Meiotic abnormalities in metaphase I human spermatocytes from infertile males: frequencies, chromosomes involved, and relationship with polymorphic karyotype and seminal parameters

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The aim of this study was to look in depth at the relationship between meiotic anomalies and male infertility, such as the determination of the chromosomes involved or the correlation with patient features. For this purpose, a total of 31 testicular tissue samples from individuals consulting for fertility problems were analyzed. Metaphase I cells were evaluated using a sequential methodology combining Leishman stained procedures and multiplex fluorescence in situ hybridization protocols. The number of chromosomal units and chiasmata count per bivalent were established and a hierarchical cluster analysis of the individuals was performed. The relationship of the seminogram and the karyotype over recombination were evaluated using Poisson regression models. Results obtained in this study show a significant percentage of infertile individuals with altered meiotic behavior, mostly specified as a reduction in chiasmata count in medium and large chromosomes, the presence of univalents, and the observation of tetraploid metaphases. Moreover, the number and the type of anomalies were found to be different between cells of the same individual, suggesting the coexistence of cell lines with normal meiotic behavior and cell lines with abnormalities. In addition, chromosomal abnormalities in metaphase I are significantly associated with oligozoospermia and/or polymorphic karyotype variants.

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INTRODUCTION

On an average, 6%–8% of infertile individuals of normal somatic karyotype present meiotic abnormalities. This percentage reaches 17.6% in patients with severe oligoastenozoospermia. These anomalies occur in cells with normal karyotype and arise from aberrant meiotic recombination, understood as a change in the number or location of chiasmata (reviewed by Egozcue et al.1). To describe these anomalies, the terms asynapsis and desynapsis have been used. Asynapsis is characterized by abnormal pairing of chromosomes together with the absence of the sex vesicle from early prophase, anomalies in synaptonemal complexes, and a great reduction in the number of chiasmata in metaphase I. Desynapsis consists of an apparently normal pairing of chromosomes in prophase I with sex vesicle presence, but with anomalies in synaptonemal complexes and a low chiasmata count in metaphase I. Synaptic abnormalities may affect one, several, or most of the bivalents, and can be displayed as asynaptic bivalents or partially asynaptic bivalents. These anomalies can involve all the cells analyzed or coexist with normal pairing cells (reviewed by Egozcue et al.1).

Meiosis is highly regulated and errors in some of its stages activate meiotic checkpoints. The pachytene checkpoint prevents cells from overcoming this step if asynapsis occurs. Some studies describe defects in the recombination and/or homologous chromosome synopsis associated with a spermatogenic arrest at this stage. The spindle assembly checkpoint regulates the transition from metaphase I to anaphase I, and acts by retaining cells at the stage of metaphase I until all bivalents are properly orientated on the spindle.12

The combination of anomalies in the process of pairing, synapsis, recombination and/or chromosome segregation, and failures in the control mechanisms leads to the presence of meiotic anomalies. These can be cytogenetically displayed in two nonexclusive ways: aberrant recombination, understood as a change in the number or location of chiasmata, or spermatocytes with numerical abnormalities. The 14% of infertile individuals show significant increases of aneuploid and/or diploid spermatocytes. Another consequence of the activation of checkpoints is the reduction in the number of cells between the beginning and the end of the spermatogenesis. It is therefore not surprising that infertile individuals with meiotic anomalies often present oligozoospermia of varying severity. The relationship between the presence of synaptic and recombination abnormalities and the arrest of the spermatogenic process and/or low sperm counts has been described by different authors.5,14
Therefore, studies of the progression of spermatogenesis in patients with fertility problems have been of interest both in the field of research and clinical diagnosis. Several cytogenetic techniques have been implemented to study testicular tissue samples in order to assess meiotic abnormalities at different stages of the process. Essentially, these techniques are addressed to the study of the synaptonemal complexes and to the analysis of the meiotic chromosomes (reviewed by Egozcue et al.3).

