Chapter 3
Chromosomal Aberrations and Aneuploidies of Spermatozoa

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Abstract Chromosomal abnormalities are relevant causes of human infertility, affecting 2–14 % of infertile males. Patients with seminal anomalies could be affected by improper meiotic recombination and increased sperm chromosome aneuploidy. Since the transmission of a haploid chromosomal asset is fundamental for embryo vitality and development, the study of sperm chromosomes has become fundamental because intracytoplasmic sperm injection allows fertilization in cases of severe male infertility.

In this chapter we summarize the data on the incidence of sperm aneuploidy, detected by fluorescence in situ hybridization (FISH), in infertile men with normal or abnormal karyotype. The possibility of reducing sperm chromosomal imbalance is also reported.

Among control males, the lowest aneuploidy rate was detected (range: 0.09–0.14 % for autosomes; 0.04–0.10 % for gonosomes). In infertile patients with normal karyotype, the severity of semen alteration is correlated with the frequency of aneuploidy, particularly for X and Y chromosomes. Among patients with abnormal karyotype, 47,XXY and 47,XYY carriers showed a high variability of sperm aneuploidy both for gonosomes and autosomes. In Robertsonian translocation carriers, the increase in aneuploidy rate was particularly evident for total sex disomy, and resulted mainly from interchromosomal effect (ICE). In reciprocal translocation carriers, a high percentage of unbalanced sperm (approximately 50 %) was detected, perhaps mostly related to ICE.

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Sperm chromosomal constitution could be analyzed to obtain more accurate information about the causes of male infertility. It would be worthwhile to evaluate the benefits of a therapy with recombinant Follicle Stimulating Hormone (rFSH) on sperm chromosome segregation in selected infertile males.

Keywords FISH • Sperm aneuploidy • Male infertility • Abnormal karyotype

Introduction

Chromosomal abnormalities are a relevant cause of human infertility, affecting 2–14 % of infertile males and have been clearly demonstrated to increase proportionally with the severity of the spermatological phenotype. Both numerical and structural chromosomal aberrations are major contributors to pregnancy loss (Egozcue et al. 2000a), perinatal death, congenital malformations, mental retardation, and behavioral anomalies (Hook 1985; Hecht and Hecht 1987), the latter accounting for the 0.8–1 % of live births (Gardner and Sutherland 2004).

Among the abnormalities, trisomy of chromosomes 13, 18, and 21 and aneuploidies of the sex chromosomes constitute the most important load of congenital abnormalities. In most cases, autosomal trisomies originate in maternal germ cells, whereas sex chromosome aneuploidies are frequently of paternal origin, occurring during spermatogenesis (Sloter et al. 2004).

The term spermatogenesis indicates the processes by which primordial germ cells, namely spermatogonia, become haploid sperm cells. Spermatogonia divide by mitosis, giving rise to primary spermatocytes, which undergo a meiotic process. Meiosis includes two successive cell divisions without DNA replication. During the first and second meiotic divisions, homologous chromosomes separate to form haploid gametes. At the end of spermatogenesis, the haploid spermatid nucleus contains 23 chromosomes with one chromatid. Soon after, they are transformed into spermatozoa by a morphogenetic process, spermiogenesis. Recently, several lines of evidence have linked unexplained male infertility to meiotic defects in pairing, synapsis, and recombination and to an increase in aneuploid sperm (Tempest and Martin 2009).

Errors during mitotic or meiotic divisions may lead to aneuploid gametes, in which autosomes or the sex chromosomes are affected. Aneuploidy, the most frequently detected cytogenetic abnormality, is defined as the condition of having fewer or more than the euploid number of chromosomes. Aneuploidies in male gametes may be caused by two main mechanisms: (1) nondisjunction of chromatid pairs during mitosis or meiosis II or nondisjunction of homologous chromosomes during meiosis I; (2) chromosome lagging near the equator at anaphase followed by chromosome loss (Ford et al. 1988).

The incidence of sperm aneuploidy increases proportionally with the severity of the male-factor sterility, including Y chromosome microdeletions, as confirmed by various studies suggesting that, in selected cases, the paternal contribution to aneuploidy in the developing conceptus could be more relevant than expected from

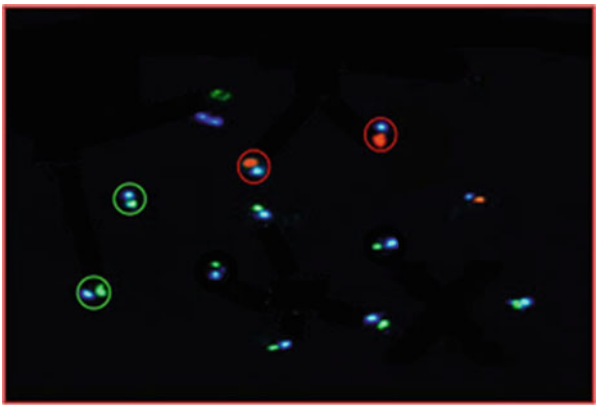

Fig. 3.1 Fish analysis using different centromeric-DNA probes for the simultaneous detection of chromosome 18 (*aqua*), X (*green*) and Y (*red*). Euploid sperm nuclei with 18,X (*green circles*), and 18,Y (*red circles*) complements are observed

general data on aborted fetuses and live births (Gianaroli et al. 2005; Magli et al. 2009; Mateu et al. 2010; Kahraman et al. 2006; Harton and Helen 2012).

This is particularly true in cases of assisted reproductive techniques, such as intracytoplasmic sperm injection (ICSI), that have improved the chances of achieving pregnancy also for patients with severe seminal anomalies (Van Steirteghem et al. 1993, 1996), despite an increased incidence of embryo aneuploidies (Verpoest and Tournaye 2006; Tesarik and Mendoza 2007). In particular, it appears that men with severe factor infertility treated by ICSI have an increased risk of generating offspring with unbalanced chromosomal constitution (Rimm et al. 2004; Wen et al. 2012).

