Revised

Meiotic abnormalities in infertile males

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Abstract

Meiotic anomalies, as reviewed here, are synaptic chromosome abnormalities, limited to the germ cells, that cannot be detected through the study of the karyotype. Although the importance of synaptic errors has been underestimated for many years, their presence is related to many cases of human male infertility.

Synaptic anomalies can be studied by immunostaining of synaptonemal complexes (SCs), but in this case their frequency is probably underestimated due to the phenomenon of synaptic adjustment. They can also be studied in classical meiotic preparations, which, from a clinical point of view, is still the best approach, especially if multiplex fluorescence *in situ* hybridization is at hand to solve difficult cases. Sperm chromosome FISH studies also provide indirect evidence of their presence.

Synaptic anomalies can affect the rate of recombination of all bivalents, produce achiasmate small univalents, partially achiasmate medium-sized or large bivalents, or affect all bivalents in the cell. The frequency is variable, interindividually and intraindividually. The baseline incidence of synaptic anomalies is 6-8 %, which may be increased to 17.6 % in males with a severe oligozoospermia, and to 27 % in normozoospermic males with one or more previous IVF failures. The clinical consequences are the production of abnormal spermatozoa, that will produce a higher number of chromosomally abnormal embryos. The indications for a meiotic study in testicular biopsy are provided.

Introduction

The incidence of constitutional chromosome abnormalities is about ten times higher in infertile males than in the general population (Zuffardi and Tiepolo, 1982; Van Assche et al., 1996). These anomalies include sex-chromosome aneuploidies, such as XXY and XYY, which are characterized by the production of germ cells that are meiotically incompetent or partially incompetent, and give rise to a more or less severe meiotic arrest (Blanco et al., 2001), or structural rearrangements, which give rise to abnormal meiotic configurations, well known since the first decades of the XXth century (Sybenga, 1975). These rearrangements may segregate abnormally during the meiotic process and produce chromosomally unbalanced spermatozoa (reviewed by: Egozcue et al., 2000a; Egozcue et al., 2003). These anomalies are addressed in several articles of this issue, and will not be dealt with here. Thus, this review will be limited to meiotic anomalies present in infertile males with a normal karyotype, and only detectable through the study of meiosis, i.e., to anomalies that have been held as marginal for a long period of time.

And yet, it has been known for many years that a variable number of infertile males may show synaptic errors which, by interfering with the normal meiotic process, may produce diploid or aneuploid spermatozoa, and affect the reproductive capacity of the carrier (review by Egozcue et al., 2000a). In fact, interest in this type of anomalies has been recently awakened by the results of immunofluorescent studies of synaptonemal complexes (Barlow and Hultén, 1996, 1998; Oliver-Bonet et al., 2003; Codina-Pascual et al., 2004; Sun et al., 2004a; Gonsalves et al., 2004), confirming older data obtained from meiotic chromosome studies (Egozcue et al., 1983) and from light and electron microscopic studies of silver-stained synaptonemal complexes (e.g., Hultén et al., 1974; Navarro et al., 1986; Vidal et al., 1987).

The first synaptic anomalies were described by Hultén at al. (1970) and by Pearson et al. (1970), and consisted in a reduction of the number of chiasmata at metaphase I (oligochiasmatic males). Later on, variants of this anomaly were described by Dutrillaux and Guéguen (1971), Skakkebaek et al. (1973), Templado et al. (1976) and Chaganti et al. (1980).

These anomalies were considered to affect from 6-8 % of infertile males in whom meiosis was analyzed (Egozcue et al., 1983; De Braekeleer and Dao, 1991), but more recently, the study of better defined groups of patients suggests that the proportion may be quite variable.

Meiotic studies in human infertile males have been very scarce in the recent past, because a testicular biopsy requires minor surgery, and also because most laboratories lacked the expertise needed to analyze meiotic configurations, especially in infertile males, in whom the number and quality of meiotic divisions may be quite low (Hultén et al., 1992; Hultén et al., 2001; Sun et al., 2004a). However, with the progressive use of intracytoplasmic sperm injection (ICSI) using spermatozoa retrieved from the testis, testicular biopsies have become quite common, and the incidence of synaptic anomalies has been confirmed by many authors, although the series are still rather short, and the categories of the patients still ill defined (Hammamah et al., 1997; Lange et al., 1997; Sarrate et al., 2004a).

