrieved sperm, a problem that may lead to increased mitotic non-disjunction and mosaicism in resulting embryos (Silber *et al.*, 2003).

#### Utility of aneuploidy assessment in sperm

There have been a number of studies which have found a high frequency of sperm aneuploidy in men who subsequently fathered chromosomally abnormal children (Blanco *et al.*, 1998b; Martinez-Pasarell *et al.*, 1999a,b; Moosani *et al.*, 1999; Soares *et al.*, 2001; Nagvenkar *et al.*, 2005). For example, in our original study of infertile men, one male had a frequency of 24,XY sperm that was 9-fold higher than controls (Martin, 1986). This man subsequently had ICSI and fathered a pregnancy that resulted in a 47,XXY fetus (Moosani *et al.*, 1999). Gianaroli *et al.* (2005) studied sperm aneuploidy and correlated it to results in blastomeres after PGD in couples. They found a higher incidence of monosomies and trisomies in embryos from microepididymal sperm aspiration and testicular sperm extraction sperm, and aneuploidy for the sex chromosomes increased proportionally to the severity of the male-factor condition. These authors suggested that it is important to include sperm FISH analysis in preliminary tests given to infertile couples, especially in the case of repeated IVF failures. These preliminary studies suggest a correlation between sperm aneuploidy frequencies and ICSI outcome. However, unpublished results from our research laboratory demonstrated that only 10% of infertile men with a normal karyotype have a 24,XY sperm frequency that is  $\geq 5$  times that of controls, and only 3% have a frequency  $\geq 10$  times that of controls. Thus, it appears that a high frequency of sperm aneuploidy is relatively rare in 46,XY infertile men.

#### Recombination analysis by single sperm PCR

Our studies, and those of others, have shown that the frequency of aneuploidy in sperm is elevated for the sex chromosomes compared with the autosomes, for both fertile (Martin *et al.*, 1991; Spriggs *et al.*, 1996; Scarpato *et al.*, 1998) and infertile men (Shi and Martin, 2000a; Hristova *et al.*, 2002). The XY bivalent normally has only one crossover in the pseudoautosomal region during meiosis. If recombination is reduced or absent for these chromosomes, they may be particularly susceptible to non-disjunction. Indeed, it has been shown that 47,XXY of paternal origin is associated with a decreased recombination frequency (Hassold *et al.*, 1991; Lorda-Sanchez *et al.*, 1992). For a direct test of whether recombination is associated with non-disjunction in human sperm, we performed single sperm PCR analysis for a sex specific locus (STS/STS pseudogene) and a pseudoautosomal locus (DXYS15) (Shi and Martin, 2001). Individual unisomic sperm (23,X or Y) were isolated using a FACStar<sup>Plus</sup> flow cytometer into PCR wells. To identify disomic 24,XY sperm, 3-color FISH analysis was performed with probes for chromosomes X, Y and 1. The 24,XY cells were identified using fluorescence microscopy, each disomic sperm was scraped off the slide using a glass needle attached to a micromanipulator and then put into a PCR well. Hemi-nested PCR analysis of the two markers was performed to determine the frequency of recombination. The frequency of recombination between the two DNA markers was 38% for the normal unisomic sperm compared with 25% for the 24,XY disomic sperm that had undergone non-disjunction. This difference was highly significant, and demonstrates that lack of recombination in the pseudoautosomal region is associated with XY non-disjunction and the production of aneuploid sperm.

#### SC analysis

The discovery that lack of recombination is associated with non-disjunction is significant because it provides a definite molecular correlate with aneuploidy. The next logical step would be to assess recombination in other chromosomes. However, this is extremely difficult and time-consuming by single sperm PCR analysis. Luckily, new immunocytogenetic techniques allow assessment of recombination and chromosome pairing by visualization of the SC in early meiosis (Barlow and Hultén, 1998; Lynn *et al.*, 2002; Sun *et al.*, 2004a, 2006b, 2007b). The SC can be analyzed throughout the stages of prophase (leptotene, zygotene, pachytene, diplotene) to assess the progress of meiosis and the fidelity of chromosome pairing and synapsis. The frequency and location of recombination sites on individual chromosomes can also be analyzed by use of antibodies to the mismatch repair protein MLH1 (Baker *et al.*, 1996; Marcon and Moens, 2003; Sun *et al.*, 2004b) combined with cenM-FISH to identify individual chromosomes (Fig. 3). This technique provided the first recombination maps for every autosome (Sun *et al.*, 2004b, 2006b).

