

CASE REPORT

THE LARGEST PARACENTRIC INVERSION, THE HIGHEST RATE OF RECOMBINANT SPERMATOZOA. CASE REPORT: 46,XY, inv(2)(q21.2q37.3) AND LITERATURE REVIEWYapan CC^{1,*}, Beyazyurek C¹, Ekmekci CG¹, Kahraman S²

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ABSTRACT

Carriers of inversions involving euchromatic regions are at risk of having unbalanced offspring due to meiotic crossover. In carriers, recombination can occur during gametogenesis and cause genetically unbalanced sperm and subsequently unbalanced embryos. Here we present segregation analysis results of an infertile male with 46,XY,inv(2)(q21.2q37.3) using fluorescent *in situ* hybridization (FISH) on sperm cells. This is the largest paracentric inversion (PAI) reported so far in a meiotic segregation analysis study. Sperm FISH revealed 28.0% recombinant spermatozoa rate for chromosome 2, which was the highest rate in PAI carriers in the literature. Our results indicate a clear correlation between the size of the inverted segment and the frequency of the recombinant spermatozoa. The results of the FISH analysis with the information of unbalanced spermatozoa rate can provide accurate counseling on the genetic risk of infertility.

Keywords: Chromosome 2; Male infertility; Paracentric inversion(s) (PAIs); Recombinant spermatozoa; Sperm; Fluorescent *in situ* hybridization (FISH)

INTRODUCTION

Balanced paracentric inversions (PAIs) are structural chromosomal rearrangements that are formed on a chromosome arm involving two breaks and rejoining of the chromosome segment after 180° rotation. In humans, the incidence of PAI ranges from 0.1-0.5% [1,2].

During meiosis, if a crossover occurs within the inversion loop, four segregational products are formed [3,4] (Figure 1). Gametes containing unbalanced chromosomes very rarely give rise to a viable zygote. However, several cases of viable recombinant offspring have been reported [5-7]. Furthermore, the relationship between infertility and PAIs reveals an association with recurrent miscarriage and infertility problems among PAI carriers [8,9], indicating the real recombination frequency in PAI must be higher than suspected from liveborn data [10]. Chromosomal analysis of gametes helps to clear up this controversial issue and provide detailed information about the percentage of viable recombinants.

Ten male carriers of PAI have been investigated to date and the frequency of recombinant spermatozoa varied from 0.0 to 12.6% [10-16]. The present case is the second with the meiotic segregation analysis performed for chromosome 2 in a male PAI carrier. In this report, we aimed to evaluate the rate of recombinant spermatozoa in a PAI carrier [46,XY,inv(2)(q21.2q37.3)] using a fluorescent *in*

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situ hybridization (FISH) technique. Furthermore, the impact of this PAI was also assessed on aneuploidies of other chromosomes to identify any possibility of interchromosomal effect (ICE) and consequences on fertility.

MATERIALS AND METHODS

Patients. A 43-year-old man and his 42-year-old wife were referred to the Istanbul Memorial Hospital In Vitro Fertilization (IVF) and Reproductive Genetics Center, Istanbul, Turkey, with primary infertility and a history of repeated implantation failures in their nine IVF attempts. Cytogenetic analysis reports indicated 46,XY,inv(2) (q21.2q37.3) (Figure 2) and 46,XX, for the man and woman, respectively. A sperm-FISH evaluation was proposed to determine the meiotic segregation profiles of the PAI.

Sperm Fluorescent *In Situ* Hybridization Analysis. Sperm samples were collected in a sterile container after 3 days of sexual abstinence. All procedures including fixation, pretreatment, probe

application, hybridization, and washing steps were carried out as described previously [17]. Hybridization was performed with two specific telomeric and centromeric probe mixtures. The first mixture included Telomeric (Tel) 2p (Spectrum Green), Tel 2q (Spectrum Orange) and Centromeric (Cen) 18 (Spectrum Aqua) as a control probe. The second mixture included Tel 2p (Spectrum Green), Cen 2 (Spectrum Orange) and Cen 18 (Spectrum Aqua) (Abbott Molecular, Abbott Park, North Chicago, IL, USA). In order to evaluate aneuploidies of other chromosomes or any possible ICE, probes specific for chromosomes 13, 15, 17, 18, 21, X and Y were also included in the study. The 13, 18, 21, X and Y probes were in our routine aneuploidy panel. Probes specific for chromosomes 15 and 17 were selected randomly in order to increase the number of chromosomes. Analysis criteria that have been described elsewhere [18] was followed.