Synaptic disorders may be related to mutations of one or more genes involved in synapsis or in DNA repair mechanisms (Edelmann et al., 1996; Hassold 1996; Grotegoed et al., 1999; Baarends et al., 2001; Judis et al., 2004), to mechanical disturbances of the synaptic process, such as heterosynapsis (which is a rescue mechanism; Saadhallah and Hultén, 1986), bivalent interlocking or nucleolar fibers connecting independent bivalents (Guitart et al., 1987), all of which can induce a

meiotic arrest resulting in the production of azoospermia or severe oligozoospermia (Saadhallah and Hultén,1986; Navarro et al., 1990), or to milder forms of the anomaly (Templado et al., 1981) that could be related to an abnormal progression of meiosis in a compromised testicular microenvironment, especially when FSH values are elevated (Speed and Chandley, 1990; Finkelstein et al., 1998; Mroz et al., 1999; Egozcue et al., 2000 b; Vendrell et al., 2003).

Methods of study

Synaptic anomalies can be analyzed through the study of synaptonemal complexes, at pachytene of meiosis I, or in meiotic chromosome preparations (metaphase I and metaphase II), using different technologies.

Analysis of synaptonemal complexes (SCs) was initially carried out by combining light and electron microscopy (Navarro et al., 1981). This allowed characterization some of the mechanical synaptic disturbances previously described, and also demonstrated the existence of interchromosomal effects (Templado et al., 1984a; Navarro et al., 1991), consisting in the presence of synaptic defects (like the one shown in the immunostained image in Fig. 1a) in individuals who carried a balanced chromosomal rearrangement. However, the technique was time consuming, and was only applied to clinical work for a short period of time.

More recently (Barlow and Hultén, 1996) the use of immunostaining of the SC elements and of the MLH1 recombination foci (Fig. 1b), and the individual identification of each SC using cenM-FISH or subtelomere labelling has contributed to a better understanding of the synaptic process and of its anomalies (Oliver-Bonet et al., 2003; Codina-Pascual et al., 2004; Sun et al., 2004a, 2004b; Gonsalves et al., 2004).

However, SCs and their MLH1 foci are better analyzed at mid pachytene, when pairing of homologues is complete, because the spreads are better, the SCs shorter, and spot counting is facilitated. But, by mid pachytene, synaptic adjustment has already taken place (Solari, 1980), the synaptic anomalies present in earlier stages may have disappeared, and thus may no be observed and not be taken into account when evaluating synaptic disturbances. The evanescence of a full inversion loop has been dramatically illustrated by Martínez-Flores et al. (2001). If such a complex structure as an inversion loop can become invisible at full pachytene, it is not difficult to imagine what may happen to small or even large synaptic splits.

Meiotic studies using classical methods (Evans et al., 1964) have been used in most cases for the diagnosis of patients with meiotic anomalies (Egozcue et al., 1983; Egozcue et al., 2000b). The technique is cheap, fast, easy to perform and reliable, but the meiotic configurations are not always easy to interpret. The quality of the preparations is usually good (Fig. 2a), and meiotic anomalies are easily identifiable by experienced personnel. Unfortunately, the use of solid staining do not allow identification of the bivalents affected. Furthermore, the number and size of the affected bivalents usually varies from cell to cell (v. ultra), indicating that the anomaly is unspecific and has different targets for reasons still unknown, but which might be more or more often related to environmental problems than to specific mutations (Mroz et al., 1999; Egozcue et al., 2000a).

To try to identify and characterize the anomalies involved, Sarrate et al. (2004b) have recently used multiplex FISH (Fig. 2), which may be combined with the sequential use of other probes (Fig. 3). This method allows identification of each bivalent in metaphase I, and characterization of the bivalents affected, but is also useful in the analysis of metaphase II figures, which are often difficult to interpret (Hultén et al.,1992, Hultén et

al., 2001), but important to analyze, because they reflect the normal segregation or the malsegregation of chromosomes in anaphase I, as a result of the synaptic anomalies present in metaphase I. Furthermore, the use of multiplex FISH (M-FISH) allows detection of structural meiotic rearrangements that may take place during spermatogenesis with an unknown frequency, in line with the few cases previously described (Templado et al., 1984b). These rearrangements are probably more frequent between the X and the Y chromosomes (unequal crossing-over) (Sarrate et al., 2004a); these exchanges could never be detected without the use of M-FISH. Unfortunately, the method is very expensive and time-consuming, and for the time being its use will have to be limited to research into this problem.

