4. RESULTADOS

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4.1.1 Artículo 1

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Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion

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Human male infertility is often related to chromosome abnormalities. In chromosomally normal infertile males, the rates of chromosome 21 and sex chromosome disomy in spermatozoa are increased. Higher incidences of trisomy 21 (seldom of paternal origin) and sex chromosome aneuploidy are also found. XXY and XYY patients produce increased numbers of XY, XX and YY spermatozoa, indicating an increased risk of production of XXY, XYY and XXX individuals. Since XXYs can reproduce using intracytoplasmic sperm injection (ICSI), this could explain the slight increase of sex chromosome anomalies in ICSI series. Carriers of structural reorganizations produce unbalanced spermatozoa, and risk having children with duplications and/or deficiencies. In some cases, this risk is considerably lower or higher than average. These patients also show increased diploidy, and a higher risk of producing diandric triploids. Meiotic disorders are frequent in infertile males, and increase with severe oligoasthenozoospemia (OA) and/or high follicle stimulating hormone (FSH) concentrations. These patients produce spermatozoa with autosomal and sex chromosome disomies, and diploid spermatozoa. Their contribution to recurrent abortion depends on the production of trisomies, monosomies and of triploids. The most frequent sperm chromosome anomaly in infertile males is diploidy, originated by either meiotic mutations or by a compromised testicular environment.

Key words: chromosome anomalies/diploid spermatozoa/male infertility/meiotic disorders/recurrent abortion

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Introduction

Human male infertility and chromosome abnormalities are often closely related. For a long time, it has been known that chromosome anomalies are much more frequent in infertile males than in the general population, because fertility clinics gather patients with chromosome abnormalities. For instance, the incidence of Klinefelter syndrome is between 30 (heterogeneous infertile population; Zuffardi and Tiepolo, 1982) and 100 times (azoospermic males; De Braekeleer and Dao, 1991) higher in sterile males attending fertility clinics, while structural chromosome reorganizations are about 10 times more frequent in infertile males and especially in intracytoplasmic sperm injection (ICSI) patients than in newborns (Zuffardi and Tiepolo, 1982; Meschede *et al.*, 1998).

Furthermore, meiotic disorders, i.e. chromosome abnormalities limited to spermatogenic cells and not detectable through the study of the somatic karyotype, are found in ~6% of patients in whom meiotic studies are performed (Egozcue *et al.*, 1983; De Braekeleer and Dao, 1991), and this figure may

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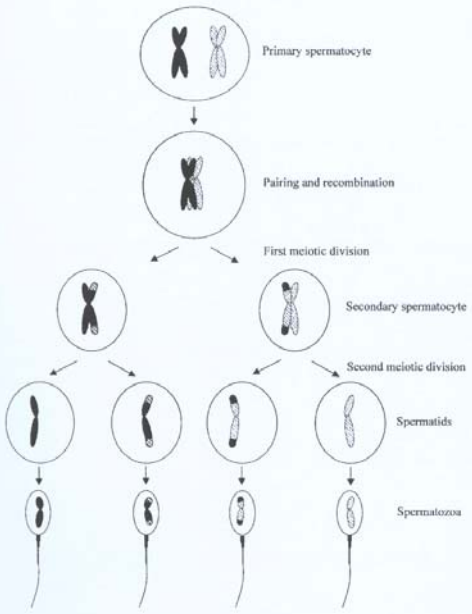

Figure 1. Diagram of meiosis. For simplification, only one chromosome pair is represented. In the first meiotic division, homologous chromosomes pair and exchange genetic information. At anaphase, whole chromosomes, with two chromatids each, move to the poles, to produce haploid secondary spermatocytes. In the second meiotic division, chromatids are segregated to produce haploid spermatids (see text).

grow to 17.5% in patients with severe oligoasthenozoospermia (OA) (Vendrell *et al.*, 1999). Meiotic disturbances are later reflected in the abnormal chromosome constitution of spermatozoa; significant increases in the rates of disomy and of diploidy have been detected by fluorescent in-situ hybridization (FISH) in patients with different degrees of OA (Downie *et al.*, 1997; Egozcue *et al.*, 1997); and patients with a severe OA are often recruited for ICSI (Aran *et al.*, 1999b; Pang *et al.*, 1999).

A correlation has also been suggested between increased frequencies of disomic and diploid spermatozoa and recurrent abortion (Giorlandino *et al.*, 1998; Rubio *et al.*, 1999). Most numerical chromosome anomalies originate at meiosis (Jacobs and Hassold, 1995); de-novo structural rearrangements are also of meiotic origin (Olson and Magenis, 1988), while inherited duplications and/or deficiencies (also known as partial trisomies and monosomies) result from the abnormal segregation of structural rearrangements at meiosis (Chandley, 1981). Meiotic pairing disorders result from mutations affecting the meiotic process (Barlow and Hultén, 1996; Edelmann *et al.*, 1996; Hassold, 1996; Hultén and Barlow, 1997; Grootegoed *et al.*, 1999) or from the influence of an abnormal testicular environment (Speed and Chandley, 1990; Finkelstein *et al.*, 1998; Rives *et al.*, 1998; Mroz *et al.*, 1999).

Meiosis is a complex process (Figure 1) that includes two successive cell divisions, without DNA replication between them. In the first meiotic division, which takes place in the diploid ($2n = 46$) primary spermatocytes, homologous chromosomes pair, and an exchange of genetic information may take place between them (recombination) to produce new genetic combinations in the offspring; at anaphase, whole chromosomes (with two chromatids each) migrate to the cell poles to produce haploid ($n = 23$) secondary spermatocytes, in which the chromosomes still have two chromatids each.

During the second meiotic division, the chromatids of each chromosome migrate to the cell poles, to produce haploid ($n = 23$) spermatids, but in these cells each chromosome has only one chromatid. The spermatids will change their shape and reorganize the location of their organelles to produce spermatozoa, but replication of the genetic material will only take place after fertilization.

Any of these steps may go wrong, giving rise to different types of chromosome abnormalities such as aneuploidy or diploidy. However, in spite of the potential problems outlined above, the use of assisted reproductive techniques (ART) has allowed infertile males to reproduce without an increased risk for inadvertent reproductive outcomes, probably because the ART employed facilitate the natural process of embryo selection. In fact, and with the exception of a slight increase of sex chromosome anomalies and of de-novo structural rearrangements after ICSI, children born with the help of ART show the same rates of chromosome errors than those found in the general population.

In this paper, we review the different types of chromosome abnormality related to human male infertility, their frequency and origin, their meiotic behaviour, their possible role in the production of abnormal spermatozoa and, eventually, their possible contribution to recurrent abortion.

Numerical anomalies

Numerical autosomal anomalies

In general, most de-novo autosomal monosomies and trisomies are not viable, and their products are eliminated during pregnancy or in the perinatal period. For this reason, most of them are found in spontaneous abortions (Jacobs, 1992; Jacobs and Hassold, 1995). The only full numerical autosomal anomalies surviving to birth are trisomy 13 (probability of survival to birth of 2.8%), trisomy 18 (probability of 5.4%) and trisomy 21 (probability of 22.1%), but only trisomy 21 may

allow survival into puberty and adulthood (Jacobs and Hassold, 1995). Most numerical autosomal anomalies originate during maternal meiosis I (trisomies 8, 13, 15, 16, 18 and 21) although some cases (up to 10%) are of paternal origin (Nicolaidis and Petersen, 1998). Defective recombination and increased recombination have been implicated in the origin of numerical anomalies (Jacobs and Hassold, 1995; Lamb *et al.*, 1996). Males with trisomy 21 are azoospermic or show a severe oligozoospermia (Speed, 1989). Meiotic studies have only been performed in one case (Johannisson *et al.*, 1983). In most metaphase I figures (88.5%), the extra chromosome was present as a univalent.

