

Fetal Loss: A Genetic Insight of the De Novo Accessory Bi-Satellited Marker of Chromosome 22P

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Abstract

Supernumerary Marker Chromosomes (SMC) follow non mendelian fashion in their inheritance, and are reported in variety of phenotypes. Although markers that contain satellites/bi-satellite variations of short arms do not confer any phenotypic alterations, it affects the fertility, vigour and interferes at non-disjunction during cell division and proves lethal to foetus. We report a couple wherein wife had Recurrent Early Pregnancy Loss (REPL) due to loss of fetal cardiac activity and husband with oligoasthenospermia. The cytogenetic analysis of the wife showed 46,XX,9qh+ karyotype and that of husband revealed 47,XY,+mar karyotype. Delineation of marker was initiated using NOR (Nucleolar Organizer Region), C-banding conventional techniques in combination with Fluorescence In Situ Hybridization (FISH) using Whole Chromosome (WCP) and Locus Specific Identifier (LSI) probes. Marker was characterized to be of chromosome 22 origin with satellites on either side of the centromere inferring it to be a bi-satellited iso-chromosome 22p with its occurrence as partial tetrasomy. Our study attempts to provide a comprehensive understanding of this cytogenetic rearrangement and its possible consequences in fertility and REPL.

Author Summary

For the successful pregnancy outcome(s), many etiological factors like uterine anomalies, endocrinological factors, chromosomal anomalies etc., play a significant role emphasizing their importance resulting in the healthy offspring/progeny. Any disturbance or ambiguity in these factors results in fetal loss. Among these, chromosomal abnormalities of the genitors and their subsequent transmission contribute to a major extent in affecting the full term pregnancy outcome causing reproductive failure. Here we present a case where in accessory bi-satellited small supernumerary marker of chromosome 22p origin in the oligoasthenospermic (infertile) male partner, results in lethality of fetus due to the errors in meiotic segregation. We discuss its mechanism of iso-chromosome formation and occurrence of partial tetrasomy of chromosome 22p due to four fold dosage of centromeric sequences where in the rest of the chromosomal region of 22q is deleted including Di-George, leading to the absence of cardiac activity in fetus and subsequently miscarriage(s) in the first trimester in the female partner.

Keywords: Miscarriage; Chromosome; Hybridization; Spermatozoa

Introduction

Recurrent Early Pregnancy Loss (REPL), also referred to as recurrent miscarriage or habitual abortion, is defined as 2 or 3 consecutive pregnancy losses in the first trimester of the gestation from the last menstrual period. Many pregnancies fail, prior to being clinically recognized and approximately 15% of all clinically recognized pregnancies result in spontaneous loss. Of all the conceptions, only 30% result in a live birth [1]. From the seminal studies by and , it has been inferred that at least 50% of clinical miscarriages result from chromosomal abnormalities [2-4]. The majority of chromosomal losses result from random numerical chromosome errors, specifically trisomy, monosomy, polyploidy etc. When a structural chromosomal anomaly is observed in one or both the partners, the likelihood of a subsequent healthy live birth depends on the chromosomes involved and the type of rearrangement.

We report a case wherein the couple was cytogenetically investigated for the reason that the female partner had three REPL and the male partner had oligoasthenospermia. The cytogenetic analysis revealed the structural anomalies in both the partners. In this report we describe the clinical conditions of both the partners and the possible mechanism(s) of the respective observed anomalies.

Case

A couple was referred for cytogenetic analysis for the REPL in

female partner – all miscarriages had occurred between 5-8 weeks of gestation, due to the absence of cardiac activity in the fetus. The Institutional Review Board of the Centre for Cellular and Molecular Biology, Hyderabad, approved this study. All hormonal profiles are in the normal limits; Male partner noted with marked oligoasthenospermia with sperm count of 6 millions/ml (normal range – 20 millions/ml), 10% active motile forms and 70% non-motile forms along with 70% normal and 30% abnormal morphological forms. As per the pedigree indications there is no family history of consanguineous marriage of the female partner side. The male partner's parents are 2nd generation cousins and also his mother had two initial miscarriages and four successful pregnancies (Figure 1A).