The clinical use of aneuploidy screening should be recommended in patients affected by Klinefelter syndrome, structural rearrangement of karyotype, severe teratozoospermia, nonobstructive azoospermia, as well as in patients with recurrent pregnancy loss or unexplained repeated in vitro fertilization (IVF) failures.

The development of the FISH technique, which uses a chromosome-specific DNA probe detected by fluorescence microscopy, and its application to the study of sperm aneuploidy has made possible the screening of a large number of germ cells in a relatively short time. In addition, the simultaneous use of different probes allows for the evaluation of aneuploidy frequency for different human chromosomes in normal (Fig. 3.1) and pathological conditions (Figs. 3.2 and 3.3) (Egozcue et al. 1997; Downie et al. 1997; Guttenbach et al. 1997a; Rives et al. 1999; Carrel 2008; McLachlan and O'Bryan 2010). Since 1990, this technique has been used to analyze chromosome aneuploidies in sperm, and many papers have been published on sperm aneuploidies even in control individuals (Downie et al. 1997; Guttenbach et al. 1997a; Egozcue et al. 1997; Rives et al. 1999). Nevertheless, FISH has its limits due, for example, to the degree of chromatin condensation since condensation efficiency is directly correlated with fluorescent signal quality (Vidal et al.

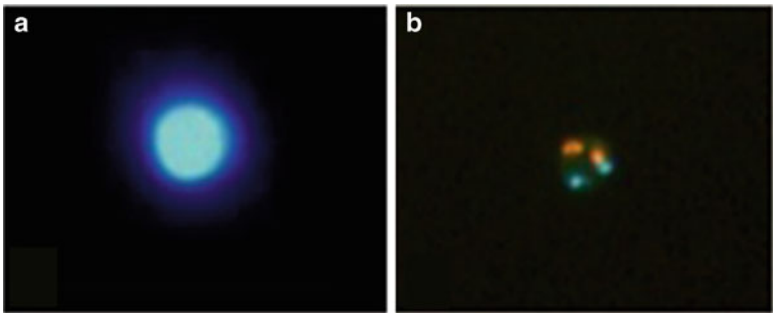

Fig. 3.2 DAPI counterstaining (**a**) and FISH analysis (**b**) showing a disomic 18,YY sperm

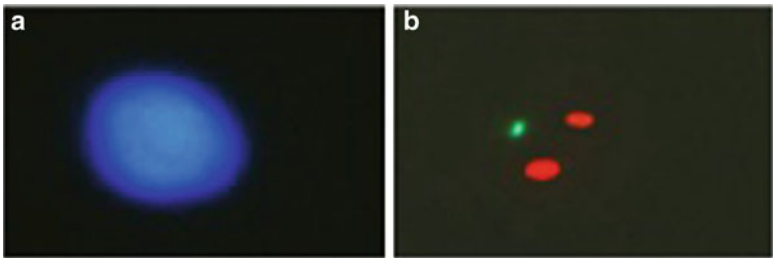

Fig. 3.3 DAPI counterstaining (**a**) and FISH analysis (**b**) showing a diploid 18,18,YY sperm

1993; Egozcue et al. 1997). Moreover, FISH does not enable one to appreciate the difference between nullisomy (the absence of a chromosome) and the absence of hybridization, despite the fact that if one of the probes gives a correct signal, the absence of a signal from the other probe may be considered evidence of nullisomy (Holmes and Martin 1993; Bischoff et al. 1994). Furthermore, FISH on decondensed sperm head only provides information about anomalies in the chromosome number, while the analysis of chromosome structural rearrangement is still complex due to the use of locus-specific probes not always able to hybridize. In these latter cases, sperm karyotyping, single-sperm polymerase chain reaction, or single-sperm typing should be applied to improve the identification of recombination in specific genome areas (Martin 2008).

Sperm karyotyping through a fusion assay was originally used, with the drawback of the technique being that it is laborious, technically difficult, and only allows for studying sperm that are able to fuse with a hamster oocyte, yielding results on a relatively small number of cells. The advent of FISH revolutionized the study of sperm chromosome constitution, mitigating many of these problems, except that it no longer allowed the whole chromosome complement to be studied in a single cell or structural aberrations to be picked up without the use of specially designed probes.

Nevertheless, not all 24 chromosomes (22 autosomes plus X and Y chromosomes) can be easily assessed in a single cell, and a limited number of fluorocromes are available; therefore 3–5 signals at most are technically feasible.

FISH Analysis in Normozoospermic Males as Reference Value for Aneuploidy Frequency

Sperm chromosomal aneuploidies have been investigated by FISH in normal donors by various authors. Unfortunately, it is not always easy to compare the results of these studies and identify a reference value, mainly because of the differences in the frequency and distribution of aneuploidies. This heterogeneity can be ascribed to differences in application of the technique, such as sperm decondensation, scoring criteria, storage of samples, types of probe, number of sperm scored, or data analysis and reporting and donor selection criteria (e.g., *normozoospermic*, *normozoospermic and fertile*, *fertile*, or *healthy* men).