Sperm chromosome studies using multicolour FISH have shown that in controls, the frequency of disomy 21 (~0.29–0.35%) is significantly higher than the incidence of disomy for other autosomes (~0.10%) (Blanco *et al.*, 1996, 1998a; Spriggs *et al.*, 1996; Downie *et al.*, 1997; Egozcue *et al.*, 1997), suggesting that chromosome 21 has a higher tendency to non-disjunction. The fathers of children with trisomy 21 had disomy figures within control limits, but the fathers of two children with Down's syndrome of paternal origin studied by Blanco *et al.* (1998a) had significantly increased frequencies of chromosome 21 disomy (0.75 and 0.78%), although this increase could not be confirmed in other cases (Hixon *et al.*, 1998).

Since, due to physical and psychosocial limitations, males with trisomy 21 do not reproduce, they do not contribute to recurrent miscarriage, although from a theoretical point of view their risk of producing trisomic offspring is only 50%, or even lower if univalent loss at meiosis I is taken into account (Sybenga, 1972).

Numerical sex chromosome anomalies

In the male, de-novo numerical sex chromosome anomalies include Klinefelter syndrome, with a 47,XXY karyotype and the 47,XYY aneuploidy. For 47,XXY patients, the possibility of survival to birth is low (55.3%), while for XYY males it is close to 100% (Jacobs and Hassold, 1995). XXYs are of paternal and maternal origin in similar proportions, and in most cases the error takes place at meiosis I. XYY males are obviously of paternal origin, and the error is produced at meiosis II or in the post-zygotic stage (Jacobs and Hassold, 1995).

47,XXY Klinefelter's syndrome

Non-mosaic XXY males are azoospermic or nearly azoospermic (Palermo *et al.*, 1998). However, the criteria to determine that a patient is non-mosaic are rather loose, and usually based on the study of a few dozen lymphocyte metaphases, as seen in Palermo *et al.* (1998). Mosaic XXY/XY patients produce spermatozoa in variable numbers. To our knowledge, no meiotic or sperm chromosome studies have been carried out in the fathers of children or adults with an XXY karyotype, probably because these cases are detected when the father has died, is too old or is not interested enough to provide the material needed for analysis but, in controls, the incidence of sex chromosome disomy is higher than that detected for any autosomal pair (0.43% versus a maximum of 0.35% for chromosome 21; Spriggs *et al.*, 1996).

Kjessler (1966) and Laurent *et al.* (1972) performed meiotic studies in 47,XXY/46,XY mosaics, and concluded that only normal germ cells can enter meiosis, but Skakkebaek *et al.* (1969) and Vidal *et al.* (1984) analysed meiotic preparations and synaptonemal complex (SC) spreads in XXY and mosaic XXY/XY patients, and suggested that at least some XXY cells could reach the primary spermatocyte stage.

Sperm chromosome studies using the human–hamster system (Rudak *et al.*, 1978; Martin, 1983) in an XXY/XY mosaic (Cozzi *et al.*, 1994) showed a significant increase of disomic XY spermatozoa (0.92 versus 0.11% in the controls from Blanco *et al.*, 1997). Sperm chromosome studies by FISH in the same individual (Chevret *et al.*, 1994) also showed an increased incidence of XY spermatozoa (2.09 versus 0.36% in controls), but not of XX, YY or diploid spermatozoa. FISH studies in decondensed sperm heads have also been performed in apparently non-mosaic XXY males by Guttenbach *et al.* (1997a), Estop *et al.* (1998a) and Foresta *et al.* (1998). Apart from the work by Estop *et al.* (1998a), where only 24 spermatozoa were analysed, results from the other two studies show increased frequencies of XY spermatozoa (8.09%; range 2.09–14.58% versus 0.11% in controls), and of XX spermatozoa (3.46%; range 0.11–6.92% versus 0.10% in controls), but not of YY spermatozoa (0.10%; range 0.003–0.21% versus 0.16% in controls) or of diploid spermatozoa (0.14%; range 0.03–0.33% versus 0.24% in controls).

Recently Mroz *et al.* (1999), based on a FISH study of spermatids obtained from XXY mice, concluded that XXY cells cannot enter meiosis, because in XXY males, a similar proportion of XY and XX spermatids should be found (as expected from the segregation of XXY cells at meiosis I), while in fact the proportion of XY spermatids was about 10 times higher (0.91% versus 0.08%). In human XXYs the proportion of XY spermatozoa is also three times higher than that of XX spermatozoa (8.09 versus 3.46%, Guttenbach *et al.*, 1997a; Foresta *et al.*, 1998). These authors suggested that the abnormal sex chromosome constitution found in spermatids from XXY mice (0.91% XY spermatids versus 0.08% in controls) should be attributed to segregation errors in XY cells placed in a compromised testicular environment, possibly related to the increased concentrations of follicle stimulating hormone (FSH) found in these animals. This hypothesis seems quite reasonable (see also Speed and Chandley, 1990), although it does not explain why other chromosome pairs are not affected. An increase in diploid spermatozoa has not been found in XXY males either (mean of 0.14 versus 0.24% in controls) when it is known that an increase in diploid spermatozoa is often found in cases where meiosis I is

compromised (Speed and Chandley, 1990; Finkelstein *et al.*, 1998; Rives *et al.*, 1998), but the number of XXY males studied is still low, and a four-fold increase in diploid spermatids was found by Mroz *et al.* (1999) in XXY mice.

The preferential pairing of homologous chromosomes at meiosis I (in this case XX versus Y, leading to an XY versus X segregation; Hultén, 1970; Chevret *et al.*, 1994) could also explain the excess of XY spermatozoa, the predominance of XY versus XX spermatozoa, the considerable excess of X-bearing spermatozoa found by Foresta *et al.* (1998) (~54% X-bearing versus 27% Y-bearing euploid spermatozoa) in their two patients, and the slight excess of X-bearing spermatids found by Mroz *et al.* (1999) in XXY mice (51.2 versus 47.1%). However, the possibility of XXY cells producing gametes seems most unlikely, even if there is some evidence that they may enter meiosis (Skakkebaek *et al.*, 1969; Vidal *et al.*, 1984). The reason is that the preferential formation of an XX bivalent mimicks the situation found in oocytes. In cells containing an XX bivalent, no sex vesicle is formed (Vidal *et al.*, 1984), X-chromosome inactivation cannot occur, and meiosis is necessarily arrested (Lifschytz and Lindsley, 1972; Forejt, 1984). Thus, the variable number of spermatozoa found in XXY patients (even if abnormal), probably originate from an XY germ line (Moosani *et al.*, 1999).