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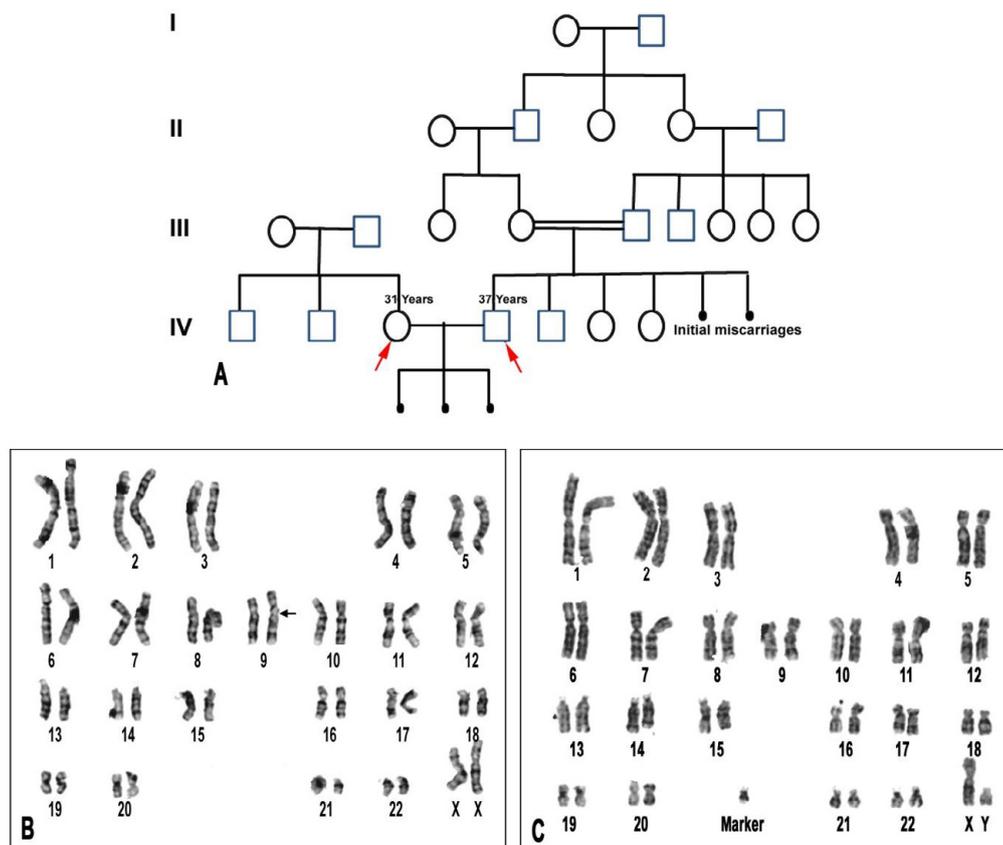


Figure 1 A: The family pedigree chart of the affected couple wherein the parents of the male partner are IInd generation cousins. GTG banding analysis. **B:** The heterochromatin rich region in the long arm of one of the chromosome 9 in the female partner 46, XX, 9qh+ karyotype. **C:** An additional marker chromosome in the male partner 47, XY+mar karyotype.

Materials and Methods

Cytogenetic analysis

Chromosomal preparations from both the partners were obtained from lymphocyte cultures from peripheral venous blood. Peripheral blood samples from the parents of the male proband were not obtained due to their non availability. Following the mandatory procedure, along with the main application the written consent from the couple was enclosed and submitted to the Institutional Ethics Committee for approval. The committee approved the study that can be useful for the future publication and research studies. Phytohaemagglutinin stimulated lymphocyte cultures were set and harvested by standard methods [5]. Standard GTG banding protocol was followed and around 40 karyotypes from each partner were analyzed at 500-band level using Cytovysion analysis platform from Applied Imaging [6].

NOR staining

Specific techniques like NOR staining was performed on the metaphases to observe the satellite regions of all the acrocentric chromosomes and more specifically the marker chromosome. A total of 20 metaphases were analyzed. Cytovysion analysis platform from Applied Imaging.

FISH analysis using WCP probes

In addition FISH was performed using Whole Chromosome

Painting probes for chromosome 21 (WCP21, Spectrum Green, Vysis Inc., USA) and chromosome 22 (WCP22, Spectrum Green, Vysis Inc., USA) according to the manufacturer's instructions.