Finally, sperm chromosome studies by FISH reflect the results of chromosome and chromatid segregation during meiosis I and II, and might help to determine the risk of producing an abnormal pregnancy in patients with synaptic anomalies. However, the number of probes that can be used is still low, and most of them do not correspond to the bivalents affected by synaptic problems.

Classification of synaptic anomalies

The synaptic anomalies described can be limited or extensive, affect one single bivalent, several bivalents or most of them, and produce totally asynaptic or partially asynaptic bivalents. They can also affect all meiotic divisions analyzed or coexist with a normal cell line, in different proportions. The most common anomalies observed in meiosis I are:

1. Precocious separation of the sex chromosomes (Fig. 2a). This anomaly (Egozcue et al., 2000a) is characterized by the absence of MLH1 recombination foci in the X and Y chromosomes in pachytene spreads. The reduction of recombination

between the sex chromosomes is correlated with a decrease in the number of recombination foci in autosomal bivalents (Codina-Pascual, unpublished).

2. Totally achiasmatic small bivalents. This anomaly is frequent, and usually affects only small bivalents (Egozcue et al., 2000a). The number of achiasmate bivalents is variable, not only from patient to patient, but also from cell to cell. Surprisingly, preliminary data obtained using multiplex fluorescent in situ hybridization (M-FISH) suggest that these achiasmate bivalents involve mainly members of the F group (pairs # 19 and 20) and not members of the G group (pairs # 21 and 22) as might have been expected (Sarrate et al., 2004a; 2004b).
3. Partially achiasmate bivalents. These are also variable in number, not only in different patients, but also in different cells from the same patient, and are usually medium sized (group C) (Fig. 1b), but may occasionally be large (groups A and B) (Fig. 2). The most common effect of the reduction of the number of recombination sites is the presence of a single chiasma in a bivalent that should usually have two or more chiasmata (Fig. 2). Preliminary studies suggest that pair # 9 may be the one most frequently involved in this anomaly. Partially achiasmate bivalents are the most common meiotic anomaly observed in infertile males.
4. Totally achiasmate bivalents. This is a very unfrequent anomaly, and affects most if not all bivalents. Chromosome fragmentation is usually present (Templado et al., 1976), and the fragments may aggregate to produce pseudochromosomes or pseudobivalents (Fig. 4).

The incidence of each one of these synaptic errors has never been estimated, although the most frequent anomalies are by far the presence of small achiasmate bivalents and the presence of medium-sized partially achiasmate bivalents.

Occasionally, and as previously described in the Orthoptera (Suja et al., 1989) asynaptic gametocytes may produce megalospermatocytes (Johannisson et al., 2003) or megalospermatids (Escalier, 2002), which is a most unusual finding, but is obviously related to synaptic errors.

Incidence

To determine the real incidence of synaptic anomalies in infertile males is difficult, because the possible influence of meiotic anomalies on the reproductive record of these patients has not been considered as it deserved. However, some published or unpublished data are available to offer an overview of this problem.

The incidence of synaptic anomalies is quite different depending on the methodology of analysis employed. By using immunostained SCs in oligozoospermic patients, Codina-Pascual (unpublished) found no significant differences in the rate of synaptic defects between patients and controls. These data underline the difficulty of using full pachytenes to establish the incidence of synaptic anomalies, due – as discussed above – to the phenomenon of synaptic adjustment. On the other hand, Gonsalves et al. (2004) found that 10% of patients with a non-obstructive azoospermia had a reduced recombination rate, while this anomaly affected 50% of patients with a “maturation arrest”. This is not surprising taking into account that patients with meiotic arrest (oligozoospermia) show a much higher incidence of synaptic anomalies (17.5%) than non-obstructive azoospermic patients (5.9%) (Egozcue et al., 2000b).

On the other hand, in 1983 Egozcue et al. studied a series of 1100 “infertile males” which included from azoospermic to normozoospermic patients. The incidence of synaptic anomalies was 6-8 %, a figure later confirmed by De Braekeleer and Dao

(1991). Later on, Egozcue et al. (2000b) studied 103 males with a severe oligoasthenozoospermia (< 1.5 x 10^6 motile sperm/ml) and found an incidence of meiotic anomalies of 17.6 %. More recently, in a still preliminary study, Egozcue et al. (2004) studied 60 normozoospermic males with a long history of sterility or with previous IVF failures, and surprisingly the incidence of synaptic anomalies was 27%. Taking into account their clinical record, out of the 103 patients studied by Egozcue et al. (2000b), 100 were sterile and three had had one abortion. In the series of 60 normozoospermic patients, Serra et al. (2004) found 17 patients with long term sterility, 21 with an embryo factor after IVF (low embryo quality, abnormal cleavage, developmental arrest,…), 11 with no fertilization at IVF and 23 with repeated IVF failures. The total adds to more than 60 patients because some of them had more than one of the problems indicated. These data are, by far, inconclusive, because they refer to short series, but underline the fact that synaptic anomalies are frequent in infertile males with a severe oligozoospermia or oligoasthenozoospermia, or in cases of normozoospermic males with previous IVF failures.