The contribution of XXY males to recurrent abortion would be directly related to the increase in abnormal (24,XY) spermatozoa producing 47,XXY progeny, independent of their mechanism of origin, because as indicated above the probability of an XXY male to survive to birth is only 55.3% (Jacobs and Hassold, 1995). Since these patients are recruited for ICSI, they would also contribute to the excess of numerical sex chromosome anomalies found in some ICSI series (In't Veld *et al.*, 1995; Liebaers *et al.*, 1995; Bonduelle *et al.*, 1996), as analysed by Martin (1996). In fact, Moosani *et al.* (1999) have already reported a 47,XXY pregnancy obtained by ICSI from a man with a normal karyotype but with an excess of XY spermatozoa, and Van Opstal *et al.* (1997) have demonstrated that six cases of sex chromosome aneuploidy following ICSI were of paternal origin, and suggested that the fathers of these children might be somatic or germinal XY/XXY mosaics. However, experience with natural and ICSI pregnancies induced by XXY males shows that virtually all their offspring are chromosomally normal.

47,XYY aneuploidy

In 47,XYY males, fertility is quite variable (Speed, 1989), and sperm counts range from normozoospermia to almost azoospermia (as in the general population). To our knowledge, no meiotic or sperm chromosome studies have been performed in the fathers of XYY individuals, probably because many of them go undetected, or when they are diagnosed the father has died, is too old or is not interested enough to provide the material needed for analysis, but in controls, the incidence of sex chromosome disomy is higher than that found for any autosomal pair (0.43% versus a maximum of 0.35% for chromosome 21) (Spriggs *et al.*, 1996).

Early meiotic studies in XYY patients (Thompson *et al.*, 1967; Melnyk *et al.*, 1969; Evans *et al.*, 1970) suggested that the extra Y chromosome might be lost before meiosis started, because these authors could not find any metaphase I figures with two Y chromosomes. However, Burgoyne (1979) suggested that the loss of the Y chromosome should take place during meiosis, because XYY spermatogonia are usually found in XYY males, and, in fact, Berthelsen *et al.* (1981) found a high percentage (45%) of pachytenes with a YY bivalent and an X univalent (as would be expected from the preferential pairing of homologous chromosomes at meiosis I; Hultén, 1970).

Furthermore, Blanco *et al.* (1997) observed 86.7% XYY spermatogonia, 60.6% XYY pachytenes and 78.1% 24,XY haploid meiocytes in an XYY male, Tettenborn *et al.* (1970), Hultén and Pearson (1971) and Luciani *et al.* (1973) observed metaphase I figures containing two Y chromosomes, and Hultén and Pearson (1971) even described the presence of 3% of spermatozoa with two Y-bodies, although quinacrine staining of the heterochromatic Yq region is quite unreliable in interphase nuclei.

The preferential formation of an X univalent and a YY bivalent (Berthelsen *et al.*, 1981; Blanco *et al.*, 1997), or the formation of an XY bivalent plus a Y univalent allow the formation of a sex vesicle (Blanco *et al.*, 1997) and the normal inactivation of the X chromosome. As a result, meiosis should proceed normally. The exception would be those cells in which pairing of extra segments (in this case the extra Y) with the X chromosome results in the formation of a trivalent, X-chromosome inactivation becomes impossible, and meiosis is arrested. This situation is similar to that found in some carriers of structural reorganizations (Lifschytz and Lindsley, 1972; Forejt, 1984).

Sperm chromosome studies using the hamster system have only been carried out in one XYY male by Benet and Martin (1988) who did not find any YY spermatozoa among the 75 sperm metaphases analysed. Sperm chromosome studies by FISH carried out in the same patient 10 years later (Martin and Rademaker, 1999) did not show an increase in YY spermatozoa, but revealed a significant increase in XY spermatozoa (0.55 versus 0.3% in controls).

Sperm chromosome studies by FISH in XYY males were first performed by Han *et al.* (1994). A review of the results published so far shows a significant increase in XY (0.35%; range 0.24–0.52% versus 0.11% in controls) and YY (0.43%; range 0.08–1.01% versus 0.16% in controls) spermatozoa, as expected, but obviously not in XX spermatozoa (0.06%; range 0.00–0.15% versus 0.10% in controls). The proportion of diploid spermatozoa (0.22%; range 0.13–0.30% versus 0.24% in controls) was not increased either. Han *et al.* (1994) found an increase of diploid spermatozoa in the bulk sample from one

XYY male, but not in the motile spermatozoa recovered by swim-up; however, Martínez-Pasarell *et al.* (1997) have shown that separation procedures do not select chromosomally normal spermatozoa.

It is interesting to note that in patients with numerical sex chromosome anomalies, the mean frequency of diploid spermatozoa is usually not increased (although it may be increased individually), in contrast to the situation in carriers of structural rearrangements, meiotic (synaptic) disorders or even in older men. This could be related to the fact that the precocious separation of the X and Y chromosomes, kept together by a single, almost terminal chiasma, may not have drastic consequences on the anaphase checkpoint at meiosis I (Hassold *et al.*, 1991) in contrast with what happens with more severe anomalies. Furthermore, the development of meiosis in a compromised environment could also contribute to the abnormal segregation of the sex chromosomes (Mroz *et al.*, 1999).

The contribution of XYY males to recurrent abortion would be directly related to the increase in XY spermatozoa, producing XXY progeny with a probability of survival to birth of 55.3%, but not to the increase in YY spermatozoa, because XYY individuals have a probability of survival to birth of 100% (Jacobs and Hassold, 1995). So far, natural and ICSI pregnancies induced by XYY males show that virtually all their offspring are chromosomally normal.

Balanced structural reorganizations

Structural reorganizations include Robertsonian translocations, also known as centric fusions, and reciprocal translocations, which are found in ~0.1% of newborns each, pericentric and paracentric inversions, found in 0.02% of newborns (with the exception of inversions affecting the heterochromatic regions of chromosomes 1, 9 and 16, which are considered as polymorphisms; see Colls *et al.*, 1997), and insertions, which are only seen occasionally (Van Assche *et al.*, 1996).

Structural reorganizations may be de-novo (20%) or familial (80%) (Jacobs, 1992), although it is obvious that all familial reorganizations started as de-novo reorganizations. With the exception of Robertsonian translocations, which are of maternal origin in 65% of cases, de-novo structural reorganizations are mainly of paternal origin (84.4% of cases) (Olson and Magenis, 1988). Since most de-novo reorganizations are balanced, their probability of surviving to birth is similar to that of chromosomally normal pregnancies. Familial reorganizations can give rise to more or less extensive duplications and/or deficiencies, depending on the behaviour of the affected chromosomes during meiosis (Sybenga, 1972). The meiotic behaviour of structural reorganizations depends on the morphology and length of the chromosome fragments involved and on the presence or absence of aggregated heterochromatin (Jalbert and Sèle, 1979), on the frequency of exchanges in the pairing and in the interstitial regions (Laurie and Hultén, 1985), and on the localization of breakpoints in G+ or G– bands (Ashley, 1988).

Short exchange fragments, acrocentric chromosomes with short arms and the presence of heterochromatin aggregates may preclude the formation of chiasmata; this gives rise to open configurations, with a higher tendency to the abnormal segregation of the chromosomes involved. Risk counselling for carriers of balanced structural rearrangements can be found in detail in Gardner and Sutherland (1989). Spontaneous de-novo structural reorganizations probably originate from DNA breaks in the spermatozoa, where DNA repair mechanisms are inactive, which are mis-repaired in the oocyte (for review see Genescà *et al.*, 1992). Spontaneous structural reorganizations may also originate in spermatogonia; in some cases, they have been detected by chance (for review see Templado *et al.*, 1984). Familial reorganizations are inherited.

