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NEW RESEARCH HORIZON ***Review***

New insights on the origin and relevance of aneuploidy in human spermatozoa

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ABSTRACT: In humans, the most common chromosomal abnormality is aneuploidy. Because the majority of aneuploid conceptuses die during the early stages of embryonic development, an accurate estimate of the frequency of aneuploidy at conception can only be assessed by directly studying the gametes. The vast majority of aneuploidies arise *de novo* as a result of sporadic chromosome missegregation in paternal or maternal meiosis. In this review, we present the basic current knowledge about the incidence of aneuploidy in human spermatozoa in the general population and in patient populations where elevated levels of sperm aneuploidy are observed. These include infertile patients, patients with abnormal somatic karyotypes, and individuals exposed to certain environmental/lifestyle hazards. The clinical impact of increased levels of aneuploidy is discussed. We then focus on the non-disjunction mechanisms that cause aneuploidy during spermatogenesis and the factors that predispose to non-disjunction in male germ cells followed by an analysis of the sex differences in the incidence of gamete aneuploidy. Recent meiotic studies using multiplex-FISH on three fertile men have revealed that the frequency of conservative aneuploidy of metaphase II spermatocytes is similar to that observed in non-inseminated oocytes of young women. These findings suggest that the differences in the incidence of aneuploidy between spermatozoa and oocytes are not due to differences in chromosome segregation errors but rather to more effective checkpoint mechanisms in spermatogenesis than in oogenesis.

Key words: aneuploidy / meiosis / non-disjunction / spermatozoa / FISH

Introduction

In humans, the most common chromosomal abnormality is aneuploidy (monosomy and trisomy). Sex chromosome aneuploidy is more common than autosomal aneuploidy, both in neonates and in spontaneous abortions. It is estimated that at least 5% of all recognized pregnancies are aneuploid (Hassold and Hunt, 2001). The only aneuploidies that are compatible with survival are the autosomal trisomies 13 (Patau syndrome), 18 (Edwards syndrome) and 21 (Down syndrome) and the sex chromosome aneuploidies, including X chromosome monosomy (the only human viable monosomy). The majority of aneuploid conceptuses die during the early stages of embryonic development, before they are detected clinically, manifesting as spontaneous abortions, infertility or sterility. This is why an accurate estimate of the frequency of aneuploidy at conception can only be assessed by directly studying the gametes. The vast majority of aneuploidies arise *de novo* as a result of sporadic chromosome missegregation in paternal or maternal meiosis in parents with normal somatic karyotypes. A low percentage of aneuploidies are mosaics that originate by post-zygotic chromosome segregation errors during embryogenesis.

The parental origin of aneuploidies has been established using molecular analyses of DNA polymorphisms. Monosomy X is of paternal origin (paternal sex chromosome loss) in 70–80% of cases (Jacobs *et al.*, 1997; Martínez-Pasarell *et al.*, 1999a), whereas 50% of cases of 47, XXY and 100% of cases of 47, XYY are paternally derived. Trisomies for autosomes and trisomy X are predominantly of maternal origin (70–100%) (Hassold *et al.*, 2007).

The two mechanisms causing chromosome segregation meiotic errors are non-disjunction and anaphase lag. Non-disjunction includes chromosome gains and losses, producing nullisomic (22 chromosomes) and disomic (24 chromosomes) gametes, while anaphase lag produces only nullisomic sperm. Non-disjunction is considered the main mechanism leading to aneuploidy in human sperm (Márquez *et al.*, 1996).

In this review, we present the basic current knowledge about the incidence of aneuploidy in human spermatozoa in the general population and in patient populations where elevated levels of sperm aneuploidy are observed. These include patients affected with infertility, patients with abnormal somatic karyotypes and individuals exposed to certain environmental/lifestyle hazards. The clinical impact of increased levels of aneuploidy is discussed. We then focus on the non-disjunction mechanisms

that cause aneuploidy during spermatogenesis and the factors that predispose to non-disjunction in male germ cells followed by an analysis of the sex differences in the incidence of gamete aneuploidy.

Sperm aneuploidy levels in males from general population

In the past 30 years, studies on aneuploidy in human spermatozoa have been carried out by either: (i) analysis of sperm karyotypes obtained after *in vitro* fusion of hamster egg and human sperm (humster assay) (Martin *et al*., 1983) or (ii) analysis of interphase sperm nuclei using multicolour fluorescent *in situ* hybridization (FISH) assays. In both studies, a conservative estimate of sperm aneuploidy (2x disomy) is used due to the impossibility to distinguish nullisomy from artifactual loss of chromosomes during slide preparation or absence of hybridization.