To provide some useful information on this topic, we compared data from 19 different studies selected on the basis of donor characteristics and techniques (Gambera et al. 2011). According to donor selection criteria, we distinguished three subgroups: *normozoospermic* – a total of 55 donors with reported normal semen parameters according to the World Health Organization (WHO) (1999, 2010) or Kruger morphology (1986) criteria but without information about fertility; *normozoospermic and fertile* – including 57 normozoospermic donors of proven fertility; *fertile* – including 37 donors with proven fertility but without information about semen parameters. In these studies sperm aneuploidies were evaluated using specific probes for all chromosomes, with the most investigated being 13, 18, 21, X, and Y chromosomes mainly because aneuploidies of these chromosomes do not preclude embryo development and survival. Overall information about sperm chromosome quantitative alteration was given as the total frequency of aneuploidies. The weighted mean of autosomal disomy for each chromosome ranged from a minimum of 0.05 % to a maximum that varied somewhat in the different groups: 0.14 ± 0.06 % in *normozoospermic and fertile*, 0.23 ± 0.08 % in the *normozoospermic* group, and 0.09 ± 0.09 % in *fertile* (Table 3.1).

Disomies for chromosomes 13, 18, and 21 have been extensively studied and provide a statistically significant background for the interpretation of results. The disomy rate for each chromosome varied only slightly in the three groups and between groups. However, chromosome 21 disomy had a significantly higher incidence in the *normozoospermic* group, suggesting greater susceptibility to nondisjunction for this chromosome.

Sex chromosome disomies were also investigated, and XY disomy frequency increased more than twice in comparison with XX and YY disomies. Therefore, it seems that errors in meiosis I, giving rise to sperm with XY chromosomes, should be more frequent than errors in meiosis II, which generate X or Y disomic sperm (Fig. 3.2). On the other hand, it must be considered that errors in meiosis I give rise to two disomic and two nullisomic sperm, while errors in meiosis II may produce a disomic sperm and a nullisomic one. The diploidy rate, evaluated by three fluorescent probes, one for autosomes and two for sex chromosomes, was not statistically different among the three groups.

Table 3.1 Aneuploidies and diploidies in fertile and infertile men ICSI candidates (Modified from Gambera et al. 2011)

	Autosomal								Sexual						
	Disomy (%)								Disomy (%)						
	1	7	8	12	13	18	21	Dipl.[a] (%)	XX	YY	XY	Tot.D[b] (%)	Dipl.[a] (%)	Tot.A[c] (%)	Tot.N[d] (%)
Sperm aneuploidy frequency in normospermic fertile men															
Normozoospermic															
Mean ± Standard deviation			*0.11 ± 0.07*	*0.05 ± 0.00*	*0.12 ± 0.04*	*0.12 ± 0.06*	*0.23 ± 0.08*	*0.15 ± 0.08*	*0.07 ± 0.05*	*0.07 ± 0.03*	*0.14 ± 0.08*	*0.15 ± 0.03*	*0.21 ± 0.11*	*1.01 ± 0.99*	*0.07 ± 0.00*
Normozoospermic and fertile															
Mean ± Standard deviation					*0.13 ± 0.05*	*0.09 ± 0.08*	*0.14 ± 0.06*	*0.12 ± 0.08*	*0.04 ± 0.02*	*0.05 ± 0.03*	*0.10 ± 0.05*	*0.34 ± 0.13*	*0.17 ± 0.11*	*0.50 ± 0.25*	*0.27 ± 0.13*
Fertile															
Mean ± Standard deviation						*0.09 ± 0.09*		*0.12 ± 0.02*	*0.14 ± 0.08*	*0.16 ± 0.10*	*0.32 ± 0.17*		*0.25 ± 0.10*	*1.01 ± 0.35*	*0.10 ± 0.03*
Sperm aneuploidy frequency in men with alterations of sperm parameters and normal karyotype															
Sperm concentration															
Oligozoospermia (5-20×10⁶)															
Mean ± Standard deviation						*0.08 ± 0.05*			*0.09 ± 0.03*	*0.09 ± 0.04*	*0.42 ± 0.22*		*0.52 ± 0.27*		
Severe oligozoospermia (<5×10⁶)															
Mean ± Standard deviation						*0.10 ± 0.09*			*0.10 ± 0.03*	*0.20 ± 0.13*	*0.57 ± 0.11*		*0.69 ± 0.36*		
Sperm morphology															
Teratozoospermia															
Mean ± Standard deviation			*0.62 ± 0.48*	*2.40 ± 4.75*	*0.51 ± 0.27*	*0.72 ± 1.32*	*0.68 ± 0.29*		*0.43 ± 0.74*	*1.05 ± 0.79*	*1.71 ± 1.91*		*0.50 ± 0.28*		
Teratoastenozoospermia															
Mean ± Standard deviation									*0.52 ± 0.45*	*0.46 ± 0.37*	*0.23 ± 0.10*		*0.06 ± 0.04*		
Oligoteratozoospermia															
Mean ± Standard deviation									*0.49 ± 0.23*	*0.44 ± 0.21*			*0.14 ± 0.15*		
Globozoospermia															

Mean ± Standard deviation					*0.95 ± 2.51*	*0.24 ± 0.26*	*0.10 ± 1.49*		*0.28 ± 0.19*	*0.16 ± 0.16*	*1.51 ± 2.18*		*0.50 ± 0.35*	
Sperm motility														
Asthenozoospermia														
Mean ± Standard deviation						*0.12 ± 0.14*			*0.09 ± 0.02*	*0.10 ± 0.01*	*0.23 ± 0.09*		*0.39 ± 0.10*	
Oligoastenoterato-zoospermia														
Mean ± Standard deviation	*0.28 ± 0.14*	*0.74 ± 0.83*	*1.63 ± 0.30*	*0.59 ± 0.24*	*0.46 ± 0.84*	*0.35 ± 0.45*	*0.54 ± 0.80*		*0.42 ± 0.80*	*0.41 ± 0.67*	*0.79 ± 3.81*		*0.45 ± 0.67*	
Genetic asthenozoospermia														
Mean ± Standard deviation						*0.10 ± 0.04*			*0.12 ± 0.09*	*0.14 ± 0.12*	*0.28 ± 0.22*		*0.43 ± 0.23*	
Sperm aneuploidy in male ICSI candidates														
Oligoastenoteratospermia														
Mean ± Standard deviation			*1.53 ± 0.29*	*0.37 ± 0.43*	*0.87 ± 0.72*	*0.64 ± 0.62*	*0.74 ± 0.80*	*0.37 ± 0.33*	*0.35 ± 0.28*	*0.40 ± 0.14*	*0.27 ± 0.11*	*1.13 ± 0.80*	*1.12 ± 1.08*	*0.89 ± 0.98*
Azoospermia														
Mean ± Standard deviation			*0.69 ± 0.22*		*0.79 ± 1.04*	*1.05 ± 0.81*	*1.49 ± 1.91*	*4.28 ± 4.97*	*0.95 ± 1.30*	*1.21 ± 1.43*	*1.64 ± 2.50*	*2.53 ± 1.54*		*3.35 ± 4.57*