To our knowledge, sperm chromosome studies in the fathers of children with de-novo structural rearrangements have only been performed in two cases. In the first case (Colls *et al.*, 1998a), the father of a phenotypically normal boy with a de-novo reciprocal t(7;9) (q22;p23) detected after amniocentesis for maternal age, the analysis by G-banding and whole chromosome painting of 309 sperm chromosome complements obtained using the hamster system did not show any relationship between the breakpoints involved in the translocation and those present in the sperm complements of the father.

In the second case (Colls *et al.*, 1998b), the father of a phenotypically normal girl with a de-novo reciprocal translocation t(11;15) (q12;q22) detected after amniocentesis for maternal age, the analysis by G-banding of 112 sperm complements, and by two-colour FISH of 313 sperm complements obtained using the hamster system, revealed a significant concentration of breaks in chromosome 11 (3.2 versus 0.4% in controls), but not in chromosome 15, suggesting that in this case the involvement of chromosome 11 in the reorganization may not have been at random.

The fertility of structural reorganization carriers is often decreased. Reorganizations are often ascertained due to sterility or recurrent abortion. As indicated above, structural reorganizations are about 10 times more frequent among infertile males than in the general population (Zuffardi and Tiepolo, 1982; Van Assche *et al.*, 1996), and are so frequent in ICSI patients (Meschede *et al.*, 1998) that it is current practice to include a karyotype in the screening of such individuals. Sperm counts range from normozoospermia to complete azoospermia, depending on the degree of meiotic arrest induced by the reorganization.

Meiotic studies in carriers of structural reorganizations have been numerous (Chandley, 1979, 1981, 1984), and the theoretical risk of transmission of normal, balanced or unbalanced chromosomes has been established (Sybenga, 1972). Synaptonemal complex studies of the behaviour of structural reorganizations during prophase I confirmed the

existing data (Vidal *et al.*, 1987; Speed and Chandley, 1990), and revealed the presence, in many cases, of extensive synaptic anomalies (Navarro *et al.*, 1986) that could be related to the more or less severe meiotic arrest seen in these patients, reflected in variable degrees of oligozoospermia. However, the need of meiotic studies to better establish the real risk of transmission of a chromosome abnormality and to provide a more accurate reproductive counselling has already been suggested (Egozcue *et al.*, 1981). Sperm chromosome studies have demonstrated that the proportion of normal and balanced versus unbalanced spermatozoa is quite variable, and often different from the theoretical results of meiotic segregation (Sybenga, 1972).

Sperm chromosome studies in carriers of structural reorganizations using the hamster system have also been numerous (Templado *et al.*, 1988a, Estop *et al.*, 1995; Blanco *et al.*, 1998b). The results have shown that the frequency of unbalanced spermatozoa was ~15% in carriers of Robertsonian translocations (theoretical risk, 66%) (Pellestor *et al.*, 1987). In carriers of reciprocal translocations, the percentages of unbalanced spermatozoa were in the range 20–77% (theoretical risk, 50%), e.g. Templado *et al.*, 1988b (20%); Pellestor *et al.*, 1989 (34–77%); Martin *et al.*, 1990 (43–52%); Estop *et al.*, 1995 (40–61%). Two exceptional cases with double translocations, involving four chromosome pairs (Burns *et al.*, 1986) and three chromosome pairs (Cifuentes *et al.*, 1998) had 87 and 86.5% unbalanced spermatozoa respectively.

Finally, carriers of paracentric or pericentric inversions had between 0% unbalanced spermatozoa, when the inverted segment was short (Balkan *et al.*, 1983; Jenderny *et al.*, 1992) and 25–30% when the inverted segment was long (Martin, 1991; Navarro *et al.*, 1993).

Sperm chromosome studies by FISH have only been performed in a limited number of cases. Martini *et al.* (1997) analysed the segregation of a reciprocal t(3;11) by triple FISH and found 55.7% unbalanced spermatozoa (which is close to the theoretical 50%), and a percentage of diploid spermatozoa similar to that seen in controls (0.11 versus 0.06%). Van Hummelen *et al.* (1998) studied a reciprocal t(1;10) by three- and four-probe multicolour FISH and found 51.9% of unbalanced spermatozoa and a significant increase of diploid spermatozoa (0.34 versus 0.16% in controls). Estop *et al.* (1998b) studied the segregation of two reciprocal translocations, t(2;18) and t(8;9) by three colour FISH and detected percentages of unbalanced spermatozoa also close to 50% (53.2 and 53.5% respectively). The proportions of diploid spermatozoa were high (0.5 and 0.6%), but the authors did not indicate whether this was or not within normal limits for their laboratory. Finally, Blanco *et al.* (1998b) analysed a reciprocal t(5;8) and found 51.8% unbalanced spermatozoa and a highly significant increase of diploid spermatozoa (1.18 and 1.23% in two separate studies versus 0.27% in controls).

The discrepancies observed between sperm chromosome studies (with frequencies of unbalanced spermatozoa often quite divergent from the expected 50%) and the low number of sperm FISH studies performed so far (with percentages of unbalanced spermatozoa close to 50%) should not be taken to indicate that when higher numbers of cells are studied in a sperm population, the percentages of abnormal cells are driven towards the theoretical mean frequency expected, because carriers of balanced traslocations often have a severe OA, and the numbers of spermatozoa studied are also low. Rather, what these data indicate is that many translocations are average with respect to the factors indicated earlier, and produce average numbers of abnormal spermatozoa (close to 50%), in contrast with unusual translocations which produce much higher or much lower numbers. This fact is important with regards to genetic counselling.

The most common Robertsonian translocations are rob(13;14) and rob(14;21). The abnormal segregation of these reorganizations can result in an almost complete trisomy 14 (which is not viable), an almost complete trisomy 13 (probability of surviving to birth of 2.8%) and an almost complete trisomy 21 (probability of surviving to birth of 22.1%). The abnormal products of other balanced reorganizations are quite variable, and their phenotypic effects range from nil to peri-implantational death. Their overall frequency in spontaneous abortions is <2.0%, and the overall probability of surviving to birth is 62.0% (Jacobs and Hassold, 1995).

Thus, the contribution of structural reorganizations to recurrent abortion will depend on the frequency of abnormal spermatozoa and on the severity of the duplications and/or deficiencies (partial trisomies and monosomies) induced by the abnormal segregation of each reorganization, ranging from almost complete trisomy or monosomy to extremely small duplications and/or deficiencies, which are difficult to detect and often have severe phenotypic consequences, but in some cases may be devoid of any phenotypic effect.