Studies of meiotic chromosomes using cytogenetic protocols allow: the analysis of cells in different spermatogenic stages; to assess meiotic arrest; to evaluate the presence of the XY body in prophase I; to analyze meiotic figures in diakinesis/metaphase I and chromosomes in metaphase II; and to determine the number and location of chiasmata to characterize the recombination process. These studies are mainly indicated in couples with recurrent miscarriages, failed in vitro fertilization–intracytoplasmic sperm injection cycles and/or oligozoospermic individuals.3

However, sample features often result in limitations in the study, either because of the small amount of material available, the few cells under division observed, or, in the case of partial arrest during prophase I, the small number of metaphase I and II spermatocytes. In addition, the meiotic chromosome features in metaphase I make difficult the identification of synaptic and desynaptic bivalents. More recent data based on the application of multiplex fluorescence in situ hybridization techniques (M-FISH), which allows the identification of all chromosomes simultaneously, overcomes some of the limitations associated with classical meiotic cytogenetic studies. Through this technique it is possible to simultaneously analyze all chromosomes in metaphase I and thus identify the chromosomes involved in abnormal meiotic behaviors.15

The aim of this study was to determine the incidence of meiotic chromosomal abnormalities in spermatocytes at metaphase I of a series of infertile individuals. This would open the possibility of studying the frequency of these abnormalities and especially to determine if they affect repetitively the same chromosomes or, on the contrary, occur at random. In addition, data from seminal parameters and somatic karyotype were used to evaluate the correlation between these parameters and the result of the meiotic study.

MATERIALS AND METHODS

Biological samples

A total of 31 testicular tissue samples from individuals consulting for fertility problems were analyzed. Samples were obtained under local anesthesia and kept in an isotonic solution at 4°C until its utilization for not more than 24 h. Somatic karyotype and seminal parameters are specified in Table 1. Protocols were approved by our Institutional Ethics Committee, and the patients gave their informed consent with regard to participation in the study.

Testicular biopsy samples were incubated in a hypotonic solution (KCl 0.075 mol l⁻¹) at 37°C and then mechanically disaggregated. The cell suspensions were fixed using methanol: acetic acid (3:1) and dropped onto dry slides. Meiotic chromosome preparations were kept at −20°C until chromosome analysis.

Meiotic study

Metaphase I cells were analyzed following a sequential methodology previously described.13 This methodology combines Leishman staining procedures and M-FISH protocols.

In brief, metaphase I spermatocytes stained by Leishman (PanReac AppliChem, Castellar del Vallès, Spain) were evaluated using an Olympus BX60 microscope (Olympus Optical España S.A., Barcelona, Spain) equipped with the capture and image analysis system CytoVision 3.6. (Applied Imaging, Newcastle, UK). The numbers of chromosomal units per metaphase I and chiasmata count per bivalent were established (Figure 1). The absence of chiasmata was reflected with the observation of univalents. For each metaphase, coordinates were noted to facilitate the location and analysis after the M-FISH protocol.

Before the application of the M-FISH protocol (Spectra Vysion Assay Protocol, Vysis Inc., Downers Grove, IL, USA), the slides were

Table 1: Somatic karyotype and sperm parameters of the individuals analyzed

| Patient code | Somatic karyotype | Sperm parameters |
|--------------|------------------|-----------------|
| 118          | 46, XY           | OA              |
| 140          | 46, XY           | AT              |
| 142          | 46, XY           | AT              |
| 284          | 46, XY           | Az              |
| 287          | 46, XYqh+        | N               |
| 289          | 46, XY           | A               |
| 291          | 46, XY           | AT              |
| 299          | 46, XY, inv9 (p11q12) | N |
| 301          | 46, XY           | A               |
| 302          | 46, XY           | N               |
| 307          | 46, XY           | A               |
| 308          | 46, XY           | OAT             |
| 309          | 46, XY           | OAT             |
| 310          | 46, XY           | OA              |
| 312          | Non evaluated    | Az              |
| 314          | 46, XY           | N               |
| 315          | 46, XYqh+, inv9 (p12q12) | Az |
| 321          | 46, XY           | N               |
| 328          | 46, XY           | OAT             |
| 331          | 46, XY           | OAT             |
| 360          | 46, XY           | OAT             |
| 361          | 46, XY           | OAT             |
| 392          | 46, XY           | OAT             |
| 6837         | 46, XY           | OAT             |
| 6854         | 46, XY           | OAT             |
| 6858         | 46, XY           | OAT             |
| 6859         | 46, XY           | OAT             |
| 6866         | 46, XY           | OAT             |
| 6867         | 46, XY           | OAT             |
| 8345         | 46, XY           | OAT             |
| 8514         | 46, XY           | OAT             |

*World Health Organization criteria (1999). A: asthenozoospermia; AT: azoospermia; OAT: oligoasthenoteratozoospermia; OA: oligoasthenozoospermia; OAT: oligoasthenoteratozoospermia; T: teratozoospermia