FISH analysis using LSI probes

FISH procedures were performed for targeted regions like ACRO-p arm (LSI ACRO-p SO) and Di-George (LSI Di-George region dual colour 22q11.2 Spectrum Orange and 22q23 Spectrum Green) from VYSIS Inc., USA, following manufacturing instructions to characterize the marker chromosome in metaphases of male partner. 25 metaphases from each hybridized probe were captured and analyzed using Zeiss Axioscope microscope dual colour filter (Zeiss, Jena, Germany). Image processing and analysis were done using ISIS platform (MetaSystems, Gmbh, Altlussheim, Germany).

Results

Cytogenetic analysis

Initial investigations using GTG banding at 500 band resolution showed an increased heterochromatin in the q arm of one of the chromosome 9 in the female partner with 46,XX,9qh+ karyotype (Figure 1B) and the chromosome complement of the male partner showed an additional marker chromosome, with 47,XY,+mar karyotype (Figure 1C).

FISH analysis with WCP probes

FISH with WCP 21 SG resulted in two green signals on both the

normal chromosomes 21 and no hybridization of chromosome 21 sequences on the marker chromosome, thus rule out the presence of chromosome 21 sequences on marker chromosome (Figure 2A). Whereas hybridization with WCP 22 SG probe showed two green signals on both the normal chromosomes 22 and also an intense green signal at the centromeric region of the marker chromosome. Hence confirming the presence of chromosome 22 sequences on marker chromosome, inferring as the marker chromosome originated partially from the chromosome 22 (Figure 2B). Further it was observed that only the centromeric region of the marker chromosome got hybridized and the

regions on either side of the centromere were left un-hybridized. Hence, these un-hybridized regions are targeted for further characterization.

FISH using LSI probes

FISH with LSI Acro-p arm SO probe Figure 2C showed all independent orange signals at 'p' arm regions of all acrocentric chromosomes and also interestingly two orange signals on marker chromosome on either side of its centromere (indicated with red arrow), thus indicated the bi-satellited appearance of the marker. Further delineation of the marker chromosome with Vysis DiGeorge Region