***In Vitro* Fertilization Study.** The couple underwent IVF treatment at the IVF and Reproductive Genetics Center at the Memorial Hospital, Istanbul,

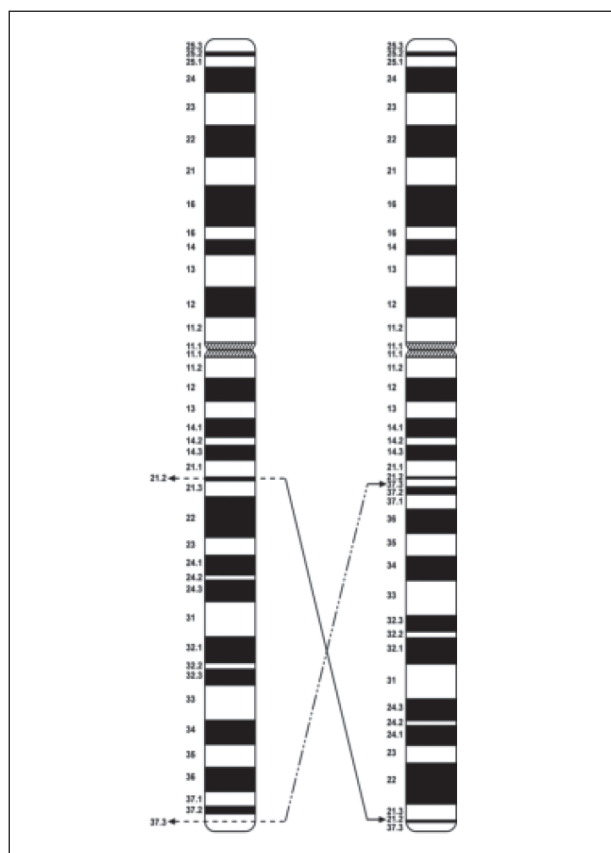


Figure 2. Paracentric inversion showing the breakpoints on chromosome 2.

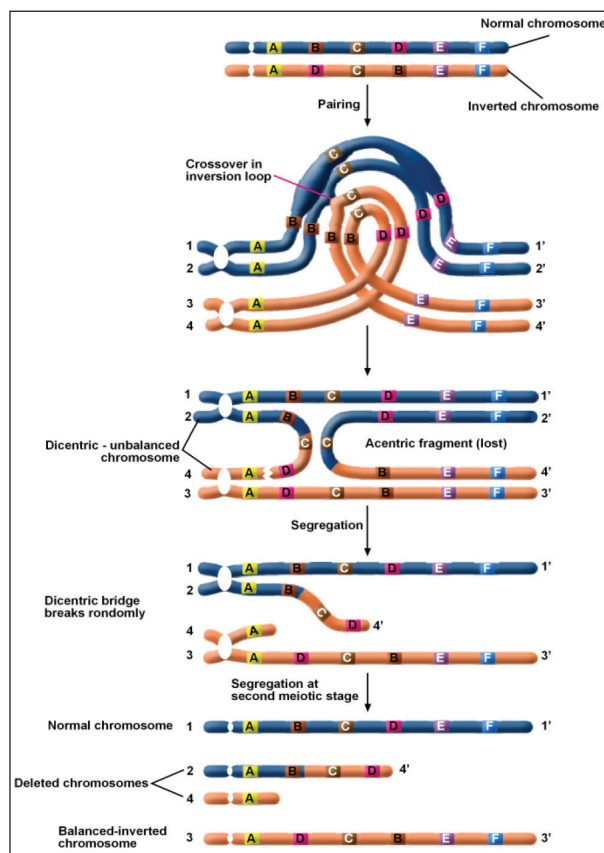


Figure 1. Meiotic segregation products of paracentric inversion.

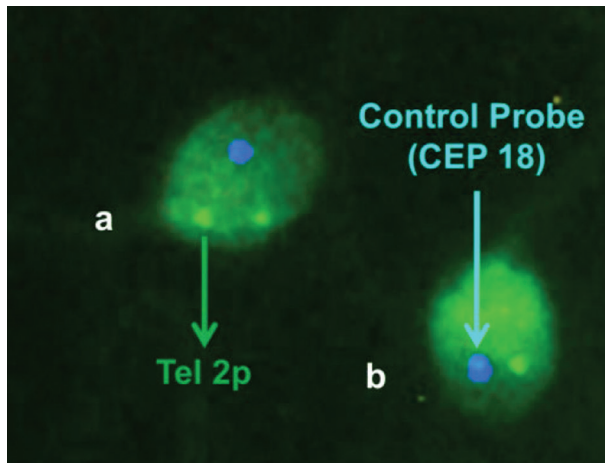


Figure 3. Images of spermatozoa with dicentric and deleted products for chromosome 2 with two green/no orange signal (a) and one green/no orange signal (b) for telomeric 2p and 2q, respectively.