Meiotic disorders

Infertile males may show a particular defect in meiotic pairing (synapsis) and recombination first described by Hultén *et al.* (1970) and by Pearson *et al.* (1970), and later confirmed by other authors (Dutrillaux and Guéguen, 1971; Skakkebaek *et al.*, 1973; Templado *et al.*, 1976; Chaganti *et al.*, 1980).These anomalies can be found in 6–8% of patients studied (Egozcue *et al.*, 1983; De Braekeleer and Dao, 1991). For some time, meiotic studies were rather scarce, because in some countries clinicians were reluctant to obtain testicular biopsies. However, with the advent of ICSI, biopsy procedures have become widespread, and more recently this high incidence of meiotic disorders among infertile males has been confirmed by many authors, although the series have been rather short, and the incidence of the anomalies not usually

indicated (Aslanis *et al.*, 1997; Guichaoua *et al.*, 1997; Hamamah *et al.*, 1997; Kristesashvili *et al.*, 1997; Lange *et al.*, 1997).

Synaptic disorders may be quite extensive, affect all meiotic bivalents, and be related to mutations of one or more genes involved in DNA repair mechanisms (Edelmann *et al.*, 1996; Hassold, 1996; Grootegoed *et al.*, 1999); they can induce a complete (18%; Egozcue *et al.*, 1983) or a partial meiotic arrest, resulting in azoospermia or severe oligozoospermia (Hultén *et al.*, 1970; Navarro *et al.*, 1990). As a result of their low frequency in humans (10% of 6–8% of patients in whom meiosis is studied; Egozcue *et al.*, 1983), meiotic mutations will only be easily detected among patients in whom synaptic disorders have been previously diagnosed through meiotic studies in testicular biopsies.

Milder forms of the anomaly (Templado *et al.*, 1981) are much more frequent (almost 90% of cases; Egozcue *et al.*, 1983), affect only a few bivalents, and could be related to a defective progression of spermatogenesis in a compromised testicular environment (Speed and Chandley, 1990; Finkelstein *et al.*, 1998; Rives *et al.*, 1998; Mroz *et al.*, 1999). In 1983, Egozcue *et al.* studied a series of 1100 infertile patients and attempted to correlate the characteristics of the semenograms with the presence or absence of meiotic anomalies, but they were unable to demonstrate any significant correlation, probably because the groups of patients were not well defined.

However, Vendrell *et al.* (1999) studied a series of 103 infertile males with a severe OA (motile sperm concentrations of $\leq 1.5 \times 10^6$/ml) and found a significant increase of synaptic anomalies (17.5 versus 5.6% in infertile males from other aetiologies studied in the same successive series of 209 patients, and versus 6–8% in the heterologous infertile populations reviewed by Egozcue *et al.*, 1983 and by De Braekeleer and Dao, 1991). The correlation increased with decreasing numbers of spermatozoa (meiotic disorders were found in 40% of patients with motile sperm concentrations of $\leq 0.5 \times 10^6$/ml).

A correlation was also found in patients with a severe OA who had increased concentrations of FSH (>10 IU/ml); in this group, the incidence of meiotic anomalies was 22.6%. In cases with azoospermia and increased concentrations of FSH the frequency of meiotic anomalies was within the limits found in the general infertile population (6–8%), probably because in azoospermic patients, and other than to an XXY karyotype, azoospermia is very often related to Y-chromosome deletions, which are found in ~17% of azoospermic males, but only in ~1.5% of cases with a severe oligoasthenozoospermia (Oliva *et al.*, 1998; Simoni *et al.*, 1998).

Sperm chromosome studies by FISH have also been performed in a number of cases (Miharu *et al.*, 1994; Guttenbach *et al.*, 1997b; Lahdetie *et al.*, 1997; McInnes *et al.*, 1998; Moosani *et al.*, 1999), especially after it became known that ICSI (which is mainly used in cases with a severe male factor) could be related to an increase of chromosome anomalies, and more specifically of sex chromosome aberrations (In't Veld *et al.*, 1995; Liebaers *et al.*, 1995). In general (Pang *et al.*, 1999) increases in autosomal and sex chromosome disomies (up to 22%, In't Veld *et al.*, 1997) and in the frequency of diploid spermatozoa (up to 40%; In't Veld *et al.*, 1997) have been detected in all series. Unfortunately, most such studies have been conducted in 'infertile males', 'males with idiopathic infertility', males with 'low-quality semen', etc., and the series have included low numbers of patients or just individual cases, and even when the study groups have been well delineated (OA or asthenozoospermia), the criteria for inclusion in each group have been quite variable.

So far, only three sperm chromosome studies by FISH in patients with well defined spermogram anomalies have been conducted (Bernardini *et al.*, 1997; Aran *et al.*, 1999b; Pang *et al.*, 1999). Bernardini *et al.* (1997) studied nine patients with OA. The mean total number of motile spermatozoa was 4.5×10^6 (range 0.01–20). Significant increases in XY and XX disomy (1.36 versus 0.60% in controls) and in DNA ploidy (1.35 versus 0.86% in controls) were found. Pang *et al.* (1999) studied nine OA patients with sperm counts of $2–15 \times 10^6$ spermatozoa/ml. Highly significant increases in sex chromosome disomy (1.6–4.9 verus 0.15% in controls), autosomal disomy (0–5.4 versus 0.05–0.2%) and diploidy (0.4–9.6 versus 0.04%) were observed. Finally, Aran *et al.* (1999b) studied 14 OA patients ($<20 \times 10^6$ spermatozoa/ml and <35% motility) and five AS patients (<35% motile spermatozoa) and found a significant increase in sex chromosome disomy (0.64 versus 0.37%) and in diploidy (1.07 versus 0.25%) in OA patients, and of diploidy (0.56 versus 0.25%) in patients with asthenozoospermia. It is interesting to note that the study by Aran *et al.* (1999b) is the only one to include, as a separate group, five OA patients (motile spermatozoa $\leq 1.5 \times 10^6$ spermatozoa/ml) with meiotic disorders detected through meiotic studies in testicular biopsies. These patients showed a significant increase in diploid spermatozoa (0.53 versus 0.25%), but not in sex chromosome or autosomal disomies.

Guttenbach *et al.* (1997a) suggested that the differences in sex chromosome and autosomal disomies and in diploidy between infertile males and controls resulted from the influence in each series of a limited number of patients with highly increased rates of abnormal spermatozoa. However, the scatter plot results of Bernardini *et al.* (1997) show a strong correlation (eight out of nine cases) between OA and aneuploidy. The data of Pang *et al.* (1999) show that the incidence of aneuploidy is higher in all nine patients studied than in controls for the 14 chromosomes studied (except for one patient for chromosome 11, two patients for chromosome 12 and one patient for chromosome 18), and the frequency of diploidy is much higher in all nine patients studied than in controls. Aran *et al.* (1999b) found a significantly higher diploidy rate in five out of 14 OA patients, and in one out of five patients studied with asthenozoospermia. Therefore, it is

possible to conclude that patients with OA have higher disomy and diploidy frequencies than controls, and that patients with OA and meiotic disorders only show higher frequencies of diploidy. As indicated, this may reflect different degrees of disturbance of the meiotic process, covering the whole spectrum, including: (i) slight disorders (with production of X and Y univalents, and perhaps of some small autosomal univalents; increase in sex chromosome disomies and eventually in some autosomal disomies); (ii) mild disorders (with synaptic anomalies in a variable number of bivalents and interference with the anaphase checkpoint at meiosis I, resulting in the production of diploid spermatozoa) possibly related to an abnormal testicular environment (Finkelstein *et al.*, 1998; Rives *et al.*, 1998; Mroz *et al.*, 1999), and (iii) severe disorders (with a complete meiotic arrest of the affected cells at the primary spermatocyte stage and, through sperm selection, no increase in disomic or diploid spermatozoa; Navarro *et al.*, 1990). The most severe anomalies are probably related to mutations affecting the meiotic process (HR51, MLH1; Barlow and Hultén, 1996; Hassold, 1996; Edelmann *et al.*, 1996, and especially HR6B which results in severe OA in homozygous male mice; Grootegoed *et al.*, 1999; the mouse protein is 100% identical with the human protein).