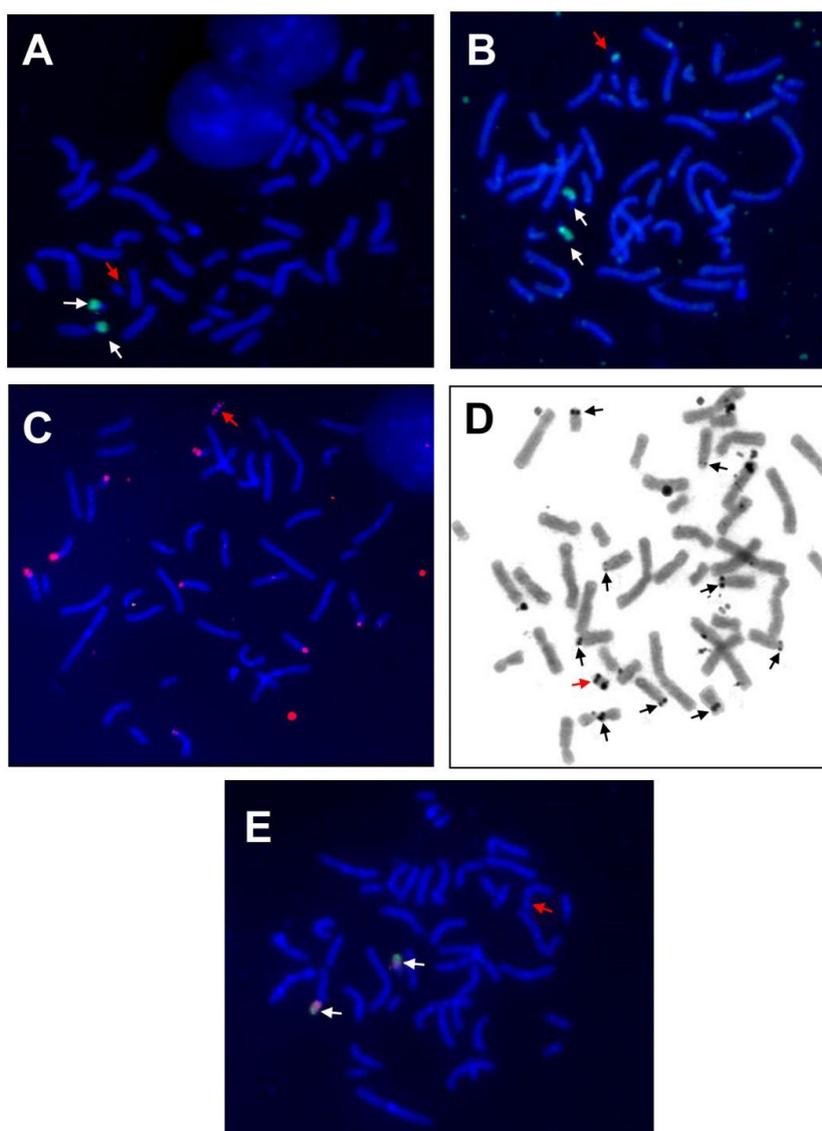


Figure 2A: WCP hybridization analysis: Using chromosome 21 probe showed two independent green signals on both the normal chromosome(s) 21 (white arrows) and no hybridization on marker chromosome (red arrow)
B: Using chromosome 22 probe showed two independent green signals on normal chromosome(s) 22 (white arrows) and also an intense green signal on marker chromosome (red arrow) thus revealed the origin of marker chromosome with sequences of chromosome 22
C: LSI hybridization analysis: Acro-p arm probe showed interestingly two orange signals of Acro-p region on marker chromosome on either side of its centromere (indicated with red arrow)
D: NOR staining showed the dark silver staining on marker chromosome on either side of the centromere (indicated with red arrow). Probe hybridization with Acro-P and NOR staining showed satellite regions on all other acrocentric chromosomes
E: LSI hybridization analysis: LSI Di-George probe showed two control signals (Green) and two LSI Di-George signals (Orange) on normal chromosome(s) 22 (white arrows) and it shows NO signal neither the control signals nor Di-George signals on marker chromosome (red arrow)

Probe - LSI TUPLE 1 Spectrum Orange/LSI ARSA Spectrum Green probe showed the positive orange (Di-George region 22q11.2) signals and green (Control 22q13) signals on both the normal chromosomes 22 and no signal was observed on the marker chromosome. Thus indicating the marker chromosome is negative for Di-George locus with its deletion about 33.3Mb from that of 22q11.2 locus till the entire 'q' arm terminal region (Figure 2E).

NOR staining

Further validation using NOR staining was to observe the regions within the "stalks" of chromosomes 13, 14, 15, 21, and 22 (the acrocentric chromosomes) [7]. Figure 2D showed the darkly stained regions at the 'p' arm regions of all acrocentric chromosomes and also the staining is observed on marker chromosome on either side of the centromere (indicated with red arrow) thereby substantiating the Acro-p FISH observations. Both FISH with Acro-p probe and NOR staining indicated the unique observation with the presence of two satellite regions on the marker chromosome. The chromosome aberration in the male partner is a marker chromosome identified as partial bi-satellited tetrasomy 22. From the above techniques the final karyotype of the male partner noted with the marker chromosome is inferred as 47,XY,+inv dup(22)(q11.1).

Discussion

Presently, a small number of accepted etiologies exist for REPL that include parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, certain uterine anatomic abnormalities and Antiphospholipid Antibody Syndrome (APS). Other probable or possible etiologies include additional endocrine disorders, heritable and/or acquired thrombophilias, immunologic abnormalities, infections, and environmental factors [8].