Figure 1: Bivalents with different number of chiasmata (arrowheads). (a) Six chiasmata; (b) five chiasmata; (c) four chiasmata; (d) three chiasmata; (e) two chiasmata; (f) one chiasma.
destained in an ethanol solution series in distilled water (70%, 80%, and 90% ethanol). Hybridized slide analysis was performed with an Olympus BX60 epifluorescence microscope equipped with a specific filter set for spectrum aqua, spectrum fred, spectrum green, spectrum gold, spectrum red, and 4',6-diamidino-2-phenylindole. Capture and image analyses were carried out with a CytoVision system (CytoVision 3.6, Applied Imaging, Newcastle, UK). To identify to which chromosome belonged each chromosomal unit, a conjoint analysis of Leishman staining and M-FISH images of the same metaphase I were done. This information was used to ascertain the specific chiasmata count to each bivalent.

**Statistical analysis**

The mean, mode, standard deviation, and range of the number of chiasmata were calculated for each bivalent. Chiasmata mean value/individual was established by adding the 23 mean values obtained for each bivalent in every single patient.

In order to identify patients with similar characteristics regarding chiasmata count, a hierarchical cluster analysis was performed. The variables used for this analysis were mean chiasmata count per each individual. These values were standardized and the Ward method was used to calculate distances. Proximity relationships between individuals are represented in a dendrogram that is a graphical representation of relative distances between individuals. Poisson regression models were established in order to analyze and quantify the differences between clusters.

To analyze whether certain karyotype or seminal parameter features affect chiasmata count, the response variable "chiasmata count" was evaluated according to these explanatory variables. Two categories were defined by karyotype: "46, XY" and "46, XY (polymorphism)" and three categories by seminal parameters: "normal," "abnormal non-oligoasthenoteratozoospermia (OAT)," and "abnormal OAT." Taking into consideration these items, all individuals were reclassified. Given the nature of the response variable "chiasmata count" (a count) it was necessary to establish Poisson regression models. For the analysis, repeated measurements were considered (different cells in each patient and 23 bivalents in each metaphase I were measured). Moreover, the problem of under-dispersion was corrected (variability was lower than the mean value because chiasmata count was always between 0 and 6).

Taking into consideration each variable separately, a model for all individuals was established. Furthermore, a model for each cluster concerning the karyotype or seminal parameters was also defined. Accordingly, the expected values of chiasmata counts/metaphase in each response variable category were calculated and the differences between them were quantified and analyzed. The quantification of these differences was obtained from the relative risk (RR) calculated from the model results (probability to show more or less chiasmata from one category to another).

Software used for the statistical analysis was SPSS v15.0.1.1 (SPSS Inc., Chicago, IL, USA), SAS v9.1 (SAS Institute Inc., Cary, NC, USA) and SPAD v4.5 (Centre International de Statistiques et d’Informatique Appliquées, Saint Mandé, France). Statistical significance was established to 0.05.

**RESULTS**

A total of 481 metaphases I were evaluated (mean: 15.5 ± 12.5; range: 1–52). All chromosomal units were identified in 85.7% (412/481) of the cells analyzed, while in those remaining some chromosomal units were not informative (14.3%; 69/481).

From the 412 metaphases I with all chromosomal units identified, 67.7% (279/412) showed all chromosomes paired forming bivalents. A percentage of 21.8% (90/412) showed 22 autosomic bivalents and the X and Y chromosomes as univalents. The remaining included metaphases classified as a hypoploidy (6.8%), as a tetraploidy (1.5%), or metaphases with totally achiasmatic bivalents (1.7%) (Supplemental Table 1).

A total of 8421 bivalents were evaluated. The mean, mode, maximum, minimum, and standard deviation of chiasmata count for each bivalent and each patient are detailed in Supplemental Table 2. In table, a zero as a minimum count of chiasmata indicates that univalents were observed in any of the metaphases evaluated.

Chiasmata mean value per metaphase and per individual was established by adding mean data obtained for each bivalent (Supplemental Table 2). From the analysis of the 8421 bivalents identified, it was possible to establish the mean value of 50.3 chiasmata per metaphase (Supplemental Table 2).

Cluster analysis was based on mean chiasmata counts per bivalent for each individual. Patient 361 was excluded from the cluster analysis because of the extremely low chiasmata count (a total of 11 chiasmata). With this adjustment, relative distances between individuals became more evident. Individuals were grouped in the dendrogram into two distinct clusters: A and B (Figure 2). Cluster A, comprising 16 individuals, presented a mean value of 53.1 chiasmata per metaphase (range between 50.2 and 55.9) and Cluster B, comprising 14 individuals, showed 47.9 chiasmata (range between 43.1 and 50.5) (Figure 2).