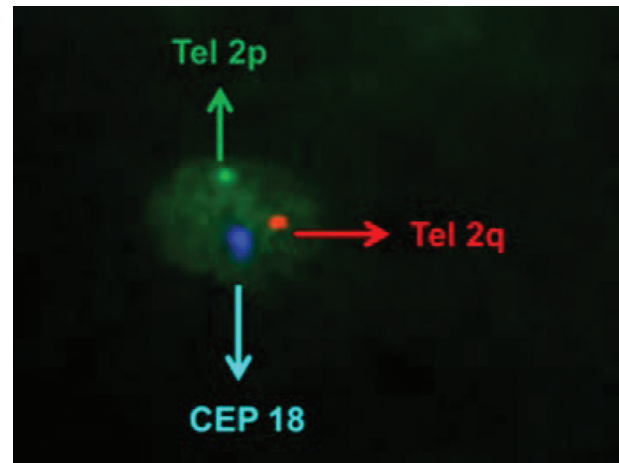


Figure 4. Images of a normal or inverted spermatozoa for chromosome 2 with one signal for telomeric 2p, 2q and the control probe.

Turkey. *In vitro* fertilization, embryological culture conditions have been described previously [19]. Written informed consent was obtained from the couple before they underwent IVF.

RESULTS

Meiotic segregation analysis of the PAI for chromosome 2 revealed that 378 spermatozoa were abnormal (28.0%) (Figure 3). The rate of normal or balanced (inverted) spermatozoa was 72.0% (Figure 4). The aneuploidy rates for chromosomes 13, 15, 17, 18, 21, and XY were 1.2, 0.92, 8.4, 1.2, 0.9 and 1.5%, respectively (Table 1).

As a result of hyperstimulation, five oocytes were picked up, four of them were matured and fertilized after intracytoplasmic sperm injection (ICSI)

[20]. Four zygotes were cultured until day 3. Two of these embryos were arrested and the other two were slow-growing embryos. Unfortunately, embryo biopsy and preimplantation genetic diagnosis (PGD) could not be performed due to poor quality of the embryos. Two grade III embryos were transferred on the third day of embryonic growth without genetic analysis. Pregnancy was not achieved in this cycle.

DISCUSSION

Although PAIs are generally considered to be harmless without phenotypic consequences in carriers [10], recombination can occur during gametogenesis, can cause genetically unbalanced gametes, and subsequently, unbalanced embryos. This could cause repeated IVF failures or repeated pregnancy loss, which is more frequently seen among inversion carriers compared to the normal population. Despite apparently being “balanced,” an inversion might disrupt a critical gene and cause an associated phenotype [21,22]. Furthermore, small cryptic deletions or duplications around breakpoints may have an effect on the phenotype.

In our case, the length of the inverted segment was the largest one reported in other PAI cases published to date. The inverted segment contained almost 43.0% of the whole chromosome and was approximately 103 Mbp in length. Subsequently, sperm-FISH analysis revealed 28.0% of recom-

Table 1. Sperm-FISH results for chromosomes 2, 13, 15, 17, 18, 21, and XY.

| Chromosome | Abnormal (%) |
|------------|--------------|
| 2 | 28.00 |
| 13 | 1.20 |
| 15 | 0.92 |
| 17 | 8.40 |
| 18 | 1.20 |
| 21 | 0.90 |
| XY | 1.50 |
| Diploidy | 1.25 |

Table 2. Results of segregation analyses of paracentric inversions.

| Inversion | Inverted Segment Size | | Number of Spermatozoa | Non Recombinants (%) | | Recombinants % | Duplicated/ Deficient Chromosomes % | Bicentric Chromosomes % | Method | References |
|----------------------|-----------------------|----------------|-----------------------|----------------------|----------|----------------|-------------------------------------|-------------------------|-----------------|------------|
| | Mb | % ^a | | Normal | Inverted | | | | | |
| inv(2)(q21.2q37.3) | 103 | 42.5 | 1350 | 72.0 | – | 28.00 | 24.0 | 4.0 | sperm FISH | this study |
| inv(9)(q21.2q34.13) | 54 | 39.0 | 1608 | 44.7 | 42.7 | 12.60 | 11.3 | 1.3 | breakpoint FISH | 10 |
| inv(5)(q13.3q33.1) | 75 | 41.0 | 4807 | 45.6 | 44.7 | 9.70 | 8.7 | 1.0 | breakpoint FISH | 10 |
| inv(14)(q23.3q32.13) | 30 | 29.0 | 7670 | 49.6 | 46.7 | 3.70 | 3.4 | 0.3 | breakpoint FISH | 10 |
| inv(2)(q14.2q24.3) | 49 | 20.0 | 496 | ? | – | 0.80 | ? | 0.8 | sperm FISH | 14 |
| inv(12)(q15q24.1) | 44 | 33.0 | 1000 | ? | – | 0.50 | ? | 0.5 | sperm FISH | 16 |
| inv(11)(q13.2q14.3) | 27 | 20.0 | 1001 | ? | – | 0.40 | ? | 0.4 | sperm FISH | 16 |
| inv(4)(p14p15.3) | 11 | 5.0 | 8158 | ? | – | 0.03 | ? | ? | sperm FISH | 15 |
| inv(7)(q11q22) | 50 | 32.0 | 94 | 36.0 | 63.0 | 0.00 | 0.0 | 0.3 | sperm karyotype | 11 |
| inv(9)(q32q34.4) | 32 | 24.0 | 282 | ? | – | 0.00 | 0.0 | 0.0 | sperm typing | 12 |
| inv(14)(q24.1q32.1) | 27 | 26.0 | 120 | 42.0 | 58.0 | 0.00 | 0.0 | 0.0 | sperm karyotype | 13 |

