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LETTER TO THE EDITOR

Sperm Biology

A comprehensive analysis of chromosomal anomalies in metaphase II spermatocytes from infertile patients

Zaida Sarrate[1], Joan Blanco[1], Oliver Valero[2], Francesca Vidal[1]

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Dear Editor,

The scarce number of secondary spermatocytes in human seminiferous tubules and the complexity of analyzing chromosomes at metaphase II are the main causes of the limited knowledge we have about chromosome characteristics at this stage. In this study, we have performed a comprehensive analysis of meiotic abnormalities in metaphase II spermatocytes combining Leishman's staining protocols and multiplex fluorescent *in situ* hybridization procedures. Results indicated that infertile individuals showed different susceptibilities to meiotic abnormalities in secondary spermatocytes without a preferentially effect on any particular chromosome.

Meiotic cytogenetic studies on testicular cells, aiming to detect abnormalities affecting the germinal line, are being applied in the diagnostic workup of infertile patients.[1] These studies provide information about the presence of the XY body at prophase I, the number and morphology of meiotic figures at diakinesis/metaphase I and chiasmata count analysis. Concerning metaphase II (MII), the low number of such secondary spermatocytes in seminiferous tubules and the complexity of the analysis due to their specific chromosome morphology (contracted and curly appearance with open chromatids) hamper the analysis of this meiotic stage and determining its relevance in the assessment of male spermatogenesis. In this study, we performed a comprehensive analysis of meiotic abnormalities in secondary spermatocytes to evaluate whether abnormalities preferentially affected some chromosomes, and to elucidate whether some individuals presented a higher susceptibility to anomalies.

Testicular tissue was collected from 24 individuals without karyotype abnormalities who had received consultations for infertility. Protocols were approved by the institutional review board of the collaborating centers, and all of the patients signed their informed consent with regard to participation in the study.

Testicular biopsies were obtained under local anesthesia and were mechanically disaggregated in a hypotonic solution (0.075 mol l^{-1} KCl). Cell suspensions were fixed using methanol:acetic acid (3:1) and dropped onto slides. Samples were analyzed following a sequential methodology combining Leishman's staining (PanReac AppliChem, Castellar del Vallès, Spain) and multiplex fluorescence *in situ* hybridization (M-FISH, Spectra Vysion™ Assay Protocol, Vysis Inc., Downers Grove, IL, USA) to identify unequivocally all chromosomes.[2] MII was categorized as "normal count," "aneuploidy," "polyploid" or "chromatid gain/loss." MII chromosome morphology was also categorized as "normal morphology" or "premature separation of sister chromatids (PSSC)."[2]

A Poisson regression model was used for the analysis of normality per chromosome. In the case of anomalies, the categories "aneuploidy," "chromatid gain/loss," and "PSSC" were analyzed with logistic regression models.

For each patient, the number of normal and abnormal MII spermatocytes was annotated. A hierarchical cluster analysis was performed to identify patients with similar characteristics. Ward's method was used to calculate distances. This analysis was only performed on individuals, in which at least four MII spermatocytes were analyzed. Contingency tables and likelihood ratio Chi-square tests were used to describe the clusters according to their abnormalities.

MII data were also analyzed according to the classification of individuals obtained from the previous metaphase I (MI) analysis.[3] The Wilcoxon signed-rank test was used to evaluate differences between clusters in the normal category and contingency tables with Fisher's exact test in the other MII categories. These analyses were carried out using the SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) software. The level of statistical significance was set to 0.05.

The majority (93.0%) of the chromosomes evaluated displayed normal MII morphology. The remaining chromosomes corresponded to PSSC (4.1%), aneuploidies (2.7%), and chromatid loss (0.2%). No significant differences were observed in the distribution of PSSC between chromosomes. Nevertheless, the incidence of this abnormality showed 30 points of difference between the minimum and maximum values. The larger chromosomes showed the highest percentages of abnormalities (chromosomes 1–9, and 12). Consistent with this observation, previous MI analyses showed that large chromosomes presented significant reductions in the number of chiasmata.[3] This reduction could be accompanied by changes in the location of chiasmata and was associated with exchanges close to the centromere,[4] resulting in cohesin perichiasmatic removal in the MI/anaphase I transition.

[1]Genetics of Male Fertility Group, Unitat de Biologia Cel·lular, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès) 08193, Spain; [2]Servei d'Estadística Aplicada, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès) 08193, Spain.
Correspondence: Dr. Z Sarrate (zaida.sarrate@uab.cat)
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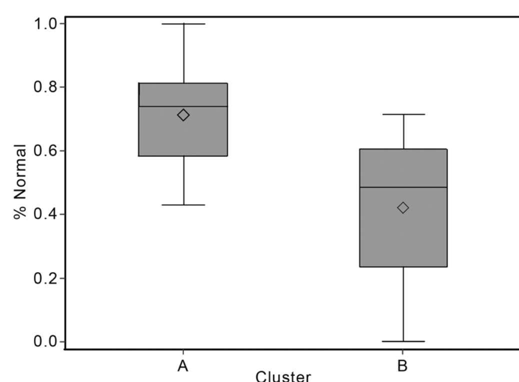

Cluster	n*	Mean	Median	Standard deviation	Minimum	Maximum
A	8	0.71	0.74	0.18	0.43	1.00
B	4	0.42	0.49	0.30	0.00	0.71

Figure 1: Graphical representation of the percentage of normal MII per cluster obtained from MI analyses. The plot represents the five-number summary (minimum, first quartile, median, third quartile, and maximum). The diamond indicates the mean. *Only individuals with at least four MII spermatocytes analyzed were included in this analysis.