Recently, Templado *et al*. (2011) reviewed aneuploidy rates in spermatozoa from FISH series of healthy donors with a total of 388 donors aged 18–80 years. This review included only FISH studies with a minimum of 5 donors, a count of 10 000 spermatozoa/probe set/individual and strict scoring criteria (see Table I). Pooled data described disomy frequencies for 18 of the 24 chromosomes of sperm complement. For individual autosomes, the mean disomy frequency was around 0.1%, ranging from 0.03 (chromosome 8) to 0.47 (chromosome 22) and the estimated incidence of total aneuploidy (2x disomy of 2.26%) was 4.5%, higher than that found in sperm karyotypes (1.8%) (reviewed in Templado *et al*., 1996, 2005). This review also noted that chromosome 21 (0.17%) and the sex chromosomes (0.27%) are more prone to non-disjunction with a 2–3-fold increase compared with the other autosomes. In agreement with these findings, these chromosomes are the most often involved in disomies in sperm karyotypes from healthy men (Templado *et al*., 1996, 2005). Moreover, in meiotic studies, chromosomes 21 and XY chromosome pairs appear separated as univalents at metaphase I spermatocytes and as disomies in metaphase II spermatocytes more frequently than other chromosome pairs (Uroz and Templado, 2012). These results were not surprising, since bivalent 21 and XY chromosomes have the smallest pairing regions and usually present a single chiasma during meiosis I (Laurie and Hultén, 1985); thus, they have greater susceptibility to non-disjunction, since chiasmata are required for the correct orientation of homologues on the anaphase I spindle (reviewed by Roeder, 1997).

In recent years, the use of assisted reproductive technologies and new drugs for erectile dysfunction offer the possibility of parenthood to men of advanced age. Thus, the interest in studying the effect of age on aneuploidy in sperm in order to determine the risk to offspring is justified. However, the studies carried out so far to determine the potential effect of age on the frequency of disomic sperm have not been able to demonstrate such an increase (reviewed in Buwe *et al*., 2005 and in Fonseka and Griffin, 2011).

No donor age effect was found for chromosomes 1, 3, 6, 7, 8, 10, 11, 12, 13, 14 or 18. For chromosomes 1, 9, 21, X and Y there is no agreement among reports, although most describe no increase of disomy with age (see Fonseka and Griffin, 2011). Future studies of healthy males selected based on their age and including men in their 60s and 70s may elucidate whether paternal age increases the frequency of disomic sperm and therefore the risk to offspring.

Aneuploidy: from normal values to clinical values

In the review of literature citations of healthy controls (Templado *et al*., 2011) chromosome 21 disomy is reported to range from 0.03 to 0.37% and that of the sex chromosomes, from 0.11 to 0.43%. The variability in the disomy values reported should encompass inter-donor variability plus variability due to technical differences in processing sperm, staining and scoring.

Several authors have studied inter-donor variations and found that some men consistently produce higher levels of aneuploidy (stable variants) at least for a number of disomies (Table II). Rubes *et al*. (2005) after testing the same 10 donors 3 times over a 5-year period, described 1 stable variant with consistent levels over the years of disomy 18 with a 3-fold increase. In 2002, Rubes *et al*. had identified two healthy donors who consistently showed increased XY (3x) and chromosome 8 disomy (3x), respectively. Likewise, Tempest *et al*. (2009), after testing 10 donors 4 times in an 18-month period, identified 1 stable variant consistently producing higher levels of disomy 13 and 2 men who displayed significant nullisomy for the sex chromosomes. At the level of meiotic cells (spermatocytes I) similar inter-individual variations have been found among fertile men. The percentage of spermatocytes I with dissociated sex chromosomes ranged from 3.2 to 43.7% in Uroz *et al*. (2011) with some men consistently showing high percentages of achiasmate homologues. Skakkebaek *et al*. (1973) had already described the percentages of sex chromosome univalents from 0 to 26% in fertile men. Reduced or absent meiotic recombination may lead to increased sperm aneuploidy frequencies and to infertility in men.

It appears that some men have a predisposition to non-disjunction that could translate into an increased risk of infertility and having children with paternally transmitted aneuploidies. Thus, the next logical question is the following.

What levels of sperm aneuploidy are necessary to result in an increase of aneuploidy in the offspring?

Several reports have focused on sperm aneuploidy in fathers of paternally inherited Down syndrome (Blanco *et al*. 1998a; Soares *et al*., 2001a), Turner syndrome (Martínez-Pasarell *et al*., 1999b; Soares *et al*., 2001b) and Klinefelter syndrome (Arnedo *et al*., 2006) (reviewed by Templado *et al*., 2011) (Table II). These studies have shown at least a doubling of sperm disomy for chromosomes 21 and XY in all the three groups of fathers. Interestingly the 2-fold moderate increases seen in these fathers of aneuploid offspring are of the same level of magnitude as those seen in the so-called stable variants described above. Not all studies of fathers of aneuploid children are consistent with these findings: neither Hixon *et al*. (1998) when studying Down syndrome fathers nor Eskenazi *et al*. (2002) when studying Klinefelter syndrome fathers reported elevated aneuploidy.