[a]Diploidy
[b]Total disomy
[c]Total aneuploidy
[d]Total nullisomy

Few extensive studies have reported high aneuploidy frequencies for chromosomes 14, 21, 22, X, and Y (Shi and Martin 2000a; Templado et al. 2005). A review of the available literature revealed only the disomy frequency of 21, X, and Y in reference subjects increased. This finding could be explained by the hypothesis that chromosomes 21, X, and Y may be prone to recombination reduction or failure during meiosis (Shen et al. 1998) because they have a single chiasmata (Sun et al. 2004), which increases the probability of incorrect segregation during meiosis I (Koehler et al. 1996; Templado et al. 2005).

A lower incidence of the mean of total aneuploidies in male *normozoospermic and fertile* subjects (0.50 ± 0.25 %) is not unexpected since normozoospermia alone does not necessarily indicate fully fertile status. Moreover, it is well known that donor age and lifestyle, including aspects such as smoking, alcohol consumption, and exposure to toxic substances, can affect semen quality. Therefore, the sperm chromosomal aneuploidy rate probably varies significantly in time; in addition, the fertile status cannot be considered constant throughout a person's life, according to several published studies on the effects of age and environmental factors on sperm aneuploidy rate (Bosch et al. 2001, 2003; Naccarati et al. 2003; Templado et al. 2011b).

FISH Analysis in Men with Alterations of Sperm Parameters

Nearly all studies investigating sperm aneuploidy in infertile men have demonstrated a significant increase in aneuploidy levels compared to their fertile counterparts (Templado et al. 2005; Sarrate et al. 2005; Miharu 2005). The majority of studies report around a threefold increase in the sperm aneuploidy rate in infertile men.

Increases in sperm aneuploidy are strongly correlated with an increasing severity of infertility: the highest level of aneuploidy was reported in men with severe oligoasthenoteratozoospermia and in cases of nonobstructive azoospermia where sperm are retrieved from testicular tissue (Vidal et al. 2001; Egozcue et al. 2003; Miharu 2005).

Furthermore, FISH data have been reported for all seminal phenotypes, including oligozoospermia (low concentration), asthenozoospermia (poor motility), and teratozoospermia (poor morphology) in infertile males with normal karyotype (Bernardini et al. 1997, 1998; Lahdetie et al. 1997; McInnes et al. 1998; Storeng et al. 1998; Pang et al. 1999; Ushijima et al. 2000; Vegetti et al. 2000; Calogero et al. 2001a; Shi and Martin 2001; Vidal et al. 2001; Egozcue et al. 2003; Rives et al. 2004; Miharu 2005; Sarrate et al. 2005; Templado et al. 2005).

In particular, a negative correlation has been reported between sperm aneuploidy rate and progressive motility (Ushijima et al. 2000; Vegetti et al. 2000; Celik-Ozenki et al. 2004; Collodel et al. 2007), normal morphology (Bernardini et al. 1998; Calogero et al. 2001a; Ryu et al. 2001; Lewis-Jones et al. 2003; Carrell et al. 2004), and nuclear maturity (Kovanci et al. 2001).

Nevertheless, seminal alterations such as oligozoospermia, asthenozoospermia, and teratozoospermia are often detected simultaneously in the same ejaculate because a damaged seminiferous epithelium produces fewer sperm, generally with abnormal morphology and, therefore, with decreased motility, as in oligoasthenoteratozoospermic (OAT) patients. A high percentage of aneuploid sperm could be produced as a result of the negative influence of any testicular pathology on spermatogenetic processes (Martin et al. 1993; Bischoff et al. 1994; Spriggs et al. 1995; Calogero et al. 2001a).

Among sperm alterations, reduced sperm concentration is reported to be the most strongly associated with chromosomal aneuploidies (Ohashi et al. 2001; Martin et al. 2003a; Nagvenkar et al. 2005) because severe quantitative impairment of spermatogenesis has been related to qualitative alterations of chromosome recombination and segregation during spermatogenesis (Egozcue et al. 2005; Miharu 2005; Sarrate et al. 2005).

Among chromosomes, gonosomes are the most susceptible to nondisjunction because X and Y are generally involved in only one crossover in the pseudoautosomal region, and thus if this process remains incomplete, normal disjunction does not occur.

Among autosomes, disomies of chromosomes 13, 18, and 21 were found to increase in OAT patients with a frequency of more than 3 times higher in comparison with *normozoospermic and fertile* controls (Table 3.1). Sex chromosomes were particularly affected by OAT condition: the mean disomy rates resulted in significantly increases in all gonosomes and mainly for XY disomy, which was approximately eight times higher than in *normozoospermic and fertile* controls (Table 3.1).

Taken together, all these data suggest that men with impaired spermatogenesis should have reduced genome-wide recombination leading to chromosome-specific sperm defects.