^a The percentage of inverted segment size was calculated for the whole chromosome.

binant spermatozoa, which was the highest rate among PAI carriers published in the literature. Our results are in line with the previous observations, supporting a clear correlation between the size of the inverted segment and the frequency of the recombinant spermatozoa (Table 2).

Some previous studies have mentioned that a significant level of unbalanced gametes would require a minimum inversion size of 100 Mbp and minimum segment proportion of 50.0% of the chromosome [23,24] for pericentric inversions (PEIs). Morel *et al.* [24], suggested that significant number of recombinants are produced when the inverted segment size is >50.0% of the total length of the inverted chromosome. For PAIs, however, there is not enough data regarding the factors affecting segregation. Our findings show that a significant rate of unbalanced gametes can be detected even if the inverted segment size was less than 50.0% of the whole chromosome (42.5%).

Recently, Bhatt *et al.* [25], mentioned that the formation of recombinants depends on the presence and number of hot spots (HSs) and the recombination frequency in the particular region. These are called HSs, high recombination rate spot (HRS) and very high recombination rate spot (VHRS). The higher the number of recombination HSs around the breakpoints, within the inverted segment, the

higher the probability of formation of double cross-overs and recombination [25]. Breakpoints were in critical regions for our case. One of them was in or around a HS (2q21.2) and the other was probably in a VHRS (2q37.3). All this knowledge contributes to explain our high recombination rate.

Interchromosomal effect has been investigated in a few previous studies with a maximum of five chromosomes including 13, 18, 21, X and Y and was demonstrated in some studies (*e.g.*, [15]) whilst not in others [12,16]. To the best of our knowledge, this is only the second study (after Anton *et al.* [15]), in which significantly high ICE has been found in a PAI carrier. In this study, an especially high aneuploidy rate for chromosome 17 was found. Inversion loops have been shown to be associated with other chromosome pairs [26]. The high rate of aneuploidy for chromosome 17 found in this study could be attributed to an ICE due to an association of inversion loop with chromosome 17 by a mechanism similar to which Batanian and Hulten [26] proposed.

In male carriers, sperm FISH studies allow counselors to offer patients better reproductive genetic advice prior to a PGD cycle. Information of unbalanced spermatozoa rate can provide tips for counseling on the genetic risk of infertility. A 5.0% technical error rate for FISH applications must be taken into consideration when counseling patients. Although imbal-

ances due to recombination could easily be detected with the FISH technique, small deletions that might occur at or near the inversion breakpoints could not be detected, unless direct sequencing of the breakpoint regions have been performed.

For the couples with an increased rate of unbalanced gametes, the PGD technique offers a healthy pregnancy, eliminating unbalanced or aneuploid embryos from transfer. With the use of more recent techniques such as array-comparative genomic hybridization (a-CGH), it is possible to select the normal or balanced embryos and also exclude ICE by analyzing all 24 chromosomes in one day [27]. However, the embryos of our patient were not suitable for biopsy since they had a poor embryonic development. It is well known that most early losses are associated with chromosomal abnormalities in sperm or egg, and result in arrested embryonic development and/or failed implantation [28]. Such a poor development might be the consequence of the high rates of recombinant gametes, variety of unpredictable unbalanced chromosome products and small microscopic deletions that could have resulted from the PAI. In addition, in our case, multiple crossover events could be likely for this large and susceptible segment to recombination.

Our results support that not every PAI is innocent as they could be the reason of high abnormality rates in sperm that might have been associated with repeated IVF failures and pregnancy losses. To date, the possibility of associations of PAI on chromosome 2 with fertility and sexual developmental problems have been discussed in several reports [14,29-31]. However, how far the impact of PAIs on poor obstetrics history should be further evaluated in order to be demonstrated as the primary cause of the infertility in this couple.

This study demonstrated the importance of sperm FISH evaluation in the genetic counseling and assisted reproductive technology (ART) practices. It also gives further evidence of a possible ICE and a possible impact of additive consequences on fertility in PAI carriers.

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Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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