Sperm aneuploidy in men with repeated spontaneous abortions and a wide range of sperm seminograms revealed that sperm sex chromosome disomy was significantly increased more than 2-fold (2.3x) compared with controls (Rubio *et al*., 1999). It seems likely that, at least in some

Table I The mean percentages of sperm disomy per chromosome in series of healthy individuals studied by multicolour FISH[a] analysis.

Percentage of disomy for chromosome in sperm nuclei[a]																
1	2	3	4	6	7	8	9	12	13	15	16	18	20	21	22[b]	Sex chr.
0.08	0.09	0.20	0.08	0.04	0.06	0.03	0.16	0.14	0.12	0.10	0.07	0.06	0.12	0.17	0.47	0.27

[a]Data collected by Templado et al. (2011) including only multicolour FISH studies with five or more donors, a count of a minimum of 10 000 spermatozoa/probe set /individual and strict scoring criteria.
[b]Only two groups have studied the frequency of disomy 22 with results extremely different: 1.21% (Martin and Rademaker, 1999) and 0.06% Soares et al., 2001b). The high frequency of disomy 22 reported in the first study could be due to the fact that the centromeric 22 probe used cross-hybridized to other chromosomes.

Table II Levels of aneuploidy in sperm of selected male populations[a].

Males population with		Sex chr. disomy	Chr. 21 disomy	Total disomy	Conservative aneuploidy	Diploidy
Stable variants	Rubes et al. (2002, 2005)	3x				5x
Aneuploid offspring	Reviewed in Templado et al. (2011)	2x	2x			
Repeated spontaneous abortions	Rubio et al. (1999)	2.3x				
Infertility	Sarrate et al. (2010)	2–3x	2–3x		3x	3–5x
Severe non-obstructive oligozoospermia	Mougou-Zerelli et al. (2011)	4x				2x
Severe oligozoospermia	Durak Aras et al. (2012)	2–6x	4x			
Non-obstructive azoospermia	Sun et al. (2008)	2–4x	4x			
Polymorphic teratozoospermia	Brahem et al. (2011a)			4–10x		2.5x
	Templado et al. (2002)	2x			2–3x	
Unclassified teratozoospermia	Tang et al. (2010)	1.5x	3x		2.5x	2x
	Gole et al. (2001)	4x				
Globozoospermia	Brahem et al. (2011a)			8–10x		4x
	Morel et al. (2004)	2–3x				
Macrocephalic head syndrome	Brahem et al. (2011a)			10–30x		23%
	Brahem et al. (2011b)					22%

[a]Expressed as an order of magnitude compared with internal controls of each study.

cases, there is an association between fathering aneuploid offspring or recurrent abortions and moderately increased levels of aneuploid spermatozoa, thus rendering the moderate increases in the rates of aneuploidy clinically significant.

Sperm aneuploidy in patients with infertility

In general, patients with infertility have significantly higher levels of sperm aneuploidy and the incidence of aneuploidy increases proportionally with the severity of the male factor infertility. It is quite likely that some subsets of infertility have an increased risk of sperm chromosome abnormalities, whereas others do not (Table II).

In a review of 319 patients who had consulted for fertility problems and who were seen at six assisted reproduction centres, Sarrate et al. (2010) found a 2- to 3-fold increase of sex chromosome disomy and disomy of chromosome 21 as well as a 3-fold elevation of aneuploidy and diploidy compared with controls. Among the abnormal seminogram parameters analysed for correlation with abnormal aneuploidy levels, a low sperm count (oligozoospermia) was correlated with increases in aneuploidy. Numerous studies cite the elevated aneuploidy of patients with oligozoospermia, in particular severe oligozoospermia (Durak Aras et al., 2012) (2–6x for sex chromosome disomy and 4x for disomy 21), and severe non-obstructive oligozoospermia (Mougou-Zerelli et al., 2011) (4x sex chromosome disomy). Elevated aneuploidy (2x–4x for sex chromosome disomy and 4x for disomy 21) has also been reported in the testicular sperm of patients with non-obstructive azoospermia (Sun et al., 2008).

For motility problems, in the review of Sarrate et al. (2010), there was no correlation between low motility and increased aneuploidy. Other studies had showed a modest correlation between the two parameters (Aran et al., 1999; Vegetti et al., 2000).

Concerning morphology, in patients with polymorphic teratozoospermia or unclassified teratozoospermia, most reports show a 2–4-fold increase of sex chromosome disomy and a 2–3-fold increase of

aneuploidy (Gole *et al*., 2001; Templado *et al*., 2002; Tang *et al*., 2010; Brahem *et al*., 2011a). However, Sarrate *et al*. (2010) did not find a correlation between teratozoospermia and aneuploidy even when analysing the 17 exclusively teratozoospermic patients. Other more severe sperm morphology abnormalities such as macrocephallic-multi-flagellated sperm syndrome (<1% of infertile male patients) display high levels of aneuploidy (10–30x) compared with controls and 20–40% rates of diploidy, triploid and tetraploidy (Perrin *et al*., 2008a; Brahem *et al*., 2011a, b). Patients with globozoospermia, also a rare condition that affects <1% of infertile patients and which is characterized by a lack of acrosome, have moderate increases of disomy according to Morel *et al*. (2004) (2–3-fold for the sex chromosomes) but Brahem *et al*. (2011a) reports a more pronounced elevation of 8–10x total disomy in these patients.