Sperm morphology is considered one of the main criteria for sperm selection before an assisted reproductive procedure. Lee et al. (1996) analyzed the chromosomal constitution of human sperm injected into mouse oocytes. Sperm with abnormal head morphology showed a frequency of structural chromosomal aberrations approximately four times higher than those with normal morphology. The statement that teratozoospermic patients have an aneuploidy rate significantly higher than controls has been confirmed by several authors using multicolor FISH analysis: the frequency of chromosome 18 disomy was approximately eight times greater than in *normozoospermic and fertile* controls (Table 3.1). Teratozoospermic samples also showed a significant increase in the frequency of disomy for sex chromosomes: some morphological abnormalities may be more closely associated with chromosome imbalance, particularly those involving the sperm head (Sun et al. 2006).

When a high percentage of macrocephalic, multinucleate, and multiflagellate sperm are detected, autosome and gonosome frequencies show an approximately tenfold increase, as reported by various authors (Table 3.1). Globozoospermia, a peculiar sperm head alteration of genetic origin, seems to be strictly associated with a higher incidence of sperm aneuploidies (Carrell et al. 1999, 2001; Moretti et al. 2005).

While a consensus exists on the role played by severe oligozoospermia and teratozoospermia on sperm aneuploidy and diploidy, whether isolated asthenozoospermia affects sperm aneuploidy is less clear. It is often difficult to group data from asthenozoospermic samples into separate categories due to the concomitant alteration of other sperm parameters, such as concentration and morphology.

Isolated asthenozoospermia has been reported by few authors (Aran et al. 1999; Bernardini et al. 2005; Collodel et al. 2007) analyzing a range of autosomes (1, 4, 8, 12, 13, 18, and 21) and sex chromosomes: on the whole, increased sperm disomy and diploidy rates were detected with respect to the controls.

In other cases of absolute asthenozoospermia characterized by systematic sperm anomalies of the flagella, such as stump tail syndrome and Kartagener syndrome, abnormal aneuploidy and diploidy rates were confirmed by different authors (Rives et al. 2005). In the case of fibrous sheath dysplasia, some studies reported that the mean frequency of diploidy (0.43 ± 0.23 %) and sex chromosome aneuploidies increased sharply in comparison to controls group (Baccetti et al. 2005; Moretti and Collodel 2006; Piomboni et al. 2007).

Sperm Aneuploidy in Infertile Male ICSI Candidates to ICSI

The advent of ICSI (Palermo et al. 1992) revolutionized the treatment of male infertility by allowing patients with severely compromised semen parameters to achieve fatherhood. Although sperm with the "best" morphological features are selected for injection into the oocyte, this is not an absolute indicator of a normal genetic constitution (Ryu et al. 2001; Burrello et al. 2004; Celik-Ozenci et al. 2004), and the transmission of chromosomal abnormalities to offspring is possible.

Various researches have shown that prenatal karyotypes of embryos obtained by ICSI have higher sex chromosome aneuploidy rates (0.6 % versus 0.2 %) and higher autosomal structural alterations (0.4 % versus 0.07 %) than the general neonatal population (Veld et al. 1997; Bonduelle et al. 1998, 2002; Van Steirteghem et al. 2002). Several clinical studies suggested a strong correlation between the aneuploidy rate of male gametes and ICSI outcome: implantation failure, decreased pregnancy, and increased miscarriage rates after ICSI have been reported in OAT male when FISH analysis demonstrated abnormal aneuploidy and diploidy frequencies.

Few studies published so far have found an effect of sperm aneuploidies on the outcome of ICSI (Colombero et al. 1999; Calogero et al. 2001b) and reported comparable fertilization rates, clinical pregnancy rates, pregnancy losses, and occurrence of neonatal malformations in males with both normal and abnormal semen parameters. However, these authors concluded that, although the overall ICSI outcome was not significantly correlated with sperm aneuploidy, a tendency to a lower aneuploidy rate was underlined in the male partner of pregnant women.

The evaluation of the influence of chromosome abnormalities in men with altered semen parameters undergoing an Assisted Reproductive Technologies (ART) procedure could be biased by semen selection methods. Many reports consistently found an increase in aneuploidy rates in subfertile men, underlining that conventional sperm separation techniques are not able to exclude aneuploid gametes from fertilizing pools (Samura et al. 1997; Pfeffer et al. 1999; Van Dyk et al. 2000). More recently, a selection technique based on hyaluronic acid (HA)–sperm binding was demonstrated as being able to recover a high percentage of euploid sperm: the advantages of HA-mediated sperm selection in relation to ICSI outcome improvement could be due to the decreasing frequency of chromosomal disomy and diploidy, which results in a four- to sixfold reduction in comparison with whole semen samples (Jakab et al. 2005; Huszar et al. 2007).

With regard to clinical practice, sperm aneuploidy screening may be recommended especially in those countries, such as Italy, where preimplantation diagnosis can be performed only in selected cases and therefore FISH on male gametes seems to be the only possible technique for determining the risk of generating unbalanced embryos.

ICSI with Testicular or Epididymal Sperm

Sperm extracted from the epididymis (MESA) or testicular tissue (TESA/TESE) have a substantially increased risk of chromosomal abnormalities, and therefore FISH investigation appears even more suitable. A high aneuploidy rate in testicular sperm recovered from nonobstructive azoospermic (NOA) patients has been widely reported (Bernardini et al. 2000; Levron et al. 2001; Burrello et al. 2002; Mateizel et al. 2002; Palermo et al. 2002; Rodrigo et al. 2004; Gianaroli et al. 2005; Vozdova et al 2012). These data have not been confirmed by Martin et al. (2000), who analyzed aneuploidy frequencies for chromosomes 13, 21, X, and Y in sperm from three men with nonobstructive azoospermia. The authors concluded that NOA patients may not have a substantially increased risk of chromosomally abnormal sperm, in comparison to healthy men. Nevertheless, in these cases it could be take into consideration that only a small number of testicular sperm are available for FISH analysis, and this could affect the accuracy of the estimated aneuploidy rate.

