

This is the **accepted version** of the journal article:

Egozcue, Josep; Sarrate Navas, Zaida; Codina Pascual, Montserrat; [et al.].
«Meiotic abnormalities in infertile males». Cytogenetic and Genome Research,
Vol. 111 N. 3-4 (sep. 2005), p. 337-342. DOI 10.1159/000086907

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7 **Meiotic abnormalities in infertile males**
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23 **Running title:** Meiotic anomalies in infertile males
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36 **Abstract**

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

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

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

56 **Introduction**

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

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

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

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

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

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

106 meiotic arrest resulting in the production of azoospermia or severe oligozoospermia
107 (Saadhallah and Hultén,1986; Navarro et al., 1990), or to milder forms of the anomaly
108 (Templado et al., 1981) that could be related to an abnormal progression of meiosis in a
109 compromised testicular microenvironment, especially when FSH values are elevated
110 (Speed and Chandley, 1990; Finkelstein et al., 1998; Mroz et al., 1999; Egozcue et al.,
111 2000 b; Vendrell et al., 2003).

112

113 **Methods of study**

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

117 Analysis of synaptonemal complexes (SCs) was initially carried out by combining light
118 and electron microscopy (Navarro et al., 1981). This allowed characterization some of
119 the mechanical synaptic disturbances previously described, and also demonstrated the
120 existence of interchromosomal effects (Templado et al., 1984a; Navarro et al., 1991),
121 consisting in the presence of synaptic defects (like the one shown in the immunostained
122 image in Fig. 1a) in individuals who carried a balanced chromosomal rearrangement.

123 However, the technique was time consuming, and was only applied to clinical work for
124 a short period of time.

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

130 However, SCs and their MLH1 foci are better analyzed at mid pachytene, when pairing
131 of homologues is complete, because the spreads are better, the SCs shorter, and spot
132 counting is facilitated. But, by mid pachytene, synaptic adjustment has already taken
133 place (Solari, 1980), the synaptic anomalies present in earlier stages may have
134 disappeared, and thus may not be observed and not be taken into account when
135 evaluating synaptic disturbances. The evanescence of a full inversion loop has been
136 dramatically illustrated by Martínez-Flores et al. (2001). If such a complex structure as
137 an inversion loop can become invisible at full pachytene, it is not difficult to imagine
138 what may happen to small or even large synaptic splits.

139 Meiotic studies using classical methods (Evans et al., 1964) have been used in most
140 cases for the diagnosis of patients with meiotic anomalies (Egozcue et al., 1983;
141 Egozcue et al., 2000b). The technique is cheap, fast, easy to perform and reliable, but
142 the meiotic configurations are not always easy to interpret. The quality of the
143 preparations is usually good (Fig. 2a), and meiotic anomalies are easily identifiable by
144 experienced personnel. Unfortunately, the use of solid staining do not allow
145 identification of the bivalents affected. Furthermore, the number and size of the affected
146 bivalents usually varies from cell to cell (*v. ultra*), indicating that the anomaly is
147 unspecific and has different targets for reasons still unknown, but which might be more
148 or more often related to environmental problems than to specific mutations (Mroz et al.,
149 1999; Egozcue et al., 2000a).

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

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

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

170

171 **Classification of synaptic anomalies**

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

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

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

182 2. Totally achiasmatic small bivalents. This anomaly is frequent, and usually
183 affects only small bivalents (Egozcue et al., 2000a). The number of achiasmate
184 bivalents is variable, not only from patient to patient, but also from cell to cell.
185 Surprisingly, preliminary data obtained using multiplex fluorescent in situ
186 hybridization (M-FISH) suggest that these achiasmate bivalents involve mainly
187 members of the F group (pairs # 19 and 20) and not members of the G group
188 (pairs # 21 and 22) as might have been expected (Sarrate et al., 2004a; 2004b).

189 3. Partially achiasmate bivalents. These are also variable in number, not only in
190 different patients, but also in different cells from the same patient, and are
191 usually medium sized (group C) (Fig. 1b), but may occasionally be large (groups
192 A and B) (Fig. 2). The most common effect of the reduction of the number of
193 recombination sites is the presence of a single chiasma in a bivalent that should
194 usually have two or more chiasmata (Fig. 2). Preliminary studies suggest that
195 pair # 9 may be the one most frequently involved in this anomaly. Partially
196 achiasmate bivalents are the most common meiotic anomaly observed in
197 infertile males.