Sperm aneuploidy in patients with abnormal karyotypes

Patients with abnormal karyotypes often present with infertility and difficulties for achieving a viable pregnancy because repeated spontaneous abortions, may have an abnormal seminogram with a lower sperm count and may have an increased frequency of aneuploidy in sperm. In general, the rates of sperm aneuploidy observed in these patients are lower than the theoretical numbers derived from the expected meiotic behaviours of the trivalents or quadrivalents resulting from the pairing of the abnormal chromosomes. It appears that in some cases the complex meiotic figures are mechanically unresolvable and sufficient to block meiotic progression (certain reciprocal translocations, complex rearrangements) and in other instances (like XXY, XYY, some Robertsonian translocations) the possibility of a selection against aneuploid sperm from an unknown meiotic checkpoint cannot be ruled out.

In Klinefelter syndrome (XXY) non-mosaics the frequency of sex chromosome aneuploidy was 25% for the first reported patient (Estop *et al*., 1998a) but averages 6% (Tempest, 2011). Thus, there are some XXY cells in non-mosaic patients that are capable of initiating and completing meiosis with a sex chromosome trivalent or, alternatively, these numbers can be explained by the presence of undiagnosed normal cells in the testes of supposedly non-mosaic men. It should be noted that the theoretical risk of sex chromosomal aneuploidy for non-mosaic Klinefelter patients is 50%. In mosaic XY/XXY individuals the average sex chromosome aneuploidy is close to 3%. The majority of patients with an XYY non-mosaic chromosome constitution in peripheral blood have elevated sex chromosome disomy which averages 4.2% (Blanco *et al*., 2001). These increased rates reached up to 19% XY disomy and 16.7% YY in a patient reported by Gonzalez-Merino *et al*. (2007).

In male carriers of reciprocal translocations the percentage of unbalanced gametes (and thus gametes with segmental or whole chromosome aneuploidy) may vary from 20 to 77% (Martin and Spriggs, 1995) but more recently it has been shown that reciprocal translocations display a more homogeneous behaviour than described earlier and which is close to 50% (Van Hummelen *et al*., 1997; Blanco *et al*., 1998b; Estop *et al*., 1998b; Anton *et al*., 2008). In a review, Benet *et al*. (2005) reported a mean of 55.3% unbalanced gametes for reciprocal translocations studied by the hamster system and a mean of 53.5% for those studied by FISH. For Robertsonian translocation carriers 66% of the gametes are expected to be unbalanced and 1–36% (average 15%) are observed (Frydman *et al*., 2001; Ogur *et al*., 2006). Finally, carriers of paracentric or pericentric inversions have 0% unbalanced spermatozoa when the inverted segment is short (Balkan *et al*., 1983; Jenderny *et al*., 1992), a few recombinants when the inverted segment is 20–50% of the length of the chromosome and when it is >50% the percentage unbalanced is 20–40% (Anton *et al*., 2005, Morel *et al*., 2007).

In addition, during the first division of the meiotic cells of carriers of chromosome balanced rearrangements, the reorganized chromosomes may show asynaptic regions that may interfere with the pairing and segregation of other unpaired segments of the remaining chromosomes, a phenomenon termed interchromosomal effect. This may result in added aneuploidy for the chromosomes not involved in the rearrangement. Fifty-four per cent of men with Robertsonian translocations, 44% of reciprocal translocation carriers and 7% of men with inversions have been reported to carry an added aneuploidy load in the form of an interchromosomal effect in the sperm, as analysed by FISH (reviewed in Anton *et al*., 2011).

Lifestyle and other exposures may have an effect on sperm aneuploidy

Exposures of the spermatozoa to aneugenic agents may be the result of lifestyle, environmental and/or occupational exposures. Across the board comparisons among studies of exposures are hampered by the fact that there is a wide range of dosages, age of donors, heterogeneity between individuals, timing and length of exposure. Frequently, the compounding effects of different lifestyle and occupational exposures are difficult to separate.

So far, the emerging picture is that whenever significant associations between an exposure and sperm aneuploidy are present, only moderate increases in the rates of chromosome disomy above the unexposed population are observed, of an order of magnitude 1.5–3x and, in the majority of cases do not raise above 2.5x. Since lifestyle and other exposures may be transient, sperm aneuploidy may vary during the life of the individual.