A higher incidence of chromosomal anomalies in epididymal than in ejaculated sperm has also been reported (Bernardini et al. 2000; Burrello et al. 2002; Palermo et al. 2002; Levron et al. 2001; Rodrigo et al. 2004).

We therefore can conclude that chromosomal abnormalities affect the ICSI outcome when sperm are obtained by MESA and TESE, decreasing the fertilization and pregnancy rates and increasing the miscarriage rate.

On the whole, embryos originated by azoospermic patients have an increased rate of chromosomal abnormalities, and therefore appropriate genetic counseling should be offered before ICSI.

Sperm Aneuploidy in Infertile Male Carriers of Chromosomal Alterations

The incidence of constitutional chromosomal abnormalities is approximately 2 % in males with combined indications of infertility (Meschede et al. 1997), 5 % in oligozoospermic, and 14 % in azoospermic men (Johnson 1998). The most common karyotype abnormalities in infertile men include numerical sex chromosome alterations and Robertsonian translocations (Shi and Martin 2001).

Numerical Sex Chromosome Abnormalities

47,XYY

The extra Y chromosome in 47,XYY males may arise by at least two mechanisms: paternal nondisjunction at meiosis II after normal chiasmata in meiosis I (84 %) or postzygotic mitotic error (16 %) (Robinson and Jacobs 1999; Rives et al. 2003a). Males with an extra Y chromosome are generally fertile, and meiotic studies carried out in these patients indicated that the extra Y chromosome is frequently lost during the premeiotic stages (Thompson et al. 1967; Chandley et al. 1976). Nevertheless, in some cases one X and two Y chromosomes have been detected during prophase I as an X univalent plus a YY bivalent (Hulten and Pearson 1971; Speed et al. 1991; Blanco et al. 1997). No increase in the frequency of any category of sex chromosomal aneuploidy was found in 47,XYY patients by Han et al. (1994). In contrast, several authors (Martini et al. 1996; Mercier et al. 1996; Morel et al. 1999; Lim et al. 1999a; Martin et al. 1999; Giltay et al. 2000; Wang et al. 2000; Moretti et al. 2007) reported a moderate increase in sex chromosome disomies.

Globally, the frequencies of sperm with an abnormal number of sex chromosomes range from 0.04% to 19 % depending on the study: the mean XY disomy rate increased sharply (4.43 ± 6.03 %) as shown by an evaluation of comparable data from different studies (Table 3.2). The general finding is that the persistence of an extra chromosome in germ cells of 47,XYY males can impair spermatogenesis, determining a low sperm count. Since most children of 47,XYY fathers have a normal karyotype, the extra Y chromosome may presumably be lost during meiosis (Shi and Martin 2000b, 2001). Nevertheless some XYY germ cells can complete meiosis and produce mature aneuploid sperm.

Recent review studies (Sarrate et al. 2005; Rodrigo et al. 2010) indicated that 3.7 % of the spermatozoa analyzed by FISH carry an extra sex chromosome and that diploid sperm ranges from 0 to 3.35 %.

On the other hand, males with a mosaic 47,XYY/46,XY showed a lower cumulative rate of sex chromosome aneuploidy in sperm than XYY patients. The mean gonosome disomy resulting from comparable data reported in the literature ranged from 0.17 ± 0.16 % for XX to 0.48 ± 0.31 % for XY (Table 3.2). As regards the risk

Table 3.2 Sperm aneuploidy frequency in infertile male carriers of chromosomal unbalance (Modified from Gambera et al. 2011)

	Autosomal disomy (%)			Sexual disomy (%)		
	13	18	21	XX	YY	XY
47, XYY						
Mean ± Standard deviation				*1.65 ± 2.31*	*1.54 ± 1.53*	*4.43 ± 6.03*
Mosaic 47,XYY/46,XY						
Mean ± Standard deviation				*0.17 ± 0.16*	*0.50 ± 0.45*	*0.48 ± 0.31*
47, XXY Klinefelter syndrome						
Mean ± Standard deviation				*4.64 ± 2.56*	*0.30 ± 0.46*	*11.1 ± 6.89*
Mosaic 47,XXY/46,XY						
Mean ± Standard deviation				*0.40 ± 0.31*	*0.42 ± 0.49*	*1.22 ± 0.71*
Robertsonian translocations						
Mean ± Standard deviation	*2.65 ± 2.73*	*0.22 ± 0.27*	*0.62 ± 0.89*	*0.65 ± 0.92 (Total sex disomy %)*	*1.20 ± 1.91 (Diploidy %)*	

assessment of the transmission of chromosomal aberration to embryos, Gonzalez-Merino et al. (2007) analyzed 47 preimplantation embryos and reported a total aneuploidy rate of 32 %.

47,XXY, Klinefelter Syndrome

Infertile males affected by Klinefelter syndrome (KS) are approximately 3 %, increasing up to 11 % among azoospermic men (Foresta et al. 1999). These subjects are rarely naturally fertile, although assisted reproductive procedures such as ICSI offer them a chance at fatherhood. The sperm phenotype among KS males is widely heterogeneous, ranging from azoospermia to normozoospermia.