The term reproductive failure includes the couples with the female partners who have experienced miscarriages and the males diagnosed with infertility [9]. Most of the common causes for recurrent miscarriages have been chromosomal abnormalities of genitors. In approximated 50% of cases, the cause of reproductive failure remains unknown. In a small number of cases, the miscarriage arises from transmission of structurally abnormal chromosomes from the parents. Chromosome abnormalities include aneuploidy and structural abnormalities, which mainly occur during errors in cell division resulting in abnormal segregation of chromosomes. Aneuploidy occurs in both eggs and sperm being the most common chromosomal abnormality with an abnormal number of chromosomes, which refers to an extra or missing chromosome. In humans aneuploidy arises from gametogenesis and results from errors in maternal and paternal chromosome segregation [10]. Structural abnormalities in both eggs and sperm include translocations, inversions, deletions and duplications. Likewise the transmission of a chromosome abnormality to an embryo can result in a low implantation rate, miscarriage, or the birth of a baby with a genetic disorder.

In this study, we report an interesting case wherein the presence of the novel chromosomal abnormality and its influence in reproductive failure has been discussed. A couple was referred for cytogenetic analysis for REPL (3 miscarriages) in female partner – all of them had occurred between 5-8 weeks of gestation, due to the absence of cardiac activity in the fetus. Hormonal profiles in the female partner are in normal range. Male partner diagnosed with marked oligoasthenospermia, showed history of consanguinity in his family. His parents are 2nd generation cousins and also his mother had two initial miscarriages, followed by four successful pregnancies.

Among the couple referred for cytogenetic investigations, the female partner had 46,XX,9qh+ karyotype, inferring the increased presence of heterochromatin region in the long arm of one of the chromosome 9. Heterochromatin polymorphisms are microscopically visible regions on chromosomes 1, 9, 16, the distal two thirds of the long arm of the Y chromosome and the satellites of the acrocentric chromosomes, with no apparent effect on the phenotype. Few previous studies report that heteromorphism of constitutive heterochromatin cause no phenotypic alterations [11]. Studies of indicated no significant difference in the heterochromatic regions between aborting and non-aborting couples [12]. Thereby indicating that 9qh+ polymorphism in the female partner does not play a significant role in REPL. Therefore we further discuss the chromosome component of the male partner 47,XY,+inv dup (22) (q11.1) emphasizing that an additional copy of chromosome 22 which was identified is occurring in partial tetrasomic condition as a bi-satellited chromosome and which is attributed to play crucial role in the occurrence of REPL.

Initially, the chromosome component of male partner showed the presence of marker chromosome. Small Supernumerary Marker Chromosomes (sSMC) are structurally abnormal chromosomes that cannot be identified or characterized by any of the routine cytogenetic banding techniques [13]. sSMC have been found for all chromosomes with different frequencies: about 30% are derived from chromosome 15, 20% from 22, 9% from 12, and only 1% from chromosome 6 [14]. Not much is known about the exact mode of sSMC formation. Specifically, when, why, and how during gametogenesis or embryogenesis an sSMC evolves is still not clear. The ideas for sSMC formation are based partly on the finding that uniparental disomy and sSMC can show up together and on the observation that sSMC can evolve by incomplete trisomic rescue. Overall, an sSMC is formed by the combination of one or more rare events happening during gametogenesis or embryogenesis [15]. In the present study the male partner has a normal phenotype and normal intellectual quotient, correlates with explanation in earlier reports and though sSMCs are largely devoid of deleterious genes, they can adversely affect vigour and fertility [16,17].

The degree of abnormality caused by such markers is related to the marker's size, staining properties, mosaicism and familial occurrence and may also be due to the presence of euchromatin, resulting in partial tetrasomy for a chromosomal segment [18-22]. These studies prompted us to further characterize the marker using FISH with a range of specific fluorescence probes, interpret the origin and occurrence of marker in the male propoita.

The marker chromosome showed satellite-like appearance resembling acrocentric chromosome on either side of the centromere (bi-satellite appearance), and was confirmed by FISH using Acro-p arm probe. Further cross-validated using NOR staining, the conventional silver-stain technique that stained both the satellites on the marker chromosome. The origin of the marker which contained chromosome 22 sequences was confirmed by FISH with the specific probe WCP22, showed strong/intense signal at the centromere region as a bi-satellite appearance- due to the isochromosome formation as also referred to 'inverted duplication' of the short arm of chromosome 22.

