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OPINION

Diploid sperm and the origin of triploidy

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Trisomy 16, the 45,X monosomy and triploidy are the more frequent chromosome anomalies in spontaneous abortions. Earlier estimations, based on frequencies of diandric triploidy at conception, resulted in a good correlation with the frequencies of diploid sperm in infertile males (up to 1.9%). Recent data have shown that most diandric triploids originate by dispermy, although 8.3% of them are produced by diploid sperm resulting from meiotic errors. Using these data, the estimated frequency of diploid sperm is still in good correlation with the percentage above. Furthermore, analysis of male pronuclei (PN) in 3PN zygotes produced by ICSI with sperm from oligo-, crypto- and azoospermic males revealed that 33.3% of them were diploid, while none of the PN produced by normozoospermic males by IVF was. The estimated frequency of diploid sperm in these infertile males is also in good correlation with the previous figures. The data suggest that most diandric triploids are produced by normozoospermic males by dispermy, while most diandric triploids produced by oligozoospermic males would result from fertilization by unreduced, diploid sperm.

Key words: diandric triploidy/diploid sperm/meiotic errors/oligozoospermia/unreduced gametes

Introduction

Trisomy 16, the 45,X monosomy and triploidy are the chromosome abnormalities most frequently found in spontaneous abortions. The association of trisomy 16 and of the 45,X monosomy with abortion is well known. However, the extremely high incidence of triploids among spontaneous abortions is often overlooked.

Diploidy, on the other hand, is the most common chromosome anomaly found in the sperm of patients with meiotic disorders (Egozcue *et al.*, 2000), in oligozoospermic males (Bernardini *et al.*, 1997; Downie *et al.*, 1997; Egozcue *et al.*, 1997; McInnes *et al.*, 1998; Aran *et al.*, 1999; Pang *et al.*, 1999; Pfeffer *et al.*, 1999; Calogero *et al.*, 2001) and in some carriers of balanced chromosome reorganizations (Egozcue *et al.*, 2000; Pellestor *et al.*, 2001) with proportions of diploid sperm ranging from 0.10 to 9.6%.

Most studies on the relationship between chromosome anomalies in sperm and the production of chromosomally abnormal embryos or offspring are usually centered on sex chromosome or autosomal disomies, and seldom on diploidy (Zaragoza *et al.*, 2000; Calogero *et al.*, 2001; Macas *et al.*, 2001; Pellestor *et al.*, 2001).

However, in the most important study published so far on the parental origin of triploidy (Zaragoza *et al.,* 2000) at least 8.3% of diandric triploids originated from diploid sperm, and a study (Macas *et al.,* 2001) on the chromosome constitution of paternal pronuclei in zygotes obtained by ICSI observed that 33.3% of the abnormal male pronuclei were diploid.

In a recent paper (Egozcue *et al.*, 2000) we underlined the important role of diploid sperm in the origin of triploidy, as well as the very high contribution of triploidy to pregnancy wastage, and suggested that a disturbance of the anaphase I (spindle assembly) checkpoint (Rieder *et al.*, 1994) related to the presence of synaptic anomalies and to the subsequent presence of erratic chromosomes could give rise to the production of diploid sperm. Although this paper was published quite recently, since then two studies have provided considerable new data on the origin of triploidy (Zaragoza *et al.*, 2000; Macas *et al.*, 2001).

The parental origin of triploidy

Zaragoza *et al.* carried out a molecular study of 91 triploid spontaneous abortions. Most triploids (60/91) were of paternal origin, 27 cases were maternal and in four cases the origin was unknown (Zaragoza *et al.,* 2000).

Regarding the 60 triploids of paternal origin, 37 cases (61.6% of diandric triploids) originated by dispermy, five (8.3%) were produced by diploid sperm, one resulted from a postmeiotic error, and in 17 cases the origin could not be determined.

Prior to the publication of the work by Zaragoza *et al.*, several studies had come to the unexpected conclusion that most triploidy was digynic. Based on estimated frequencies of triploidy at conception of 4.5% (Munné and Cohen, 1998) to 8.8% (Jacobs, 1992; Egozcue *et al.*, 2000) or even higher (12.4 %) (Eiben *et al.*, 1990) and on an erroneously low proportion of diandry of only 25%, we (Egozcue *et al.*, 2000) came to the conclusion that 1.12–2.2% of triploidy was diandric; although these figures underestimated diandry, they were in good correlation with the proportion of diploid sperm found in infertile males (up to 1.90%) (Egozcue *et al.*, 2000).

Using the new data of Zaragoza *et al.* (2000), which show that at least 8.3% of diandric triploids originate from diploid sperm, the proportion of diploid sperm can be estimated at 0.73%, which is still in good correlation with the percentages of diploid sperm found in infertile males (up to 1.90%). Although in this case the estimated percentage is slightly lower, one has to take into account that it corresponds to a general population of males, which also includes more normozoospermic than oligozoospermic individuals.