198 4. Totally achiasmate bivalents. This is a very unfrequent anomaly, and affects
199 most if not all bivalents. Chromosome fragmentation is usually present
200 (Templado et al., 1976), and the fragments may aggregate to produce
201 pseudochromosomes or pseudobivalents (Fig. 4).

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

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

209

210 **Incidence**

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

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

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

230 (1991). Later on, Egozcue et al. (2000b) studied 103 males with a severe
231 oligoasthenozoospermia ($< 1.5 \times 10^6$ motile sperm/ml) and found an incidence of
232 meiotic anomalies of 17.6 %. More recently, in a still preliminary study, Egozcue et
233 al. (2004) studied 60 normozoospermic males with a long history of sterility or with
234 previous IVF failures, and surprisingly the incidence of synaptic anomalies was
235 27%. Taking into account their clinical record, out of the 103 patients studied by
236 Egozcue et al. (2000b), 100 were sterile and three had had one abortion. In the series
237 of 60 normozoospermic patients, Serra et al. (2004) found 17 patients with long
238 term sterility, 21 with an embryo factor after IVF (low embryo quality, abnormal
239 cleavage, developmental arrest,...), 11 with no fertilization at IVF and 23 with
240 repeated IVF failures. The total adds to more than 60 patients because some of them
241 had more than one of the problems indicated. These data are, by far, inconclusive,
242 because they refer to short series, but underline the fact that synaptic anomalies are
243 frequent in infertile males with a severe oligozoospermia or
244 oligoasthenozoospermia, or in cases of normozoospermic males with previous IVF
245 failures.

246

247 **Clinical consequences**

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

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

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

267 2. Fertilization, pregnancy, implantation and abortion rates: No significant
268 differences were detected when comparing infertile males with synaptic
269 anomalies and controls (Arán et al., 2003) but the work gave no indication about
270 the birth rate.

271 3. Normal embryos: Patients with synaptic anomalies produced more
272 chromosomally abnormal embryos than controls. In a recent study based on data
273 from preimplantation genetic screening (PGS) of embryos from individuals
274 with synaptic anomalies (Arán et al., 2004), 42.5% of the embryos were
275 abnormal, and of these, 17.6 % had complex chromosome abnormalities. These
276 figures are similar to those more recently compiled in our laboratory (69 cycles,
277 41.45% of abnormal embryos of which 16.86% with complex anomalies).

278 4. Embryo cleavage: In carriers of synaptic anomalies, embryo division was
279 significantly delayed (Vendrell et al., 2003).

280

281 **Indications for a meiotic study**

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

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

293

294 **Acknowledgements**

295 Research supported by project DGR-2001 SGR-00202 (Generalitat de Catalunya,
296 Spain), project SAF 2003-04312 (Dirección General de Investigación, Ministerio de
297 Ciencia y Tecnología, Spain) and project PI 020258 (Fondo de Investigaciones
298 Sanitarias, Spain).

299

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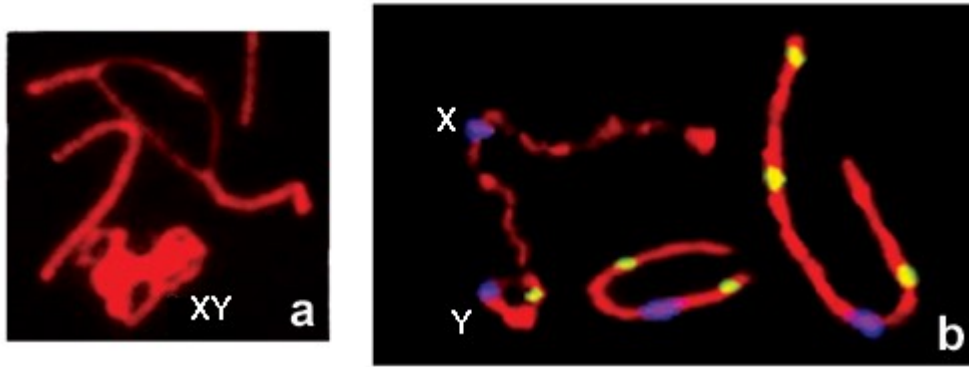


Figure 1. a) Medium-sized synaptonemal complex showing a long asynaptic region b) One medium-sized synaptonemal complex and one small immunostained with SCP3 (red) showing MLH1 recombination foci (yellow) and the centromere (CREST; blue).The sex chromosomes are indicated (XY).