Studies in a number of laboratories have demonstrated that normal healthy men have  $\sim 50$  recombination foci per pachytene cell, with a wide range in the mean number of recombination sites per cell and in the number of sites in individual pachytene cells (Barlow and Hultén, 1998; Lynn *et al.*, 2002; Sun *et al.*, 2004b, 2006b). This number of recombination sites is very

similar to chiasma counts at diakinesis (Hultén, 1974; Laurie, 1985; Laurie and Hultén, 1985) and also to the corresponding genetic length obtained from linkage data (Kong *et al.*, 2002).

### *SC analysis in translocation carriers*

Reciprocal translocations are known to generate meiotic disturbances that affect both quantitative and qualitative sperm production. The chromosomes involved in a reciprocal translocation must pair as a quadrivalent, which is clearly visible at the SC level during pachytene (Oliver-Bonet *et al.*, 2005a; Sun *et al.*, 2005b). Meiotic studies of infertile men carrying chromosomal rearrangements have shown that quadrivalent configurations have different degrees of asynapsis around the break points. It has also been observed that these regions sometimes interact with the sex body. Associations between an autosome and the sex body can be visualized as continuous proteinaceous filaments that connect the two together. This association has been suggested to be the cause of infertility in carriers (Gabriel-Robez *et al.*, 1986). It has been hypothesized that this may be due to the activation of X-linked genes, or a spreading of inactivation to the autosomes. Study of the SC permits analysis of these two hypotheses.

The X and Y chromosomes pair in only a small region, with the rest of the chromosomes remaining unpaired. Meiotic sex chromosomes are inactivated with many proteins locating to the sex body (Oliver-Bonet *et al.*, 2005b). It has been hypothesized that the association of an autosome with the sex body causes reversal of the sex body inactivation and allows expression of some genes, with lethal results for the cell (Lifschytz and Lindsley, 1972). Others have suggested a spreading of sex body inactivation toward the autosomes connected to the sex body (Jaafar *et al.*, 1993). Recent studies have shown normal timing and progression of condensation through the pachytene stage for the sex body in a carrier of a t(Y;1) translocation (Sun *et al.*, 2005b) and for the sex body associated with the quadrivalent in a t(13;20) carrier (Oliver-Bonet *et al.*, 2005b). These results, together with the fact that there is a strong relationship between sex body inactivation and XY condensation (Fernandez-Capetillo *et al.*, 2003), do not support the model of gene activation on the X chromosomes. In addition, the observation that autosome arms invading the sex body show gradual heterochromatinization, mimicking the behavior of the sex chromosomes, suggests that spreading of inactivation to the autosome is taking place.

It has been suggested that transcriptional repression might not be an exclusive mechanism of the sex chromosomes, but rather a general mechanism that acts to silence any asynapsed region in the cell (Baarends and Grootegoed, 2003). Such a general mechanism has been described in *Neurospora crassa* (Shiu *et al.*, 2001), and in the mouse (Turner *et al.*, 2005). It is possible that a similar mechanism is operative in humans and that the spreading of sex body inactivation toward the translocated chromosome is a consequence of asynapsed regions within the quadrivalent attaching to the sex body. In this case, genes important in meiosis might be repressed with resultant destruction of the cell.

The meiotic process in two translocation carriers with different fertility outcomes, one normozoospermic and the other azoospermic, have been compared with interesting results (Oliver-Bonet *et al.*, 2005a). A significant number of quadrivalents were attached to the sex body in the azoospermic carrier, whereas such an

association was never detected in the normozoospermic carrier. In addition, this normozoospermic patient displayed heterologous synapsis within the quadrivalent. Thus, it appears that unpaired regions within the quadrivalent are likely to be detected by the pachytene checkpoint, so asynapsed regions seek each other out and try to pair, in order to escape the checkpoint and avoid apoptosis of the cell. The other option to shelter unsynapsed regions from the checkpoint is association with the sex body, but this situation may lead to anomalies disrupting the proper segregation of the chromosomes.