Diploid pronuclei and the origin of triploidy

Of even more interest are the recent data of Macas *et al.*, who analysed the chromosomal constitution of 163 paternal complements from 163 tripronucleate (3PN) zygotes obtained by ICSI using the sperm of oligozoospermic (90 cases), cryptozoospermic (28 cases) and azoospermic (20 cases, with sperm obtained from the epididymus or the testes) patients (Macas *et al*., 2001). The data were compared with those obtained in136 paternal complements from 68 3PN zygotes produced by IVF from normozoospermic males.

The first important observation is the high proportion of dispermy, which was not specifically indicated in the paper, but can be deduced from the high incidence of dispermy in spontaneous triploids (61.6%) (Zaragoza *et al.*, 2000), from the even higher incidence of dispermy induced by IVF conditions, and from the high number of paternal pronuclei (136) found in the 68 IVF zygotes analysed. This is in good agreement with the data of Rosenbusch *et al.* (1997), who found an excess of XYY triploid zygotes over the theoretical expectations, indicating a possible increase of dispermy related to IVF, and with those of Zaragoza *et al.* (2000), who found that in spontaneous abortions dispermy is the most frequent cause of triploidy (Rosenbusch *et al.*, 1997; Zaragoza *et al.,* 2000).

However, in the oligo-crypto-azoospermic group, in which the zygotes had only one paternal pronucleus (as established by careful analysis of the oocyte and the polar body characteristics), four of the 12 abnormal male pronuclei (33.3%) were diploid. This impressive proportion of diploidy included three diploids in seven abnormal, Y-containing pronuclei, and one diploid in five abnormal X-containing pronuclei.

On the other hand, not a single diploid male pronucleus was found in the control population of pronuclei obtained by IVF from normozoospermic males.

These data confirm that in patients with low numbers of sperm, the presence of diploid sperm is the most constant and frequent chromosome anomaly (Egozcue *et al.*, 1997).

The proportion of diploid pronuclei was of two diploids in eight abnormal pronuclei (25%) in oligozoospermic males, one diploid in two abnormal pronuclei (50%) in cryptozoospermic males and one diploid in two abnormal pronuclei (50%) in azoospermic males.

The overall frequency of diploid male pronuclei was 1/73 (1.3%) in X-bearing pronuclei and 3/90 (3.3%) in Y-bearing pronuclei. Even if the numbers are still low, a proportion of 1.3–3.3% diploid sperm is in good correlation with the proportion of diploid sperm in infertile males (up to 1.90%).

Although in this case the estimated frequency of 3.3% diploid sperm is slightly higher, one has to take into account that it corresponds to a population of oligo-crypto-azoospermic males.

Conclusions

From the comparative analysis of previously published data (Egozcue *et al.,* 1997; Zaragoza *et al.,* 2000; Macas *et al.,* 2001) it is possible to come to the following conclusions regarding the origin and distribution of triploidy: (i) Over the total number of triploids, 65.9% are diandric (of paternal origin), 61.6% by dispermy, 8.3% by fertilization of an oocyte by a diploid sperm and 1.6% as a result of mitotic errors. In 17 cases (28.3%) the origin of the anomaly could not be established (Zaragoza *et al.*, 2000); (ii) The proportion of diploid sperm in infertile males (Egozcue *et al.*, 1997) (0.10–1.90%) is in good correlation with other estimated frequencies: 1.12–2.2% (Egozcue *et al.,* 2000); 0.73% as calculated by us from the work of Zaragoza *et al*. (Zaragoza *et al*., 2000); and, in male pronuclei, 3.3% (Macas *et al.,* 2001). In the former case, the lower figure (0.73%) could result from a population with a majority of normozoospermic males, while in the latter case the higher figure could be related to a population made exclusively of oligo-crypto-azoospermic males.

Normozoospermic males produce diandric triploid zygotes mainly by dispermy. Oligo-crypto-azoospermic males produce diandric triploid zygotes mainly through the fertilization of normal oocytes by diploid sperm.

From a clinical point of view, it is obvious that patients with a severe oligoasthenoteratozoospermia have a higher risk of producing triploid embryos after ICSI (Macas *et al.*, 2001). This information should be taken into account in relation to the reproductive history and future management of these couples. A sperm chromosome study by fluorescence in-situ hybridizaton (FISH) may be indicated in some cases. Unfortunately, at present, and using standard sperm selection procedures, such as Percoll separation, the proportion of diploid sperm can only, at best, be reduced by half (Kovanci *et al.*, 2001). Diploid sperm can only be completely eliminated by flow sorting (Vidal *et al.*, 1999), a technique that results in a drastic decrease of sperm counts and that, as a result, cannot be used in patients with reduced numbers of sperm.

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