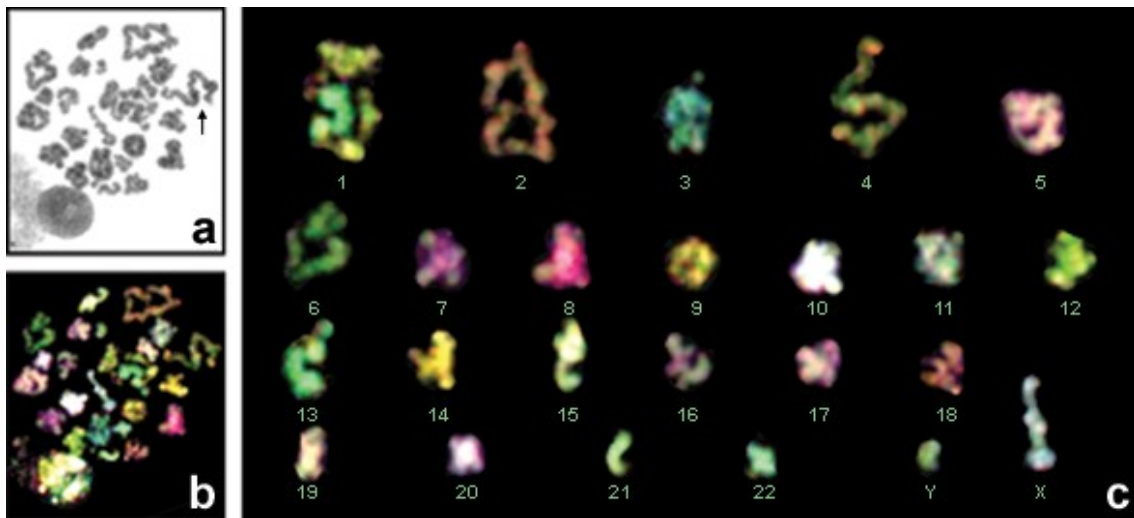


Figure 2. a) Leishman-stained metaphase I figure showing the precocious separation of the sex chromosomes, a large partially asynaptic bivalent (arrow) and a difficult-to-resolve superimposition (center). b) M-FISH of the same figure; the sex chromosomes are identified, the large, partially asynaptic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

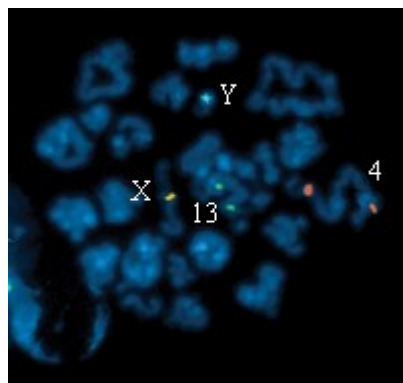


Figure 3. The previous Metaphase I recycled for multiprobe FISH using a combination of a centromeric probe for chromosome 4 (orange), a centromeric probe for chromosome X (red), a probe identifying the heterochromatic region of chromosome Y (blue) and locus specific probe for chromosome 13 (13q14;green).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

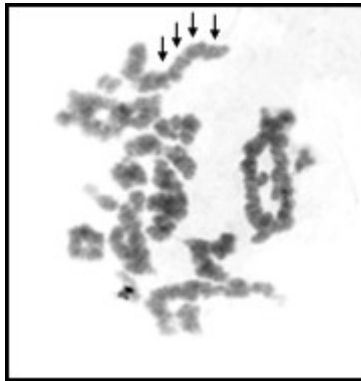


Figure 4. Metaphase I (Giemsa stain) with mostly asynaptic bivalents. Chromatin aggregates (arrows) produce pseudo-chromosomes and pseudo-bivalents.