Moderate increases are seen for cigarette smoke, a known aneugenic agent. Chromosome 13 disomy was reported to increase 3x (Shi *et al*., 2001), 1.5x for XX (Robbins *et al*., 1997), 2x for YY disomy (Rubes *et al*., 1998) and 2x for XY disomy (Naccarati *et al*., 2003). Shi *et al*. (2001) observed a lack of association between smoking and sex chromosome aneuploidy. The consequences of alcohol consumption on sperm aneuploidy are not clear due to a scarcity of studies. Robbins *et al*. (2005) reported raised XY disomy (1.38x) in workers exposed to boron who were taking alcohol. In a previous study, Robbins *et al*. (1997) detected a significant linear association between increasing alcohol intake and XX disomy in a cohort of Chinese men. The effects of occupational exposure to benzene were studied by Xing *et al*. (2010). They reported from a slightly elevated disomy YY (1.2x) in low level exposure to a 2.8x of XX disomy for those exposed to higher concentrations of benzene. Disomy XY and total sex chromosome disomy were affected as well but at an intermediate range.

Pesticides are released to the environment in large quantities so that most individuals have some degree of exposure. General pesticide exposure (Härkönen *et al*., 1999; Smith *et al*., 2004) did not result in changes in the rates of sperm aneuploidy, whereas fenvalerate exposure raised sex chromosome disomy 1.9-fold and disomy of chromosome 18, 2.6 times (Xia *et al*., 2004). Carbaryl, similarly, raised sex chromosome

disomy to 1.7x and chromosome 18 disomy to 2.2x (Xia et al., 2005). Polychlorinated biphenyl and *p, p′*-DDE both raised sex chromosome disomy very slightly (McAuliffe et al., 2012).

Nutritional folate, zinc and antioxidant intake were evaluated by Young et al. (2008). Interestingly, a slight reduction in the frequency of XX was seen for those taking folate (−0.75x), whereas oral zinc and antioxidants did not affect sperm aneuploidy.

Clinical relevance of aneuploidy

Overall, studies show that there are a number of clinical conditions in the male that have the potential to consistently affect sperm aneuploidy. Moderate increases (2–3-fold) are seen in fathers of children with aneuploid offspring, who themselves have normal somatic karyotypes, men who are stable variants for aneuploid sperm, in some forms of infertility, in men with repeated spontaneous abortions and after some lifestyle and environmental exposures. Higher increases are reported in some XXY and XYY men, in carriers of structural rearrangements and in severe forms of infertility (polymorphic teratozoospermia, globozoospermia and macrocephalic head syndrome). Higher frequencies of aneuploidy, which might be the result of severe meiotic impairment, seem to correlate with more severe infertility. Serious long-term somatic and reproductive health consequences may include increased risks of aneuploidy-related somatic diseases, infertility and of having children with paternally transmitted aneuploidies, such as Klinefelter, Turner, triple-X and XYY syndromes and of having offspring with partial or full aneusomic karyotypes.

Non-disjunction mechanisms in human male meiosis

In spermatogenesis, meiosis is the cell division leading to haploid spermatozoa through two consecutive chromosome segregations after a single round of DNA replication (Fig. 1A). During the first meiotic division (meiosis I) segregation of homologous chromosomes to opposite poles occurs, whereas sister chromatids remain together. To guarantee a correct chromosome segregation in meiosis I, the proper pairing, synapsis and recombination of homologous chromosomes ensuring the presence of at least one chiasma per bivalent, the gradual loss of cohesion between sister chromatids and the monoorientation of the sister kinetochores are necessary (reviewed by Brar and Amon, 2008). During the second meiotic division (meiosis II), the sister chromatids segregate to opposite poles due to the loss of centromeric cohesion (Tóth et al., 2000), as in mitosis.

Anomalies during meiotic segregation in man can be investigated directly, by analysing the chromosome number and configuration in germ cells, or indirectly, using centromeric heterozygosity/homozygosity to infer the stage of meiotic non-disjunction in trisomic conceptuses (Lamb et al. 1996; reviewed by Hassold et al., 2007). Although direct meiotic studies are the best approach for studying meiotic non-disjunction, the number of published studies is still low because of the difficulty in obtaining a testicular biopsy, especially in fertile men.

The meiotic processes that were first associated with non-disjunction in infertile men were synapsis and recombination (Hultén et al., 1970; Pearson et al., 1970; Templado et al., 1976, 1981). The major recombination error leading to non-disjunction in meiosis I is the presence of two separated univalents at MI (Fig. 2). It corresponds to homologous chromosomes which did not recombine, and thus are not joined by chiasmata during MI. Indirect studies related lack of recombination to the genesis of trisomic conceptuses both in cases of Down syndrome (Savage et al., 1998; Oliver et al., 2009) and Klinefelter syndrome (Hassold et al., 1991; Thomas et al., 2000). Pachytene studies in infertile men also showed a positive correlation between achiasmate bivalents and increased levels of aneuploid spermatozoa (Ferguson et al., 2007; Sun et al., 2008).