Clinical consequences

The clinical consequences of synaptic anomalies are difficult to evaluate, because as stated before this is a field that has been mostly ignored by clinicians and researchers. However, some general data are available concerning the possible clinical consequences of synaptic anomalies.

1. Abnormal sperm: in the only five patients with synaptic anomalies in whom sperm chromosomes were analyzed by FISH (Arán et al., 1999), using probes for chromosomes 18, X and Y, diploidy (0.53 %) was significantly increased

when compared to controls (0.25 %; P< .01). No increases of sex chromosome or autosomal disomies were observed. However, Marina (unpublished) has compared the results of meiotic studies and sperm chromosome studies by FISH in 60 patients with different spermograms. In 18 cases (30 %) meiosis and FISH were normal, and in 17 cases (28.3 %) meiosis and FISH were abnormal, for a total of 58.3 % of coincidence. However, in 25 cases (41.6 %) FISH results were normal, but meiotic results were abnormal. Since, as discussed above, many of the meiotic anomalies observed cannot be detected by the set of probes employed (13, 18, 21, X and Y), sperm chromosome studies by FISH do not cover, at present, a chromosome spectrum wide enough to detect all the effects of synaptic anomalies. Another possibility might be the selective elimination of aneuploid cells as suggested by Blanco et al. (2001; 2003).

2. Fertilization, pregnancy, implantation and abortion rates: No significant differences were detected when comparing infertile males with synaptic anomalies and controls (Arán et al., 2003) but the work gave no indication about the birth rate.
3. Normal embryos: Patients with synaptic anomalies produced more chromosomally abnormal embryos than controls. In a recent study based on data from preimplantation genetic screening (PGS) of embryos from individuals with synaptic anomalies (Arán et al., 2004), 42.5% of the embryos were abnormal, and of these, 17.6 % had complex chromosome abnormalities. These figures are similar to those more recently compiled in our laboratory (69 cycles, 41.45% of abnormal embryos of which 16.86% with complex anomalies).
4. Embryo cleavage: In carriers of synaptic anomalies, embryo division was significantly delayed (Vendrell et al., 2003).

Indications for a meiotic study

In general, most meiotic (SCs, meiotic chromosomes) or meiotically related (sperm FISH) studies have been carried out in ill defined populations, such as "infertile males", "ICSI candidates", etc. Only a few of them have included patients with well known spermogram characteristics. By progressively narrowing the pathological spectrum, the best candidates for a meiotic study would be:

1. Infertile males with a normal karyotype and unexplained infertility, and among them,
2. Infertile males with normozoospermia and long-term sterility, or IVF failures (embryonic factor, no fertilization, repeated IVF failures), or
3. Infertile males with a severe oligozoospermia ($< 5 \times 10^6$ sperm/ml) or a severe oligoasthenozoospermia ($< 1.5 \times 10^6$ motile sperm/ml).

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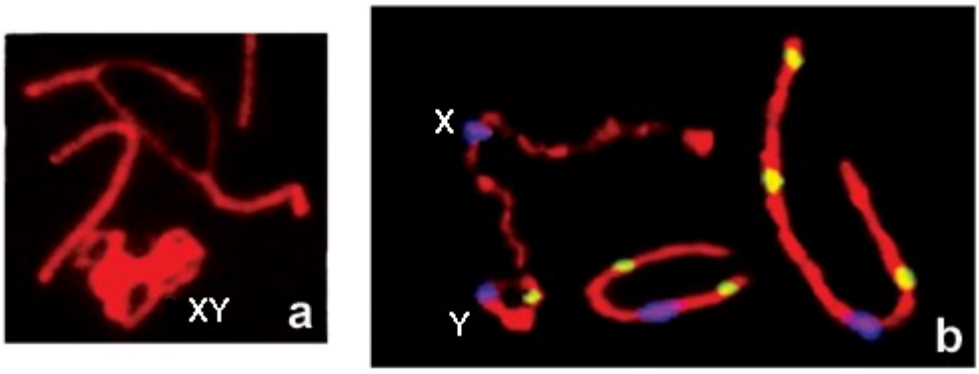

Figure 1. a) Medium-sized synaptonemal complex showing a long asynaptic region **b)** One medium-sized synaptonemal complex and one small immunostained with SCP3 (red) showing MLH1 recombination foci (yellow) and the centromere (CREST; blue).The sex chromosomes are indicated (XY).