In fact, Martin (1996) has recently proposed, as we did in 1983 (Egozcue *et al.*), that many infertile 46,XY males are affected by synaptic anomalies during meiosis, resulting in a more or less severe meiotic arrest and in the production of aneuploid spermatozoa. However, recent data suggest that meiotic anomalies produce more diploid than aneuploid spermatozoa. Regarding the effect of meiotic disorders on recurrent abortion, the above classification is self-explanatory. Autosomal trisomies or monosomies are mostly non-viable; pure meiotic disorders do not usually produce the most common aneuploidies seen at birth. Sex chromosome aneuploidies are more viable, and have a probability of survival to birth of 55.3% for XXY, 70% for XXX, 100% for XYY and 3% for XO (Jacobs and Hassold, 1995). Triploids only exceptionally survive to birth. As a result, most infertile males with meiotic disorders are sterile or have a history of recurrent abortion. In a preliminary work, Aran *et al.* (1999a) have shown that in a series of 74 ICSI patients, those with meiotic disorders had a lower pregnancy rate (27.3 versus 36.8%), a lower implantation rate (16.0 versus 19.6%) and a higher abortion rate (33.0% versus 7.1%) than patients with a normal meiosis.

Abnormal spermatozoa and the origin of aneuploidy and triploidy

The relationship between the production of abnormal spermatozoa and the origin of aneuploidy has been discussed above for each particular case. Leaving aside the fact that most numerical anomalies are of maternal origin, if no data other than the incidence of disomic spermatozoa in the general population were available, one would expect that in humans the most common numerical anomalies of paternal origin would be sex chromosome aneuploidies, with an XXY, XXX or XYY constitution (0.43% disomic spermatozoa; incidence of about 0.5–1 cases/1000 births each) and trisomy 21 (0.29–0.35% disomic spermatozoa; incidence of 1–2 cases/1000 births) (Jacobs and Hassold, 1995).

Sex chromosome disomies are also more frequent in infertile males, and especially in OA patients, who are among the natural candidates for ICSI. From this, one would expect the incidence of sex chromosome anomalies to be increased in the offspring of these individuals. In fact, this increase (from 0.2% in the general population to ~1% in ICSI children) has been well documented (Bonduelle *et al.*, 1996). Other autosomal aneuploidies are much less frequent (one case in 7000 births for trisomy 18; one case in 10 000 births for trisomy 13); duplications and/or deficiencies (partial trisomies and monosomies) are only occasionally seen, and usually result from the abnormal segregation of a structural rearrangement in a carrier father or mother.

On the other hand, the production of diploid spermatozoa (Figure 2) is the most common anomaly to all chromosome aberrations related to human male infertility, and its role in the origin of diandric triploidy worth exploring. The frequency of triploids in humans is unusually high. Jacobs (1992) estimated its incidence at 1.01% of clinically recognized pregnancies, which corresponds to a frequency close to 9% for all conceptions (Jacobs, 1992; Egozcue *et al.*, 1997). Munné and Cohen (1998) found a frequency of triploids of 4.5% in binucleated embryos (to exclude dispermy) obtained by ICSI.

No significant increases in the incidence of diploid spermatozoa have been found in patients with autosomal or sex chromosome numerical anomalies. On the other hand, increased frequencies of diploid spermatozoa are found in some carriers of balanced chromosome reorganizations (Van Hummelen *et al.*, 1998; Blanco *et al.*, 1998b), and are a common finding in patients with meiotic disorders, with frequencies from 1.07 to 9.6% (Bernardini *et al.*, 1997; Aran *et al.*, 1999b; Pang *et al.*, 1999) that in some cases may reach impressive proportions (25%, Pieters *et al.*, 1998; 40%, In't Veld *et al.*, 1997; or even close to 100%, Aviram-Goldring *et al.*, 1997, Bergère *et al.*, 1997). Older men (aged >55 years) also show significant increases in diploid spermatozoa (0.31–0.5%) (Bosch *et al.*, 1998), although an age effect on diploidy was not detected by Griffin *et al.* (1995).

If cases of triploidy originated by dispermy are excluded, diandric triploids result from the fertilization of a normal oocyte by an unreduced spermatozoon. Although earlier studies suggested that most triploids were of paternal origin (Eiben *et al.*, 1996), recent data suggest that most triploidy results from digyny (McFadden and Pantzar, 1996). A simulation based on a frequency of triploids of 8.8% (estimated from recognized abortions plus peri-implantational

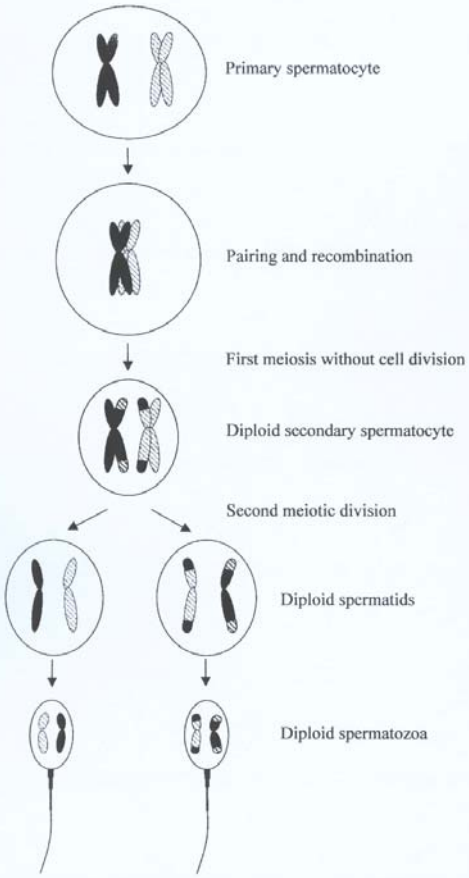

Figure 2. Production of diploid spermatozoa. If the chromosomes do not align properly on the metaphase plate, they may be unable to migrate to the poles at anaphase, the cell is unable to divide, and a single, diploid secondary spermatocyte is produced, giving rise to two diploid spermatozoa.

losses; Jacobs, 1992; Egozcue *et al.*, 1997) or of 4.5% (based on data from binucleated ICSI embryos; Munné and Cohen, 1998) and a frequency of diandry of 25% (2.2 or 1.125% of triploidy respectively) results in a good correlation with the frequencies of diploid spermatozoa found in infertile, OA males (0.10–1.90%) and even in carriers of structural reorganizations (0.10–1.42%), but not with the frequency of diploid spermatozoa found in controls (0.2–0.3%).

