CASE REPORTS

A MALE (15;15) ROBERTSONIAN TRANSLOCATION CASE WITH 11 PREVIOUS CONSECUTIVE RECURRENT SPONTANEOUS ABORTIONS

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ABSTRACT

Robertsonian translocation is one of the major chromosomal rearrangements and constitutes 18% of all genetic abnormalities with an incidence of 1/1000 in the general population. Re-arrangements between homologous chromosomes are very rare and mainly manifest as monosomic or trisomic offspring. A couple was referred to our center with a history of eleven consecutive spontaneous abortions. The father was diagnosed as having 15;15 Robertsonian translocation. Fluorescence in situ hybridization analysis (FISH) was applied on sperm cells and resulted in only nullisomy and disomy for chromosome 15 that leads to monosomy or trisomy 15 in case fertilization occurs. Therefore the couple was counselled extensively on the risk of a future pregnancy. Furthermore they were informed that they would not benefit from preimplantation genetic diagnosis and sperm donation and adoption could be the only solution.

Keywords: Homologous Robertsonian translocation, Recurrent abortion, Sperm FISH

INTRODUCTION

Robertsonian translocations are recognized to be the most common structural chromosomal abnormalities in the population with an incidence of 1.23/1000 live births \(^1\). These chromosomal translocations are mainly observed in group D chromosomes including 13, 14, 15, and group G including 21 and 22. The D/D translocation is the most frequent type, including a high predominance of 13;14 translocation \(^2\). In Robertsonian translocations, the pericentric regions of two acrocentric chromosomes fuse to form a single or two centromeres. The resulting balanced karyotype has only 45 chromosomes including the translocated one, which is a result of a fusion of the long arms of two chromosomes. Malsegregation of Robertsonian translocation, results in trisomy or monosomy of complete chromosomes \(^3\). In this study, we report a couple with a male 15q;15q Robertsonian translocation, the couple had 11 recurrent spontaneous abortions and one perinatal death. This homologous male 15q;15q translocation is in analogy of 13q;13q \(^4,5\) and 21q;21q \(^6,8\) translocations and mainly originated de novo without any chance of having a normal offspring. Male gamete segregation would give a nullisomic or disomic sperm for chromosome 15 and produce monosomic or
trisomic embryos after fertilization. The effect of the marker chromosome on repeated abortions and the place of preimplantation genetics and genetic counselling are discussed in this study.

CASE REPORT

A couple both 41 years of age was referred to our in vitro fertilization (IVF) center due to the history of repeated spontaneous abortions. The mother had twelve pregnancies, eleven of which ended in spontaneous abortions during the first trimester and one live born male infant died after six hours. The infant weighed 2400 g and had right hemiplegia; unfortunately, no genetic investigation and autopsy were performed.

Chromosome Analysis

For karyotype analysis, 2 ml peripheral blood was sampled from both partners in 5 ml lithium heparinized tubes. A blood sample of 0,5 ml was added to 5 ml HAM’S F-10 medium (Biochrom, Germany) which contains 0,8 ml Fetal Bovine Serum Albumin (Sigma, USA), 0,04 ml L- Glutamin, 0,04 ml Penicillin-Streptomycine and 0,1 ml Phytoshemagglutinin was added. During 72 hours incubation at 37 ºC, 50 µl ethidium bromide was added at 68th hour and 100 µl colcemide (0,05 mg/ml) added 3 hours later to harvest the cells at early metaphase and the cells were prepared for GTG banding (Giemsa Trypsin G-banding).

Sperm Parameters

The sperm analysis showed normal sperm parameters with 52x10⁶ sperm cells/ml, a progressive total motility of 67% and a normal morphology in 8%.

Sperm Preparation and Fluorescence in situ hybridization analysis (FISH)

Semen specimens for sperm FISH analysis were allowed to liquefy at 37 °C under 5% CO₂ and evaluated within 30 min of collection. Specimens were analyzed for sperm concentration, percentage of motile spermatozoa forward progressive motility and morphological analysis (according to Kruger’s strict criteria) using a Papanicolau stain (Spermac, Fertipro, Beernum, Belgium). Following analysis, the semen specimens were mixed with sperm rinse (Vitrolife Mölundalsvagen, Sweden) and concentrated by centrifugation (Labofuge 400, Heraeus, Germany) under 3000 rpm for 10 min. After centrifugation, supernatant was discarded. Precipitated pellet was mixed with 0,2ml sperm rinse.