In cluster A, mean chiasmata count from more than half of the bivalents (14/23) was higher than mean values obtained from all individuals (Figure 3). The same bivalents showed significantly lower mean chiasmata counts in cluster B. Most of these bivalents were formed by medium and large chromosomes.

Poisson regression models were established in order to analyze and quantify the differences between clusters A and B. Differences
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were statistically significant (F = 102.61; P < 0.0001). Expected values of chiasmata counts per metaphase were 50.1 chiasmata in cluster A and 45.5 chiasmata in cluster B. From this estimation, it was possible to establish that the chiasmata count in cluster A was 10% higher than in cluster B (RR: 1.102; P < 0.0001).

Regarding the modeling according to karyotype and seminal parameters, the differences between the categories of each variable were significant for both karyotype (F = 14.92, P = 0.0001) and seminal parameters (F = 9.50, P < 0.0001). Similarly, the expected values of chiasmata counts per metaphase were also calculated. The expected values of chiasmata counts in "46, XY" category were 48.4 chiasmata and 45.2 chiasmata in "46, XY (polymorphism)" category. Regarding the seminal parameters, a total of 49.8 chiasmata were expected in the "normal" category, 48.3 chiasmata in the category of "abnormal non-OAT," and 46.7 chiasmata in the "abnormal OAT" category. From the estimated values, it was possible to establish that, on average, it would be expected to find 7% fewer chiasmata in the category "46, XY (polymorphism)" than in the category "46, XY" (RR: 1.070, P = 0.0001). Moreover, analyzing the different categories of seminal parameters, it was established that it would be expected to find 7% fewer chiasmata in the category "abnormal OAT" than in the category "normal" (RR: 1.067, P = 0.0002), and 3% fewer chiasmata in the category "abnormal OAT" than in the category "abnormal non-OAT" (RR: 1.034, P = 0.0082). There were no significant differences in expected values of chiasmata between "normal" and "abnormal non-OAT" categories (RR: 0.969, P = 0.1254).

DISCUSSION
Metaphase features

Most of the cells showed the expected 23 chromosomal units. However, in a significant percentage of metaphases, different types of abnormalities were observed. The presence of univalents was the abnormality most frequently observed. This chromosomal separation could affect one, two, or all bivalents of the same metaphase. These two situations correspond to the classical categories of "desynapsis of individual bivalents" and "complete desynapsis." Several studies suggest that defects in the processes of pairing, synopsis, and/or recombination of homologous chromosomes during prophase I would lead to achiasmatic chromosomes. Small autosomic chromosomes (F and G group) and sex chromosomes often show one chiasmata. Therefore, it is expected that changes that negatively affect synopsis and/or recombination processes generate a greater presence of univalents of chromosomes belonging to these groups. This meiotic behavior is also consistent with the sperm FISH studies that described higher aneuploidy rates in sex chromosomes and G group chromosomes than in other chromosomes.

It is important to note that numerical analysis of metaphases only shows the variations in the number of chiasmata involving chromosomal separation. However, it is clear that reductions do not exclusively affect bivalents formed by small or sex chromosomes. In medium and large chromosomes, factors that negatively affect the formation of chiasmata are mainly translated into a reduction in exchanges number. In this sense, the present study reinforces this behavior showing chromosomal separation mainly manifested in small or sex chromosomes and variations in the number of chiasmata in bivalents formed by medium and large chromosomes (Figure 3).

A significant percentage of tetraploid metaphase I were also identified. Other authors also reported the presence of tetraploid spermatocytes, both at the pachytene stage and at metaphase I. These results support the existence of tetraploid meiotic cells and demonstrate that human spermatocytes, although presenting double the number of chromosomes, can proceed along synopsis and recombination.

In some metaphases, the lack of bivalents or chromosomes (usually the Y-chromosome) was observed. The fact that no single metaphase I showed an extra bivalent or chromosome (complementary products) suggests that most of these reductions could be artifactual and due to the methodology used to obtain the meiotic cell spreads. In fact, this is a well-known and assumed consequence of the "air-drying method," although it is still recognized as the best procedure to obtain good quality cytogenetic preparations.