From work in the Orthoptera it has been known for years that the lack of inactivation of an X chromosome during meiosis in a species with an X_1X_2Y sex-determining system (Callan and Jacobs, 1957; Antonio *et al.*, 1993) or the presence of erratic B-chromosomes (Suja *et al.*, 1987, 1989) can result in a meiotic arrest and in the production of macrospermatids (in fact, diploid spermatids), because in both cases the hyperphosphorylation of the inner plate of the trilaminar kinetochore (Li and Nicklas, 1997) interferes with the normal attachment and orientation of the bivalents, and division is arrested at the anaphase I checkpoint (Rieder *et al.*, 1994). Heterosynapsis may rescue meiosis (Saadallah and Hultén, 1986) and the anaphase arrest may be overcome (Eichenlaub-Ritter, 1994), but the absence of cytokinesis results in the production of diploid secondary spermatocytes which, in turn, will originate diploid spermatids and diploid spermatozoa.

In mouse females, experimental treatment with Ca^{2+}-ionophores triggers anaphase I in the absence of cytokinesis, producing diploid oocytes (Soewarto *et al.*, 1995). However, since oocytes lack the anaphase I checkpoint, spindle anomalies result in the production of multiple aneuploidies rather than diploidy (Eichenlaub-Ritter *et al.*, 1999). In contrast with the situation in males where high FSH concentrations are correlated with meiotic anomalies that mainly result in the production of diploid spermatozoa (Aran *et al.*, 1999b; Vendrell *et al.*, 1999) in the female persistently high FSH concentrations result in the production of aneuploid oocytes. Unreduced oocytes mainly originate from the non-extrusion of the first polar body in a compromised environment (immaturity or overmaturity; Badenas *et al.*, 1989; Català *et al.*, 1988; hypoxia; Van Blerkom *et al.*, 1997). These factors may interfere with the organization of the spindle near the periphery of the oocyte, or may induce the centripetal migration of the spindle apparatus.

In infertile males with meiotic disturbances, all these factors can participate in the production of diploid spermatozoa. Meiotic mutations (Barlow and Hultén, 1996; Edelmann *et al.*, 1996; Grootegoed *et al.*, 1999) or a compromised testicular environment (Finkelstein *et al.*, 1998; Rives *et al.*, 1998; Mroz *et al.*, 1999) can delay synapsis and prevent some erratic bivalents or individual chromosomes (univalents) from reaching anaphase on time. Heterosynapses between unpaired regions of some chromosomes, frequently seen in carriers of structural reorganizations and in patients with a synaptic failure, and the interlocking of bivalents, which requires a breakage-and-repair mechanism, can have the same effect (Guitart *et al.*, 1987). And, of course, the presence of extra chromosomes or of unpaired chromosome regions can interfere with the inactivation of the X chromosome which is needed for meiosis to proceed correctly (Lifschytz and Lindsley, 1972; Forejt, 1984).

Thus, any severe meiotic disorder, either primary or secondary to a somatic chromosome abnormality, can affect the anaphase I checkpoint and result in the production of

diploid spermatozoa. This could explain why diploid spermatozoa are so frequently found in infertile males, and are the main and more constant anomaly observed whenever serious disturbances of the meiotic process occur.

Abnormal spermatozoa and recurrent abortion

Sperm chromosome studies using the hamster system were carried out by Rosenbusch and Sterzik (1991) in 10 males from couples with habitual abortion. The results demonstrated the existence of an increase in unstable chromosome lesions (breaks and fragments).

Sperm chromosome studies by FISH are also scarce. Giorlandino *et al.* (1998) analysed two couples with first trimester recurrent abortions. The study of ~600 spermatozoa in each of the two males revealed increased (10–20-fold) autosomal (chromosomes 15 and 18) and sex chromosome disomies and nullisomies.

Rubio *et al.* (1999) studied 12 couples with first trimester recurrent abortions. In five cases, the male partners had normal semenograms, although in two of them motility was borderline. In seven cases (included in an oocyte donation programme for low response to gonadotrophins) the male partners had OA (n = 5), oligozoospermia (n = 1) or asthenozoospermia (n = 1). Studies of ~1500 spermatozoa per individual in the 12 males revealed a significant increase in sex chromosome disomy (0.84 versus 0.37% in controls), but not in the rates of autosomal disomy or of diploidy. However, sperm FISH analyses in the subset of seven males with abnormal semenograms revealed a higher incidence of sex chromosome disomy (1%) and a significant increase of diploidy (0.43 versus 0.25% in controls).

Finally, Aran *et al.* (1999a) have shown that patients with meiotic disorders, and with increased diploidy frequencies (0.53 versus 0.25% in controls) also had increased abortion rates after ICSI (33.3 versus 7.1% in cases with normal meiosis). Thus, existing data suggest a direct involvement of abnormal spermatozoa in cases of recurrent abortion of presumably paternal origin. Unfortunately, a study of sperm chromosomes in males from couples in which the paternal origin of abortions had been determined by molecular techniques has not been carried out.

Conclusions

Chromosome abnormalities are an important factor in the aetiology of human male infertility, and although ~5% of cases of recurrent abortion are of genetic origin (Stirrat, 1990; Fignon *et al.*, 1995), chromosomal anomalies also represent an important contribution to pregnancy wastage.

In gestations obtained when the male partner carries a sex chromosome abnormality, prenatal diagnosis is highly recommended (but cannot be mandatory). Although in these cases preimplantation genetic diagnosis (PGD) would be desirable, the risk of losing the embryo(s), and the lower pregnancy rates obtained after PGD, preclude its use in cases in which a gestation may already have been very difficult to obtain.

In some carriers of structural chromosome anomalies, meiotic studies (now that testicular biopsies are performed at ease), or the relatively more expensive and time-consuming sperm chromosome analyses by FISH could help to better establish a reproductive prognosis and to recommend PGD, at least in cases with a high genetic risk and with a history of recurrent abortion.

Patients with meiotic disorders can only be diagnosed through the study of meiosis. Since these anomalies are especially frequent in cases with a severe OA, meiotic studies or sperm chromosome analyses by FISH seem to be indicated in this group.

Hopefully, the diagnostic procedures at hand, and their use in increasing numbers of patients should help to emphasize the importance of chromosome abnormalities in the aetiology of human male infertility.

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4.1.2 Artículo 2

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Increased Incidence of Meiotic Anomalies in Oligoasthenozoospermic Males Preselected for Intracytoplasmic Sperm Injection

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Purpose: *Based on data from the literature, to detect the possible presence of an increased frequency of meiotic anomalies in oligoasthenozoospermic (OA) patients preselected for intracytoplasmic sperm injection.*
Methods: *Meiotic studies in as many successive patients with a clinical indication for a diagnostic testicular biopsy as needed to complete at least 100 cases with a severe OA (motile sperm concentration $\leq 1.5 \times 10^6$/ml).*
Results: *An increased incidence of meiotic anomalies was found in 102 patients with a severe OA (17.6%) compared to the mean for 105 patients with other etiologies in the series (5.7%) or the mean for patients reviewed in the literature (6.5%).*
Conclusions: *Patients with a severe OA have a higher incidence of synaptic anomalies. This may result in the malsegregation of chromosomes at meiosis I, producing abnormal sperm, and could explain the high incidence of sterility and some cases of abortion (in two thirds of the couples with abortions the husband had meiotic anomalies) in this group.*

KEY WORDS: Diploidy; meiotic anomalies; oligoasthenozoospermia; sterility; synaptic anomalies.