The extra X chromosome in males with KS may arise by a paternal nondisjunction at meiosis I in approximately 50 % of cases (Hall et al. 2006). During spermatogenesis, the extra sex chromosome appears to be eliminated (Shi and Martin 2001). On the other end, many studies carried out in 47,XXY males detected marked increases in sex chromosome disomies and diploid sperm (Guttenbach et al. 1997b; Estop et al. 1998; Foresta et al. 1998, 1999; Okada et al. 1999; Rives et al. 2000; Morel et al. 2003; Ferlin et al. 2005; Sarrate et al. 2005; Templado et al. 2011a). The mean disomy rate increased sharply for XX (4.64 ± 2.56 %) and XY (11.1 ± 6.89 %),

with an average incidence of 6.3 % (Table 3.2). Diploid sperm in these patients also increased, ranging from 0.03% to 4.2 %, as did autosomal aneuploidies, reaching 6.2 % for chromosome 21 (Templado et al 2011a).

In patients with mosaic KS, the frequency of sperm aneuploidy varied according to the percentage of 47,XXY cells. Various FISH studies (Chevret et al. 1996; Martini et al. 1996; Lim et al. 1999b; Okada et al. 1999; Rives et al. 2000; Ferlin et al. 2005) have demonstrated an increased frequency of sex chromosome disomy, ranging from 0.40% to 1.22 % for XY (Table 3.2).

Chromosomal Translocations

Balanced chromosomal translocations are characterized by breakpoints in two chromosomes and repair of the chromosomal fragments with transpositions of genetic material between them, without loss of genetic material.

Male carriers of these structural alterations generally have a normal phenotype while showing a reduced fertility and an increase in spontaneous miscarriage and birth defects.

Robertsonian Translocations

Robertsonian translocations are the most common chromosomal anomaly among infertile men, characterized by the centric fusion of two acrocentric chromosomes (13, 14, 15, 21, 22) and resulting in a 45 chromosome karyotype. The most frequent reorganization are t(13q;14q) and t(14q;21q), with an estimated frequency of 0.97 and 0.20 %, respectively (Frydman et al. 2001). Before the report of Plymate et al. (1976), testicular function defects were only associated with sex chromosome abnormalities (Paulsen et al. 1968). Since 1976, many studies have shown that carriers of chromosome anomalies, especially translocations, have an altered spermatogenesis characterized by severe oligozoospermia (Chandley et al. 1976; Veld et al. 1997; Ogawa et al. 2000). In addition, unusual ultrastructural sperm anomalies related to immaturity were observed in carriers of Robertsonian translocation (Baccetti et al. 2002). Spermatogenetic alterations could be a consequence of a chromosomal anomaly: the pairing of the reorganized chromosomes during meiotic prophase I gives rise to a trivalent configuration that is prone to segregate in an alternate way, producing normal or balanced sperm (Sybenga 1975; Vidal et al. 1982; Luciani et al. 1984). Unbalanced sperm are generated by an adjacent segregation pattern, and they could be responsible for miscarriages or aneuploid offspring (Egozcue et al. 2000b).

In Robertsonian translocation carriers, FISH analysis demonstrated a percentage of normal or balanced sperm ranging from 73.6 up to 91 % (Escudero et al. 2000; Morel et al. 2001; Anton et al. 2004; Roux et al. 2005; Nishikawa et al. 2008). Contrasting results showing a high percentage of unbalanced sperm derived from adjacent segregation, ranging from 3 % to 36 % (reviewed by Harton and Tempest 2012).

Reciprocal Translocations

Exchanges of genetic material between two or more chromosomes characterize the reciprocal translocation. A wide range of different situations is included in this structural chromosomal anomaly, each of them unique in individual families, depending on the chromosome involved, the size of the translocated regions, and the probability of recombination within these regions (Harton and Tempest 2012). Reciprocal translocations are the most frequent (1/600) structural chromosomal anomalies in humans (Estop et al. 1997). Among infertile males these chromosomal reorganizations are approximately ten times more frequent than in the general population (Van Assche et al. 1996), and a high level of unbalanced gametes are reported in various studies ranging from 29 % up to 81 % (Harton and Tempest 2012), with an average of 50 % (Shi and Martin 2001).

The meiotic behavior of reciprocal translocations depends on the chromosomes involved in the rearrangement, the position of the breakpoints, the presence of crossovers in the translocated chromosomes, and the morphological characteristics of the rearranged chromosomes. During meiosis I, segregation of the quadrivalent formed among the translocated chromosomes and their normal homologs produces a variety of balanced and unbalanced gametes. In the alternate segregation pattern, where homologous centromeres move to opposite poles, chromosomally balanced or normal gametes are produced. Unbalanced gametes are produced by the other segregation patterns, specifically adjacent I, adjacent II, and 3:1 segregation.

Alternate segregation is the most common meiotic behavior, occurring with a frequency of 44–51 %; adjacent I segregants have a frequency of 16–40 %, while adjacent II segregants have a lower mean frequency of approximately 9 % (Shi and Martin 2001), which varied inversely with the length of the shorter centric segment (Faraut et al. 2000). Finally, 3:1 segregants occur with a mean frequency of 11 % (Shi and Martin 2001) even if, in some cases, 3:1 segregation is the preferential pattern (Jalbert et al. 1980; Estop et al. 1999; Van Assche et al. 1999) with an unusually high rate up to 23.5 % as reported in four different reciprocal translocation carriers (Nishikawa et al 2008).

An analysis of familial cases confirmed that segregation patterns were specific for a given translocation, as demonstrated by detection of the same profile of meiotic segregation mode in each family (Rousseaux et al. 1995; Cora et al. 2002; Anton et al. 2004; Morel et al. 2004; Wiland et al. 2007).

Interchromosomal Effect

The possibility that chromosome rearrangements could interfere with the meiotic behavior of chromosomes not involved in translocation led to the concept of interchromosomal effect (ICE), postulated for the first time in humans by Lejeune (1965).

Meiotic segregation of sex chromosomes and autosomes was investigated directly on sperm nuclei by FISH by various authors (Rousseaux et al. 1995; Blanco et al. 2000; Vegetti et al. 2000; Morel et al. 2001; Anton et al. 2004), and the results

suggested that ICE was generally restricted to translocation carriers with abnormal semen parameters.