Figure 3: Summary of the mean number of chiasmata/bivalent. *Statistically significant differences between the mean population value and mean value in cluster A and B (F = 102.61; P < 0.0001). BV: bivalent.
Abnormality features: variability and chromosomes involved

In this study, the number of chiasmata showed remarkable ranges in both intra-individual (11–61 chiasmata) and inter-individual (43.1–55.9 chiasmata) comparisons. Published studies also showed significant differences in chiasma counts either in infertile patients or in fertile/control subjects (Table 2). This variability evidences the presence in all patients of a proportion of abnormal and normal metaphase I suggesting that the processes leading to anomalies do not uniformly affect all cells.

Inter-individual variability is higher in infertile patients than in fertile individuals/controls (Table 2). These differences indicate that the infertile population is more heterogeneous regarding chiasma counts. In fact, this result is not surprising considering the high variety of etiologies that are associated with infertility.11,32

In this study, hierarchical cluster analysis of the infertile population resulted in the separation of this population into two clusters. The mean value of the chiasma count in cluster A was higher than most of the population mean values of fertile or infertile/control individuals described in the literature. In contrast, mean chiasma count in cluster B was lower (Table 2 and Figure 3). These results indicate that mean values described in previously published studies could be the result of grouping patients with different characteristics regarding the number of chiasmata.

From the comparison between cluster results in our study and previously published data, it was possible to consider that cluster A includes individuals with chiasma counts close to normality. In contrast, it was considered that cluster B, presenting mean chiasma counts lower than the published data, showed a significant reduction in the number of chiasmata. As would be expected, the variability between individuals was higher in cluster B. Reinforcing this interpretation, and from the results of clusters modeling, it would be expected to find 10% more chiasmata in cluster A than in cluster B. In addition, the mean expected value of chiasma in cluster A was higher than the mean of cluster B. In summary, it was possible to conclude that 48.4% of infertile individuals analyzed in this study (15/31, 14 individuals of cluster B and the individual 361) showed an abnormal meiotic behavior.

This reduction in the number of chiasmata in cluster B was due to the presence of lower chiasmata counts in bivalents of medium and large chromosomes (Figure 3). In contrast, there were no differences in bivalents of small chromosomes or sex chromosomes. These results could be interpreted as that the reduction mainly affects the medium and large chromosomes, and is random in the others. However, it should be considered that:

1. Medium and large size chromosomes, probably due to the presence of a great number of chiasmata,22 are rarely observed as univalents.
2. Univalency is more frequent in small chromosomes and the XY pair.
3. Synaptic and recombination abnormalities in pachytene and misaligned chromosomes in the metaphase plate have been linked to meiotic checkpoints activation and cell elimination.

Therefore, it is possible that the activation of meiotic checkpoints eliminates cells with univalents (preferentially constituted from small chromosomes and the XY pair). This selective cell elimination would reduce the incidence of cells with univalents in the cluster B achieving levels that could be interpreted as “basal” (similar to cluster A). This reasoning would explain the absence of differences in the number of chiasma in bivalents of small chromosomes or sex chromosomes between clusters.

However, several authors described that the reduction in the number of chiasmata in infertile patients affects mainly small bivalents or bivalents formed by sex chromosomes (reviewed by Egozcue et al.). In these bivalents, the reduction in the number of chiasmata is often associated with the presence of univalents, and therefore with a variation in the number of chromosomal units. This numerical alteration is an observation that is most evident and probably for this reason is most frequently described in the literature.

In relation to sex chromosome pairing, pachytene stage studies show that the higher the recombination rate of the individual, the larger number of XY pairs with one crossover point observed.33 Therefore, Codina-Pascual proposed that the recombination in the XY pair could be an indicator of the recombination degree in the spermatocyte, and therefore also of the individual.

Reported results of meiotic studies at metaphase I are more controversial. While some authors also describe the relationship between chiasma count at metaphase I and the presence of unpaired sex chromosomes,34,35 other studies do not show any relationship.22 In the series of 31 individuals evaluated in this study, no relationship was observed between these two parameters. Although cluster B showed a reduction in the number of chiasmata, the sex chromosomes showed no differences between clusters.