However, only studies analysing simultaneously MI and MII spermatocytes by chromosome identification techniques are able to accurately detect the origin and mechanisms of segregation errors during meiosis I. Two studies are of relevance: one in an infertile patient with a high incidence of univalents at MI (Uroz et al., 2008) and another in a series of three fertile men (Uroz and Templado, 2012) using multiplex-FISH (M-FISH). In these studies, chromosome segregation errors, achiasmate non-disjunction and premature separation of sister chromatids (PSSC) were the two main mechanisms that caused aneuploidy during meiosis I, with a similar contribution (Uroz et al., 2008; Uroz and Templado, 2012).

Univalents at MI may segregate at random, leading to whole chromosome non-disjunction in half of the cases (achiasmate non-disjunction) (Fig. 1B). Alternatively, they may undergo PSSC (Fig. 1C). This last mechanism, first described in woman by Angell (1991), is characterized by the presence of an extra or missing chromatid at MII. Studies in human oocytes identified also achiasmate non-disjunction and PSSC as the two main non-disjunction mechanisms generating aneuploidy in meiosis I (reviewed by Pellestor et al., 2006; Fragouli et al., 2011). PSSC seems to have its origin in the premature biorientation of sister chromatid centromeres of univalents during meiosis I to avoid spindle assembly checkpoint (Kouznetsova et al., 2007).

An abnormal chiasma location may favour non-disjunction in meiosis I, called "true" non-disjunction (Fig. 1D). Accordingly, a recent study on aneuploidy of single spermatozoa by whole-genome sequencing described that, on average, sperm cells with aneuploid autosomes exhibit significantly fewer crossovers than normal cells (Lu et al., 2012). A single distal chiasma (characteristic of cells with a decreased number of chiasmata) can be resolved prematurely causing homologous chromosomes to behave as univalents at anaphase I (McDougall et al., 2005).

Non-disjunction in meiosis II implies the segregation of the two sister chromatids to the same pole. Classically, it was thought that the inability to lose cohesion between centromeres of sister chromatids or a bad orientation in the metaphase plate could lead to segregation of the two chromatids together, designated as 'true' non-disjunction (Fig. 1F). However, the presence of a proximal chiasma (typical in cells with an increased number of chiasmata) could lead to a premature loss of centromeric cohesion between chromatids (Rockmill et al., 2006), with their subsequent segregation at random, leading to aneuploid gametes in half of the cases (Fig. 1E). This has been observed in human MII oocytes (balanced PSSC, Angell, 1991). We have observed MII spermatocytes from fertile men containing chromosomes with separated sister chromatids as well (Uroz and Templado, 2012).

Sex differences in the contribution to aneuploidy

Most of *de novo* numerical abnormalities are maternal in origin (Hassold et al., 1996), with advanced maternal age being an important