In six carriers of Robertsonian translocations t(13;21) and t(14;22), the interactions between chromosome rearrangements and ICE were studied by evaluating aneuploidy and diploidy frequencies of chromosomes 18, X, and Y: the mean percentage of sex chromosome disomy as well as the frequency of diploid sperm were significantly higher than in controls (Baccetti et al. 2005)

Therefore, the increase in sperm aneuploidies among Robertsonian translocation carriers could be related to ICE, as suggested by many studies (Blanco et al. 2000; Vegetti et al. 2000; Morel et al. 2001; Baccetti et al. 2002, 2005; Anton et al. 2004; Ogur et al. 2006; Chen et al. 2007). However¸ a negative effect of an altered testicular environment on the meiotic process cannot be excluded in any of these studies because none of the enrolled subjects with translocations was classified as normozoospermic.

The question of ICE in reciprocal translocation carriers is still controversial. Some authors did not report any evidence of ICE in several reciprocal translocation carriers (Van Hummelen et al. 1997; Honda et al. 1999; Estop et al. 2000; Rives et al. 2003b; Oliver-Bonet et al. 2004). Some of the analyzed patients had normal semen parameters, and therefore the authors suggested that ICE in translocation carriers could be restricted to patients with abnormal semen parameters (Vegetti et al. 2000; Pellestor et al. 2001).

On the other hand, many reports detected an ICE in different reciprocal translocation carriers (Blanco et al. 2000; Oliver-Bonet et al. 2001, 2002; Baccetti et al. 2003; Douet-Guilbert et al. 2005; Wiland et al. 2007; Vozdova et al. 2008).

All reports support the occurrence of ICE in particular cases of structural chromosome reorganization, depending on the type of reorganization and on the chromosome or chromosomal region involved. However, the increase in aneuploidy and diploidy rates in infertile translocation carriers could be feasibly due to altered semen quality, as previously reported for infertile males of normal karyotype with oligoasthenoteratozoospermia.

Sperm Aneuploidy and Hormone Treatment

As highlighted so far, errors in sperm chromosome segregation are often observed in infertile males, and this is especially negative for candidates for assisted fertilization, increasing the failure rate and risk of generating offspring with chromosome imbalance. Therefore, it would be useful to develop methods for reducing the rate of aneuploidy in sperm.

Follicle stimulating hormone (FSH) is known for its role in the initial development of Sertoli cells and their stimulation to control spermatogenesis. FSH can therefore be used to improve spermatogenesis and fertilizing competence of oligozoospermic males, increasing both spermatogonial population and sperm production (Acosta et al. 1991, 1992; Foresta et al. 1998, 2002, 2005; Baccetti et al. 1997,

2004; Ben-Rafael et al. 2000). The administration of FSH can be useful in hypogonadotropic hypogonadism and when sperm alterations associated with normal gonadotropin levels suggest functional gonadotropin deficit.

In selected male patients with serum FSH less than 8 mIU/mL and a frequency of sperm aneuploidies greater than 0.6 % according to FISH analysis, 3 months of recombinant FSH therapy improved sperm quality and significantly decreased the frequency of sperm chromosomal alterations. The average percentage of total aneuploidies dropped by 31.8 %. The general improvement in sperm chromosome segregation was predominantly due to the decrease in diploidies and sex chromosome disomies (Piomboni et al. 2009).

The effect of FSH therapy on spermatogenesis may be explained by findings indicating that gonadotropins act as survival factor for spermatogonia and spermatocytes regulating the intrinsic and extrinsic apoptotic pathways, by which germ cells die in normal adult seminiferous epithelium (Ruwanpura et al. 2008).

Conclusions

Multicolor FISH in decondensed sperm nuclei using probes for sex chromosomes and autosomes, particularly chromosomes 1, 13, 18, and 21, allows an accurate evaluation of the incidence of sperm aneuploidy and is an appropriate way to analyze several thousand cells as well as a few cells in the case of severe oligozoospermic or azoospermic patients undergoing testicular biopsies. This technique, developed in the 1990s, may be applied for clinical or research aims. By pooling all published data from FISH analysis on sperm nuclei, it has become a useful tool in reproductive counseling for infertile couples (Gambera et al. 2011).

Using multicolor FISH, errors in chromosomal segregation have been found in sperm from normozoospermic or fertile men with a mean incidence ranging from 0.6 % to 1.45 %. Moreover, the percentage of numerical chromosomal aberrations increases in relation to sperm phenotype as in OAT men, suggesting that the risk of chromosome malsegregation events increases depending on the severity of testicular failure. This is also true for infertile males with abnormal karyotypes, which can produce a high percentage of gametes with unbalanced chromosomes. Sperm carrying chromosome abnormalities generally have a reduced fertilization potential; however, the development of assisted reproductive techniques such as ICSI revolutionized the treatment of male infertility, enabling these patients to procreate but increasing the risk of generating embryos with chromosomal unbalances.

Therefore, on these bases, information about meiosis and the incidence of eventual meiotic abnormalities should be useful in couples undergoing assisted reproduction for male infertility factor.

No technical procedure of sperm selection can guarantee a choice of gamete without chromosomal imbalance; in that case, knowledge of the chromosomal constitution of the male gametes in selected cases might suggest the need for a preimplantation or prenatal genetic diagnosis.

Further information on the relationships between sperm chromosome unbalance and human male infertility could help to promote a correct diagnostic and therapeutic approach to infertile couples, even when the cause of infertility is unknown, as in idiopathic diagnosis.

As regards the progressive improvement of the technique, in the future, the introduction of automated systems for multicolor FISH scoring would save time in the evaluation of results, which actually implies many hours of microscope viewing, which could depend on interoperator variability.

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