An isochromosome is a condition in which one arm is missing and the other duplicated in a mirror-image fashion, consisting of either two short or long arms and resulting in unbalanced chromosome constitution [23]. Duplication of a chromosome segment usually occurs by unequal crossing over between homologous chromosomes or sister chromatids. Duplications can also result from abnormal meiotic segregation in a translocation or meiotic crossing over

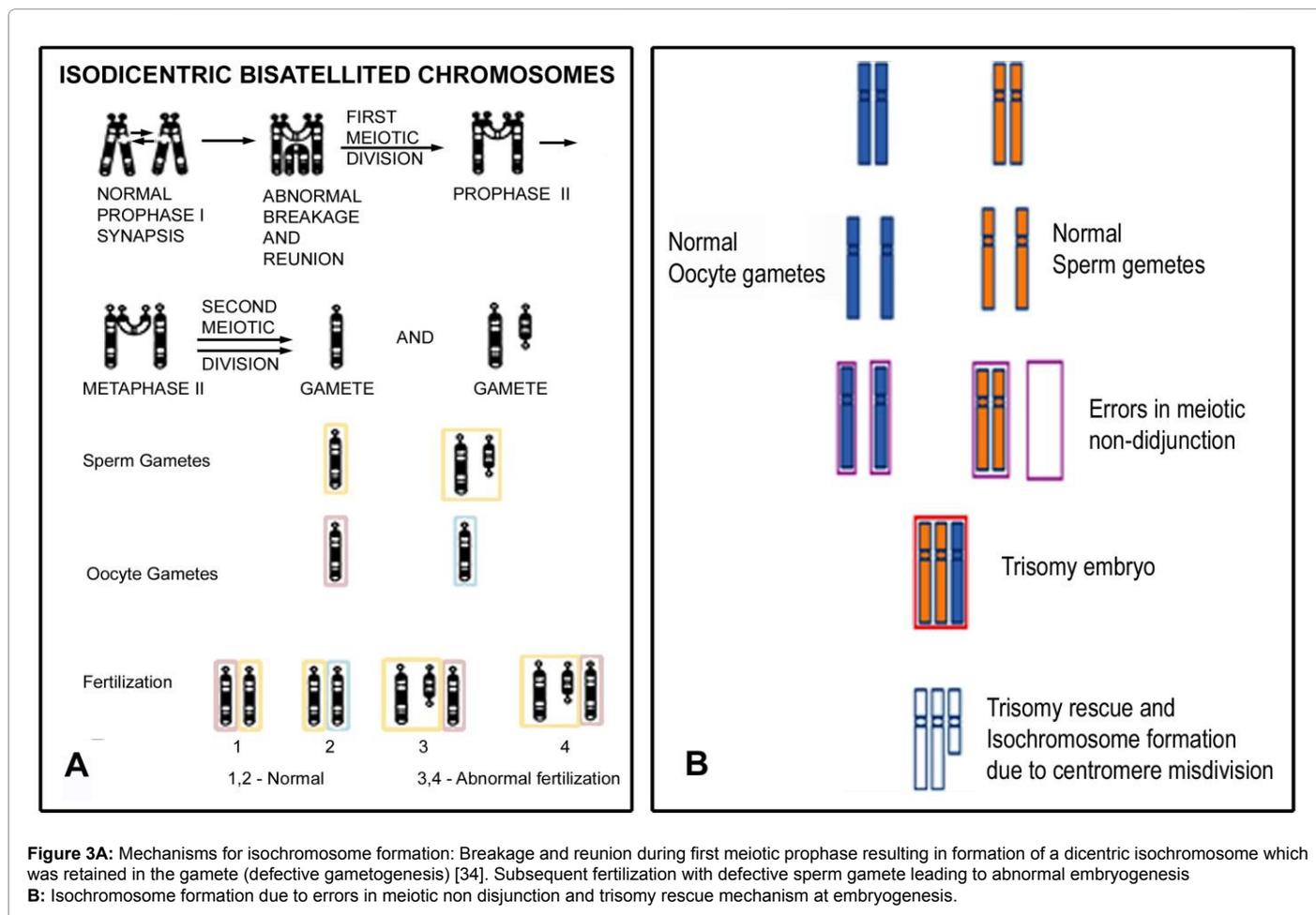
in an inversion carrier. In general, duplications are less harmful than deletions but they inevitably are associated with some clinical abnormalities. The degree of clinical severity is correlated with size of the duplicated segment [24, 25].