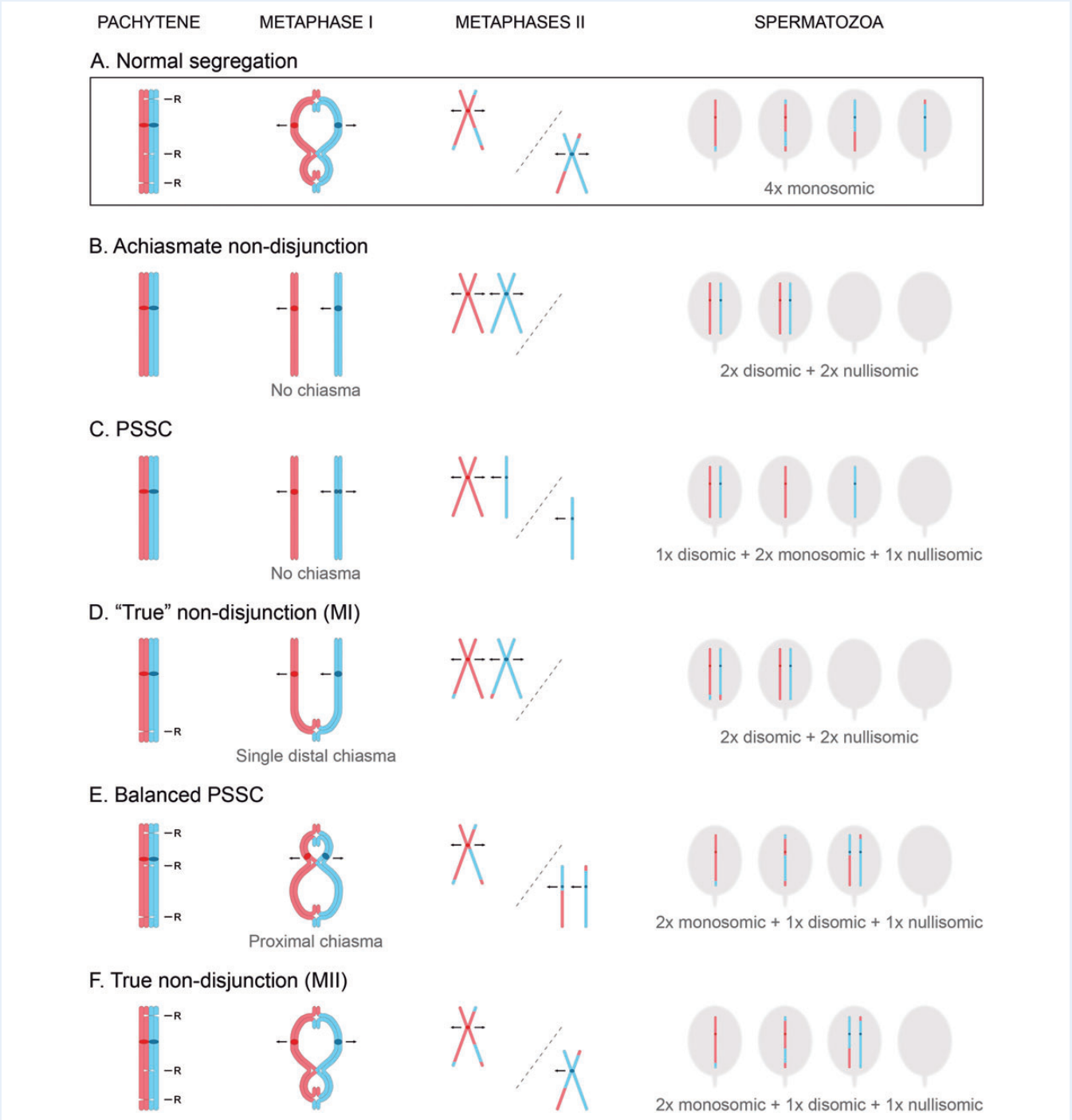

Figure 1 Non-disjunction mechanisms in human male meiosis. (**A**) Normal chromosome segregation during human male meiosis. (**B**) Achiasmate non-disjunction involves the random segregation of two homologous chromosomes at meiosis I due to the absence of chiasma. (**C**) Premature separation of sister chromatids (PSSC) arises by sister chromatid segregation from a univalent in meiosis I. (**D**) 'True' non-disjunction at meiosis I originates probably by joint segregation of a lineal bivalent showing a single distal chiasma. (**E**) Balanced PSSC is characterized by the random segregation of two separated sister chromatids at meiosis II as a result of a proximal chiasma. (**F**) 'True' non-disjunction at meiosis II originates by the joint segregation of the sister chromatids that maintain their cohesion.

contribution. The origin of the aneuploidy increase with maternal age is still uncertain and could involve errors in different stages of oogenesis (Hassold and Hunt, 2009). However, even young women are major contributors to embryo aneuploidy compared with men. We will focus on the similarities and differences in meiotic processes between men and women under 35 years old.

The incidence of conservative aneuploidy in oocytes is around 15–20% (reviewed by Pellestor *et al.*, 2006), which is much higher than

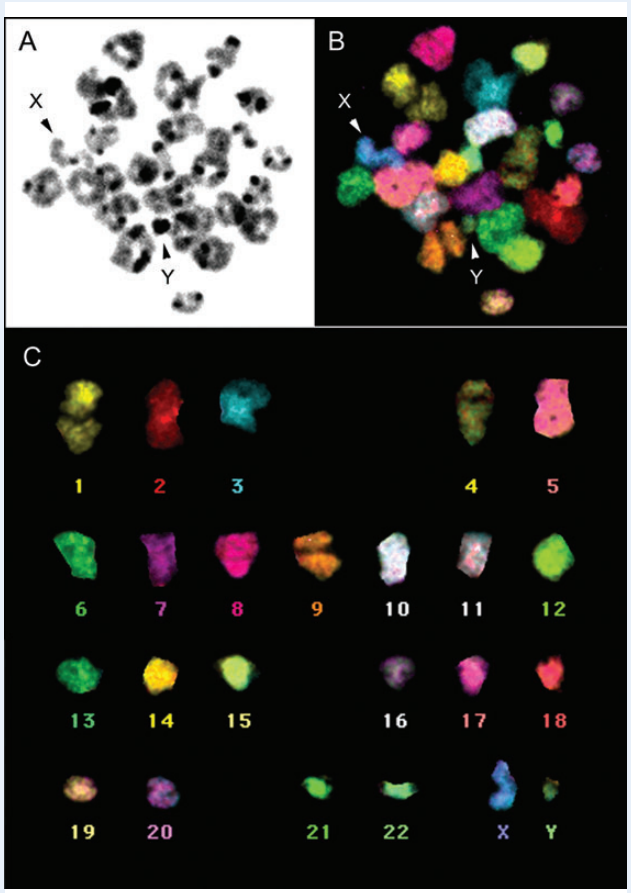

Figure 2 Human metaphase I spermatocyte showing separated sex chromosomes. (**A**) Metaphase I counterstained with DAPI. (**B**) Metaphase I and (**C**) its karyotype hybridized with the M-FISH technique.