INTRODUCTION

In recent years, the widespread use of intracytoplasmic sperm injection (ICSI) in couples with a severe male factor has led to an increased frequency of chromosome abnormalities in children conceived using this method (1). Although occasionally one of the parents carried a balanced chromosome rearrangement, in most cases de novo anomalies were detected. Several authors have suggested that this increased frequency of chromosome abnormalities in ICSI children could be related to a generalized disruption of the meiotic process (2, 3).

The first cases of recombination deficiency resulting in a disruption of meiosis in humans were described almost 30 years ago (4, 5). The most common anomaly was described as desynapsis of individual bivalents, affected a limited number of meiotic bivalents, and was characterized by the presence of variable numbers of univalents or of oligochiasmatic or achiasmatic homologues (6). The incidence of meiotic errors in heterogeneous populations of infertile males was established at 4–8% (7, 8).

Attempts to correlate the presence of meiotic anomalies and the characteristics of the semenograms failed to disclose any significant relationships, probably because the populations studied were ill defined. However, meiotic anomalies showed a tendency to accumulate in oligoasthenozoospermic (OA) males, although at the time the term OA was used to define a number of variable situations.

To determine whether patients with extremely poor semen parameters had an increased frequency of meiotic anomalies, we carried out meiotic studies in testicular biopsies from 103 male patients with a severe oligoastheozoospermia preselected for ICSI.

MATERIALS AND METHODS

The study was carried out in as many successive patients with a clinical indication for a diagnostic testicular biopsy as needed to complete a series of at least

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100 patients with a severe oligoasthenozoospermia (motile sperm concentration $\leq 1.5 \times 10^6$ sperm/ml in at least two successive semenograms) preselected for ICSI. Testicular biopsies are included in the protocol of study of infertile and sterile patients at the Institut Dexeus, and as such its use has been approved by the institutional ethics committee. Written informed consent was obtained from all patients.

Inclusion in the OA group was based only on seminal parameters. The other patients were selected for ICSI for heterogeneous reasons, including OA with higher sperm numbers and/or motility, different types of obstructive (variable anatomical levels and etiologies), or nonobstructive (traumatic, infectious, idiopathic, cryptorchidic) azoospermia, previous in vitro fertilizaton (IVF) failures, recurrent abortion, idiopathic infertility, and so forth. Somatic chromosome studies in peripheral blood also were offered, but not all patients accepted.

Unilateral testicular biopsies were obtained under local anesthesia. Meiotic preparations were made using the technique of Evans *et al.* (9). All preparations were coded, scored to locate meiotic divisions at 10×, and analyzed blindly by two different observers. The blind was only disclosed at the end of the study.

Statistical analyses were carried out using the χ^2 test.

RESULTS

The final series included 103 patients with OA as defined, and 106 patients of different etiologies. In the OA series 70 out of 103 patients were karyotyped, and in the heterogeneous sterile population karyotypes could be obtained from 61 of the 106 patients. Somatic chromosome studies detected an XXY/XY mosaic in the OA group and an azoospermic XXY patient and a carrier of a reciprocal t(8;17) with recurrent abortions in the heterogeneous group. The mosaic patient and the translocation carrier were eliminated from the corresponding groups, because it is known that such patients are prone to meiotic anomalies, leaving a total of 102 patients in the OA group and 105 patients in the heterogeneous group.

Results were classified as: 1—normal meiosis; 2—spermatogenic arrest, when prophase I figures and occasional spermatozoa but no metaphase I or metaphase II figures were found; 3—absence of spermatogenic cells, when only Sertoli cells were observed; and 4—meiotic anomalies, when synaptic errors were present as univalents and/or oligochiasmatic or achismatic homologues. Meiotic errors were found in 18/102 OA patients and in 6/105 patients in the heterogeneous group (Table I). This is a significant increase (as a result of the relatively small numbers of patients) over the mean frequency in the heterogeneous group (17.6% vs. 5.7%; $\chi^2 = 7.24$, DF = 1, $P < 0.01$) and a highly significant increase (17.6% vs. 6.5%; $\chi^2 = 21.38$, DF = 1, $P < 0.005$) over the mean of the published heterogeneous series (7). If only the karyotyped patients are taken into account, the incidence of meiotic anomalies is similar: 13/69 = 18.8% in OA patients and 3/60 = 5.0% in the heterogeneous group.

The anomalies observed in the OA group were two cases of complete desynapsis (synaptic anomalies affecting all bivalents), nine cases of desynapsis affecting a variable number (usually 2–6) medium-sized or large bivalents, and seven cases with the presence of a variable number of small univalents in their metaphase I figures. In the heterogeneous group, four patients had synaptic anomalies affecting two to four medium-sized or large bivalents and two patients had small univalents in their metaphase I figures.

In the OA series, 100 patients were sterile and 3 had one abortion; of these, two had meiotic anomalies. In the heterogeneous group, 91 patients were sterile and 15 had at least one abortion (mean of 1.8 abortions per couple).

DISCUSSION

Our results have shown that in patients with a severe OA, as defined by present criteria, the incidence of meiotic anomalies is much higher than that previously found in heterogeneous populations of infertile males, as had been already suspected (7). In fact, this tendency has been confirmed in relatively short series of patients by meiotic analyses (10), and several studies using fluorescence in situ hybridization (FISH) on decondensed sperm heads have shown that OA males have a higher incidence of autosomal and sex chromosome aneuploidies and of diploid sperm (2, 3, 11, 12), as

Table I. Results of Meiotic Studies in 207 Successive Patients

	OA (n = 102)(%)	Heterogeneous (n = 105)(%)
Normal	63 (61.7)	63 (60.0)
Sperm. arrest	21 (20.5)	21 (20.0)
No sperm. cells[a]	-- (-----)	15 (14.2)
Meiotic anom.[b]	18 (17.6)	6 (5.7)

[a] Only observed in some cases of azoospermia.
[b] Univalents or oligo/achiasmatic homologues.

should be expected from the disturbance of the anaphase I checkpoint (13) by the presence of erratic chromosomes or bivalents.

Andrologic studies carried out in our patients have shown the existence of a correlation between the incidence of meiotic anomalies and decreasing numbers of motile spermatozoa, and also between the incidence of meiotic anomalies and increased levels of FSH (14); the relationship between disturbed meiosis and increased levels of FSH or other environmental anomalies also has been suggested by several authors (15, 16). Finally, preliminary results on the reproductive performance of some of these patients after ICSI indicate that patients with meiotic anomalies have a decreased pregnancy rate and an increased abortion rate when compared to infertile individuals with a normal meiotic process (17).

Since these patients produce sperm with complex chromosome abnormalities (11) and an increased rate of diploid spermatozoa (12), and their embryos produced by ICSI show slower division rates, as shown in our laboratory (unpublished), some cases of sterility resulting from a severe male factor could be related to an increased peri-implantational wastage. In fact, although it is generally accepted that about 50% of abortions from clinically recognized pregnancies have chromosome anomalies, it also has been shown that over 80% of peri-implantational losses have chromosome aberrations (18).

These observations stress the need for genetic testing (either through meiotic studies, sperm FISH, or both) and for genetic counseling in patients with a severe OA or with major sperm phenotype aberrations (14, 17, 19, 20).

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