In a normal individual with 46 chromosomes, consequences of an isochromosome formation result in partial monosomy and partial trisomy. In the present report, the unique karyotype identified in the male partner has 47 chromosomes, with two normal homologues of chromosome 22 along with its isochromosome as well, thus indicating a tetrasomic state for the short arm of chromosome 22. The origin of these isochromosomes can be explained as- most likely they result from exchange between homologues during meiosis, or from breakage and reunion of sister chromatids near the centromere [26] (Figure 3A). Centromere misdivision during meiosis II is also considered to be a possible, though a less likely, mechanism. The presence of chromosome 22 in partial tetrasomic condition like an iso-chromosome because of the duplication of short arm of chromosome 22 may have occurred due to meiotic segregation errors at non-disjunction. The possible explanation could be that trisomy of chromosome 22 might have occurred at meiotic non- disjunction and, q arm of the chromosome might have got lost (trisomy rescue) and followed by the inverted duplication of the p arm leading to bisatellited iso-chromosome formation (Figure 3B). The most common isochromosome involves the long arm of the X-chromosome, which is frequently seen in individuals with Turner syndrome [27]. Most X-isochromosomes are

actually dicentric in nature. Inactivation of one centromere makes this abnormal chromosome more stable during cell division. Unbalanced isochromosomes are always associated with clinical abnormalities owing to their inherent genetic imbalance. The male carrier has an increased risk for oligospermia or complete azoospermia and often has to be ascertained through clinical investigations for infertility [25].

It is well known that 'q' arm of chromosome 22 has Di-George locus critical for cardiac activity [28]. In the present report iso-chromosome formation is leading to loss of 'q' arm of chromosome 22 and thereby disruption of Di-George locus (Figure 2E). Further, fertilization of these defective gametes may cause variations in chromosome rearrangements, in fetus susceptible to REPL, due to the absence of cardiac activity.

In our report the presence of additional bi-satellited iso-chromosome is of chromosome 22 origin; and that this rearrangement alters the synapsis of homologous chromosomes during meiosis; and also it is likely that the presence of abnormally distributed chromatin interferes with meiotic division and thus reduces sperm production eventually leading to infertility [29]. Spermatozoa bearing abnormal chromosomes may cause abnormal embryonic development, with poor cardiac activity, which can in turn, cause early pregnancy loss [30,31]. However earlier reports suggest the presence of this small bi-satellited chromosome 22 with the impaired spermatogenesis and male infertility due to the four fold dosage of centromeric sequences - this holds true for the male partner with marked oligospermia [32]. The presence of this small abnormal chromosome entity in the



karyogram does not show any phenotypic abnormality or any other IQ related developmental variations in the individual as he is a well-qualified working professional. The first human chromosome that was completely sequenced is chromosome 22, containing approx 500 to 600 genes that provide instructions for making proteins [33]. Number of disease conditions is associated with chromosome 22 that include 22q11 deletion syndrome, 22q13.2 deletion syndrome, Ewing sarcoma, Rubenstein-Tyabi syndrome etc., Deletions in 22q11 region of chromosome 22 cause various problems that include heart defects, cleft lip/palate, immune system abnormalities, characteristic facial features and learning disabilities. Certain combinations of these features are called Di-George or velocardiofacial syndrome. Individuals with this disorder have a 50 percent chance of passing the chromosomal abnormality on to their offspring with each pregnancy.

Conclusion

The present case report emphasizes the significance of cytogenetic analysis in elucidating such defects which can be of immense help to the clinicians in treatment for infertility, REPL presented with poor fetal cardiac activity in addition to the above- mentioned disease conditions associated with any defects of chromosome 22. Advanced molecular cytogenetic techniques and extensive molecular investigations offer a better characterization of such sSMC's. In such cases, prenatal chromosomal evaluation is a prerequisite in order to avoid or minimize the risk of propagation of chromosomal abnormalities into next generation.

Authors' Contributions

VPLO performed all the FISH experiments and NOR staining, VM performed GTG banding, PB referred the patient(s) for cytogenetic analysis, LRK reviewed and approved the manuscript.

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