Table III Comparison of male and female frequencies of conservative aneuploidy and common disomies in oocytes II from young women (<35 years old), spermatocytes II and spermatozoa.

	Oocytes II[a]	Spermatocytes II[b]	Spermatozoa[c]
Cells analysed	134	248	>10 000/ chromosome
Consevative aneuploidy	13–19%	13.7%	4.5%
Common disomies	22, 16	21, XY	21, XY

[a]Sandalinas *et al.* (2002); Garcia-Cruz *et al.* (2010).
[b]Uroz and Templado (2012).
[c]Reviewed in Templado *et al.* (2011).

that observed in sperm nuclei (4.5%) or sperm karyotypes (1.8%) (reviewed in Templado *et al.*, 2005, 2011). However, it should be noted that these frequencies compare germ cells at different gametogenic stages. Furthermore, most of the oocytes analysed were from women over 35 years of age in IVF programmes or ICSI failures. The genesis of numerical abnormalities during meiosis requires errors in chromosome segregation and the malfunction of meiotic checkpoints (both pachytene checkpoint and spindle assembly checkpoint) (reviewed by Roeder and Bailis, 2000; Malmanche *et al.*, 2006, Polanski, 2013). Conventionally, it was believed that both errors are more common in female meiosis than in male meiosis (reviewed by Hunt and Hassold, 2002). However, recent meiotic studies (Uroz and Templado, 2012) have revealed a frequency of conservative aneuploidy of metaphase II spermatocytes (14%) similar to that observed in non-inseminated oocytes of young women (13–19%) (Sandalinas *et al.*, 2002; Garcia-Cruz *et al.*, 2010) (Table III). These findings suggest that the differences between the paternal and maternal contribution to aneuploidy are not due to differences in chromosome segregation errors but rather to more effective checkpoint mechanisms in spermatogenesis than in oogenesis. Indeed, studies on infertile men described that synaptic and recombination errors caused not only abnormal chromosome segregation, but also meiotic arrest. Arrest may occur at different stages of spermatogenesis, and its effect on sperm production is variable. A partial arrest blocks the progression of some germ cells and results in oligozoospermia, whereas a complete arrest affects all germ cells and leads to azoospermia (Hultén *et al.*, 1970; Gonsalves *et al.*, 2004; reviewed by Egozcue *et al.*, 2005).

In our study of three fertile men, the frequencies of aneuploidy in MII spermatocytes were three times higher than those seen in spermatozoa (Uroz and Templado, 2012) (Table III), indicating the possible existence of a checkpoint monitoring aneuploidy during male spermiogenesis. To our knowledge, no such a checkpoint mechanism in relation to numerical chromosome abnormalities has been described. However, evidence of spermiogenic arrest has been widely reported in infertile males, suggesting the existence of a feasible checkpoint either in spermatid stage (Guichaoua *et al.*, 2005) or in spermatozoa (Rodrigo *et al.*, 2004; Óvári *et al.*, 2010). Meanwhile, there is no evidence of the existence of a post-meiotic checkpoint in women, which would explain the greater maternal contribution to trisomic embryos.

In conclusion, moderate increases of aneuploidy in spermatozoa, which may or may not affect fertility, are seen in men who are stable variants, patients with offspring with the most common aneuploidy conditions, patients with repeated spontaneous abortions, patients with some types of infertility and patients who may have been exposed to aneugenic agents. Patients with higher aneuploidy values and who almost universally suffer from infertility may have karyotypes with sex chromosome aneuploidy, karyotypes with structural chromosome rearrangements or may have rare alterations of the seminogram. The main meiotic abnormality leading to aneuploid spermatozoa is the presence of univalents at metaphase I, affecting mainly chromosomes 21 and XY. As a consequence, the two main non-disjunction mechanisms in male meiosis are achiasmate non-disjunction and PSSC, as previously described in female meiosis. The incidence of aneuploidy in spermatozoa cannot be directly compared with that of the oocytes because of the different gametogenic stages. Limited reports on the incidence of aneuploidy in spermatocytes II and oocytes reveal striking similarities, thus, we suggest that further analyses of metaphase II oocytes from young women and metaphase II spermatocytes using techniques of chromosome identification are needed. The disomy frequencies in spermatocytes II are significantly higher than in human spermatozoa, suggesting the existence of a post-meiotic checkpoint which monitors and arrests spermatocytes II with numerical abnormalities.

Authors' roles

All authors contributed to conception and design of the review, drafting the manuscript and revising it critically for important intellectual content, and final approval of the version to be published.

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Conflict of interest

None declared.

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