This would result in a loss of cohesion between sister chromatids and trigger the premature loss of cohesion in the second meiotic division as evidenced by the PSSC observed in the MII analyses.[5]

Concerning aneuploidies, none of the chromosomes were preferably affected. Most of the aneuploidies were nullisomies (97.4%), although sex chromosome disomies were also observed (2.6%). The absence of the expected complementarity between disomy and nullisomy values suggests a possible chromosomal loss during the anaphase I stage.[6] In addition, the absence of chiasmata in MI has been documented to lead to errors in chromosome orientation at the metaphase plate. Consistent with this, it deserves to be mentioned that sex chromosomes were involved in most of the MII aneuploidies and were also observed as the most frequently separated chromosomes during MI of the same individual.[3]

Chromatid loss has also been observed in some chromosomes. As previously described, the presence of chiasmata near the centromere promotes the premature separation of sister chromatids during meiosis I.[5] Studies of mouse oocytes have shown that the bi-orientation of achiasmatic chromosomes in the spindle can result in the premature separation of chromatids during anaphase I.[7] Both mechanisms can promote the segregation of one chromosome with one of the two chromatids from the homologous pair to the same pole and the remaining chromatid to the opposite one.[8] This asymmetrical distribution is thought to be a main cause of aneuploidies in human oocytes[9] and has been previously described in 4.6% of MII spermatocytes from an infertile male.[10] However, our results indicate that this phenomenon is not common in males as there were no MII spermatocytes showing chromatid gains, and chromatid loss was infrequent.

At the metaphase level, 116 MII spermatocytes were evaluated. A percentage of 61.2% were normal, 18.1% showed chromosomes with PSSC, 14.7% were aneuploid, 3.4% had a lost chromatid, and 2.6% were diploid.

Cluster analysis was performed on data from the 12 individuals after evaluating more than four metaphases ($n = 96$ MII spermatocytes). Individuals were classified into three clusters. Cluster 1 included four individuals with at least one aneuploid metaphase. Cluster 2 comprised five individuals of which four presented PSSC. Finally, cluster 3 included three individuals with at least one metaphase with PSSC and one MII,46,XY. Statistically significant differences were only observed between clusters in the aneuploidy ($P = 0.002$) and MII,46, XY categories ($P = 0.001$). Although these results should be taken with care considering the data available, they showed that some abnormalities are predominantly present in certain individuals rather than in others.

Finally, the MII data were compared with those from a previous study of MI spermatocytes from the same patients[3] that were grouped according to chiasmata count (cluster A = normal count; cluster B = lower count). The percentage of normal MII was 71.0% in cluster A versus 42.0% in cluster B, suggesting a relationship between low chiasmata count and MII abnormalities, although the difference was not statistically significant ($P = 0.062$) (**Figure 1**).

To conclude, infertile individuals show different susceptibilities to meiotic abnormalities in secondary spermatocytes. These anomalies do not preferentially affect a particular chromosome and are consistent with the chromosomal abnormalities described in MI.

AUTHOR CONTRIBUTIONS

ZS was involved in experimental procedures, data collection, and assembly; ZS, FV, OV, and JB were involved in data analysis and interpretation, and manuscript writing. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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REFERENCES

1 Egozcue J, Sarrate Z, Codina-Pascual M, Egozcue S, Oliver-Bonet M, *et al*. Meiotic abnormalities in infertile males. *Cytogenet Genome Res* 2005; 111: 337–42.
2 Sarrate Z, Blanco J, Egozcue S, Vidal F, Egozcue J. Identification of meiotic anomalies with multiplex fluorescence *in situ* hybridization: preliminary results. *Fertil Steril* 2004; 82: 712–7.
3 Sarrate Z, Vidal F, Blanco J. Meiotic abnormalities in metaphase I human spermatocytes from infertile males: frequencies, chromosomes involved, and the relationships with polymorphic karyotype and seminal parameters. *Asian J Androl* 2014; 16: 838–44.
4 Lamb NE, Feingold E, Savage A, Avramopoulos D, Freeman S, *et al*. Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. *Hum Mol Genet* 1997; 6: 1391–9.
5 Rockmill B, Voelkel-Meiman K, Roeder GS. Centromere-proximal crossovers are associated with precocious separation of sister chromatids during meiosis in Saccharomyces cerevisiae. *Genetics* 2006; 174: 1745–54.
6 Nath J, Tucker JD, Hando JC. Y chromosome aneuploidy, micronuclei, kinetochores and aging in men. *Chromosoma* 1995; 103: 725–31.
7 Kouznetsova A, Lister L, Nordenskjöld M, Herbert M, Höög C. Bi-orientation of achiasmatic chromosomes in meiosis I oocytes contributes to aneuploidy in mice. *Nat Genet* 2007; 39: 966–8.
8 Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2001; 2: 280–91.
9 Pellestor F, Andréo B, Anahory T, Hamamah S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. *Eur J Med Genet* 2006; 49: 103–16.
10 Uroz L, Rajmil O, Templado C. Premature separation of sister chromatids in human male meiosis. *Hum Reprod* 2008; 23: 982–7.

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