### *SC analysis in infertile men*

Studies of infertile men have demonstrated a number of meiotic errors. Most of these meiotic studies have been performed on men with NOA, but some men with obstructive azoospermia and oligoasthenoteratozoospermia have also been studied. Approximately one-half of the men with NOA have no meiotic cells (Gonsalves *et al.*, 2004; Sun *et al.*, 2005a; Topping *et al.*, 2006, 2007b). In those with meiotic cells, the progression of meiosis is altered, with significantly more cells observed in the early stages of prophase (leptotene and zygotene; Gonsalves *et al.*, 2004; leptotene and zygotene; Ferguson *et al.*, 2007; Sun *et al.*, 2007b). This suggests problems in the development of the lateral and transverse elements of the SC, leading to difficulties in pairing and synapsis of homologous chromosomes.

Sun *et al.* (2005a,c, 2007b) determined that NOA men have a significant increase in the proportion of pachytene cells with unsynapsed regions. Also, a number of studies have demonstrated that NOA males have a significant reduction in the frequency of MLH1 foci compared with controls (Gonsalves *et al.*, 2004; Sun *et al.*, 2005a, 2007b), although two studies have not observed this reduction (Codina-Pascual *et al.*, 2005; Topping *et al.*, 2006). Achiasmate bivalents (with no crossovers) have been observed with a significantly elevated frequency in NOA males compared with controls (leptotene and zygotene; Gonsalves *et al.*, 2004; Sun *et al.*, 2005a, 2007b; Ferguson *et al.*, 2007). For example, Sun *et al.* (2007b) observed that 29% of pachytene cells had at least one bivalent with no recombination foci, compared with 5% in controls. This is a dangerous situation, since without a crossover there is no mechanism to ensure orderly chromosome segregation at metaphase I. This could lead to engagement of the pachytene checkpoint and meiotic arrest or sperm aneuploidy.

A minimum number of recombination sites (crossovers) for correct alignment and segregation of the chromosomes is one per chromosome arm (except for the short arms of the acrocentric chromosomes, which rarely recombine). Achiasmate chromosomes (those without a recombination site) are rare in humans, except for the sex chromosome pair, which has a visible recombination site only 56–93% of the time (Codina-Pascual *et al.*, 2005; Sun *et al.*, 2006a; Ferguson *et al.*, 2007). However, when achiasmate chromosomes are observed in autosomes, they occur most frequently for chromosomes 21 and 22 (Codina-Pascual *et al.*, 2006; Sun *et al.*, 2006a). This is interesting, since it parallels data on aneuploidy in human sperm. Both studies on human sperm karyotypes (Martin and Rademaker, 1990) and FISH analysis (Williams *et al.*, 1993; Spriggs *et al.*, 1996; Blanco *et al.*, 1998b; Scarpato *et al.*, 1998) have demonstrated that chromosomes 21 and 22, and the sex chromosomes have the highest

frequency of aneuploidy. This strengthens the association between lack of recombination and aneuploidy. However, these studies on meiotic recombination and sperm aneuploidy have not been performed on the same men. A more robust test of the association between meiotic recombination and aneuploidy would be to correlate both analyses in the same men.

When we performed just such a study in vasectomy reversal patients, we found no correlation between meiotic recombination in individual chromosomes and sperm aneuploidy for the same chromosome (Sun *et al.*, 2008). We hypothesized that our population of fertile men may not have reached the threshold of meiotic abnormalities necessary to demonstrate the relationship between recombination and aneuploidy, since achiasmate bivalents were rare in this group of men. Ferguson *et al.* (2007) found a significant inverse correlation between meiotic recombination for the sex chromosomes and XY disomy in sperm. They also found a significant relationship between the frequency of achiasmate chromosome 21 and sperm disomy 21. This analysis was performed in a mixed group of control donors (vasectomy reversals) and infertile men. When we studied men with NOA, we also demonstrated that a low frequency of meiotic recombination in the sex bivalent was significantly correlated with a high frequency of aneuploidy for the sex chromosomes (unpublished results). Thus, it is possible that men with more dramatic meiotic abnormalities are infertile because of loss of meiotic cells at the pachytene checkpoint, and also face an increased risk of aneuploid sperm because of non-recombinant cells that escape the checkpoint but are still susceptible to errors of chromosome segregation. Egozcue's group in Barcelona has performed elegant research in this area. They suggest that the best candidates for a meiotic study would be: infertile males with a normal karyotype and unexplained infertility, and among them, infertile males with normozoospermia and long-term sterility, or IVF failures (embryonic factor, no fertilization, repeated IVF failure), or infertile males with a severe oligozoospermia ( $<5 \times 10^6$  sperm/ml) or a severe oligoasthenozoospermia ( $<1.5 \times 10^6$  motile sperm/ml) (Egozcue *et al.*, 2005).

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