Table 2: Series of infertile and fertile/control males in which chiasma counts were analyzed

| Infertile male series | Number of individuals | Chiasma mean per series | Chiasma per cell (range) | Chiasma mean per individual (range) |
|-----------------------|-----------------------|------------------------|-------------------------|------------------------------------|
| McDermott 197327       | 15                    | 54.4                   | 45-62                   | 52.2-57.0                          |
| Skakkebaek et al. 197331 | 16                  | 48.7*                  | 39-64*                  | 42.5-55.0*                         |
| Chandy et al. 197628   | 87                    | 48.9*                  | -                       | 39.3-56.3*                         |
| Lamont et al. 198129   | 10                    | 49.0                   | -                       | 45.5-53.1                          |
| Laurie and Hultén 198532 | 7                   | 51.3*                  | 40-60*                  | 49.6-53.7*                         |
| Individuals in the present study | 31 | 50.3                  | 11-61                   | 43.1-55.9                          |

| Fertile/control male series | Number of individuals | Chiasma mean per series | Chiasma per cell (range) | Chiasma mean per individual (range) |
|-----------------------------|-----------------------|------------------------|-------------------------|------------------------------------|
| McDermott 197327            | 36                    | 52.9                   | 43-62                   | 47.2-55.5                          |
| Skakkebaek et al. 197331    | 6                     | 51.2*                  | 43-57*                  | 49.0-54.6*                         |
| Lamont et al. 198129        | 16                    | 48.5                   | -                       | 42.6-53.2                          |
| Uroz et al. 201133          | 17                    | 50.3                   | -                       | 47.4-53.4                          |

*Number of chiasmata evaluated for autosomal bivalents only

Relationship with descriptive variables

Concerning the relationship with seminal parameters, OAT individuals showed lower chiasma counts than individuals with normal seminal parameters or individuals with abnormal non-OAT seminal parameters. This result is consistent with the results described in several published studies. The relationship between the presence of synaptic and recombination abnormalities, arrest in the spermatogenetic process, and low sperm counts, has been described by several authors.4-6,13 This relationship could be explained by the activation of checkpoints in pachytene and metaphase I/anaphase I, which would block and remove cells with meiotic abnormalities.35 Depending on the severity of the affection and the effectiveness of control mechanisms, a total or partial arrest of spermatogenesis could occur resulting in azoospermia or a more or less severe oligozoospermia.

Regarding the influence of the karyotype on the chiasma count, individuals with polymorphisms analyzed in this study presented a
reduction in the number of chiasmata/metaphase compared to normal somatic karyotype individuals.

The most common chromosomal polymorphism in humans is the pericentric inversion of chromosome nine (9q13). Different studies relate the pericentric inversion of chromosome 9 with reproductive problems.30–32 Another polymorphism that is considered a variant of normal somatic karyotype are those affecting heterochromatic regions of chromosomes 1, 9, 16, and Y. The incidence of these polymorphisms in infertile men is higher than in the general population.33–35 Studies in synaptonemal complexes of these individuals show that the polymorphic heterochromatic region present more synaptic anomalies than the non-polymorphic equivalent regions.36

Both the presence of inversions and the presence of large heterochromatic regions can hinder meiotic progression, and lead to the formation of asynaptic regions during the first meiotic division.44 These regions may interfere in the pairing and segregation of other unpaired segments, a phenomenon that is called the inter-chromosomal effect.45 This phenomenon is usually associated with carriers of structural chromosomal abnormalities, but also with carriers of polymorphic variants.

Although the number of individuals with polymorphic karyotype analyzed in this study is low and do not include all polymorphic variants, our results suggest the presence of inter-chromosomal effects in individuals with a polymorphic karyotype, which is manifested by a reduction in the number of chiasmata. This reduction indicates anomalies in pairing and synaptic processes that could lead to the activation of control mechanisms. These would act by blocking and eliminating cells with abnormalities, which might affect the number of resulting spermatozoa.

CONCLUSION
Infertile individuals show a considerable percentage of meiotic abnormalities in metaphase I indicating anomalous meiotic behavior. The reduction in the chiasmata number, the presence of univalents, and the observation of tetraploid metaphases, are the abnormalities most frequently observed. Chiasmata number reduction mainly affects medium- and large-sized chromosomes. Moreover, the number and the type of abnormalities are different between cells of the same individual suggesting the coexistence of cell lines with normal meiotic behavior and cell lines with abnormalities. In addition, chromosomal abnormalities in metaphase I are significantly associated with oligozoospermic individuals and/or patients with variant polymorphisms in their karyotype.

AUTHOR CONTRIBUTIONS
ZS was involved in experimental procedures, data collection and assembly; ZS, FV and JB were involved in data analysis and interpretation, manuscript writing and final approval of the manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the Asian Journal of Andrology website.

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