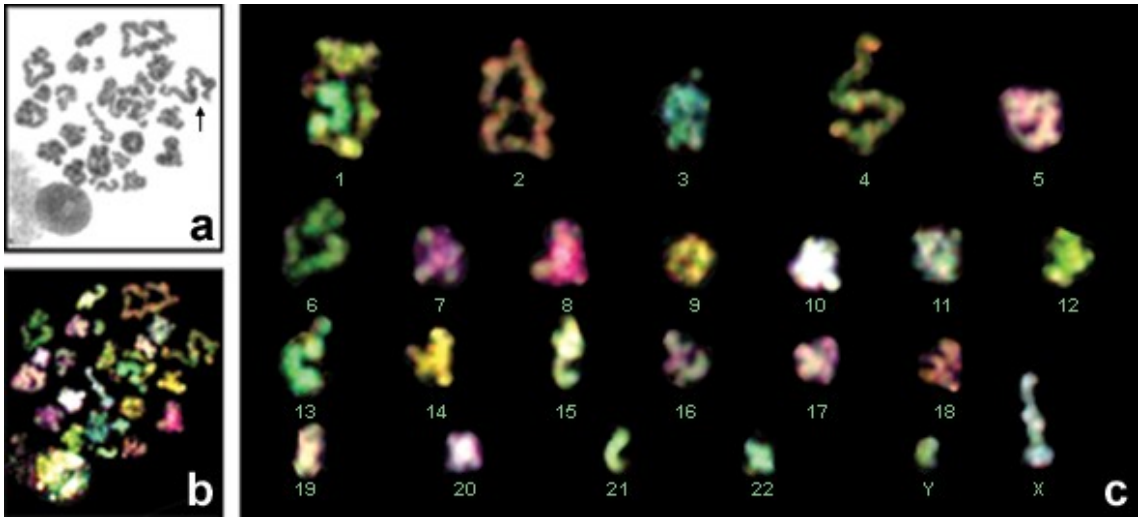

Figure 2. a) Leishman-stained metaphase I figure showing the precocious separation of the sex chromosomes, a large partially asynaptic bivalent (arrow) and a difficult-to-resolve superimposition (center). **b)** M-FISH of the same figure; the sex chromosomes are identified, the large, partially asynaptic bivalent corresponds to pair # 4, and the difficult-to-resolve superimposition includes pairs # 1 and 13.

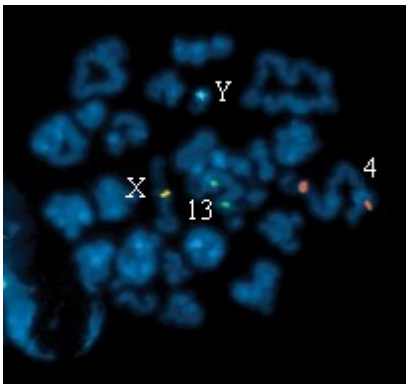

Figure 3. The previous Metaphase I recycled for multiprobe FISH using a combination of a centromeric probe for chromosome 4 (orange), a centromeric probe for chromosome X (red), a probe identifying the heterochromatic region of chromosome Y (blue) and locus specific probe for chromosome 13 (13q14;green). The centromeres of the partially asynaptic bivalent # 4 are wide apart, indicating that asynasis is proximal.

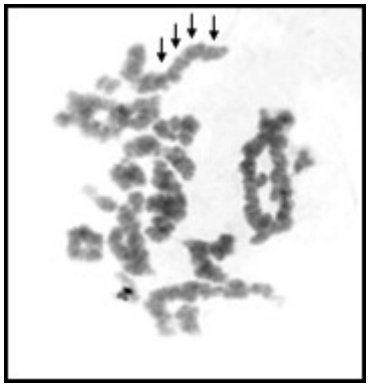

Figure 4. Metaphase I (Giemsa stain) with mostly asynaptic bivalents. Chromatin aggregates (arrows) produce pseudochromosomes and pseudobivalents.