The sperm cells were fixed in methanol:acetic acid (2:1) for FISH analysis. Sperm slides were washed with 2xSSC 15 min and the sperm heads were decondensed by using 1M Tris pH: 9,5, which contains 25 mM dithiothreitol (DTT), for 1 min. Washing steps were performed again with 2xSSC and PBS for 5 min respectively.

After the washing with different alcohol concentrations, FISH was performed using the standard protocol¹⁹ and employing a hybridization mixture containing CEP 11 alpha satellite probe in spectrum green (D11Z1; Vysis, Inc., USA) and telomeric 15q probe in spectrum orange (D15S396; Vysis Inc., USA). The slides were observed under a fluorescence microscope (Olympus Optical Co.; Japan) with the appropriate filters to visualize the fluorochromes and by using a computerized image capturing system (Isis in situ imaging system, Version 3.4.0; MetaSystems GmbH, Germany).

Chromosome analysis revealed male 45,XY,der(15;15)(q10;q10) and normal female 46,XX karyotypes. The FISH analysis of the sperm sample using the centromeric probe of chromosome 11 for an internal control and telomeric probes for chromosome 15 demonstrate 50% of nullisomy and 50% of disomy 15 in the ejaculate. Translocated disomic sperm cells were observed with two telomeric signals for chromosome 15 and one centromeric signal for chromosome 11 while the nullisomic ones contained only one centromeric signal for chromosome 11 (Fig. 2).

Fig. 1: Robertsonian translocation 15;15
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A male (15;15) Robertsonian translocation case with 11 previous consecutive recurrent spontaneous abortions

DISCUSSION

This paper demonstrates that Robertsonian translocation did not affect the sperm production and sperm parameters. Nullisomy or disomy of chromosome 15 did not affect the fertilization capacity of this sperm, because the couple declared 12 pregnancies in the past. By performing sperm FISH, we confirmed that this male would produce only monosomic or trisomic offspring of chromosome 15 and therefore there was no reason for the couple to refer to preimplantation genetic diagnosis since a trial of a natural conception would be fruitless.

In general non homologous Robertsonian translocations are higher in infertile men and they are associated with infertility. The translocation between the D/D group is the most frequent one and studies of both spontaneous abortions and live births indicate a high predominance of 13;14 translocation. Translocations of Dq;Dq were about six times more frequent than Dq;Gqs. Meiotic segregation in Robertsonian translocation carriers can produce similar numbers of chromosomally normal and balanced gametes. However, another study indicates that this ratio is different at 14;21 translocation carriers and the incidence of the normal gametes is higher than the balanced ones.

This particular case of 15;15 Robertsonian translocation is very rarely seen in the postnatal or prenatal period and it is associated with infertility like in all Robertsonian translocations. However, it did not affect the fertility of the father in our case. Although it was not possible to analyze the parents of the translocation carrier, normal phenotype of the patient suggested a de novo mutation at the post zygotic stage via fusing two homologous chromosomes to form a monocentric balanced translocation.

Fig. 2a: Disomic sperm cells for chromosome 15 (D15S396, spectrum orange; D11Z1, spectrum green)  
2b: Nullisomic sperm cells for chromosome 15 (D15S396, spectrum orange; D11Z1, spectrum green)

Couples carrying balanced re-arrangements with severe segregation and suffering embryo blockage, implantation failure or abortions are referred to assisted reproduction technology. When assisted reproduction is pursued, preimplantation genetic diagnosis (PGD) is an option that permits the embryo selection to increase the implantation rate and avoids the risk of spontaneous abortion.

Robertsonian translocation carriers in non homologous chromosomes have the ability to produce normal gametes and to have an unaffected child. However, it is not possible to have an unaffected child in cases with Robertsonian translocations in homologous chromosomes as there is no chance to produce normal or balanced gametes. Translocation formation prevents the normal segregation of chromosome 15 at meiosis during spermatogenesis.

The segregation results in the formation of disomic or nullisomic gametes and this phenomenon leads to offspring with abnormal karyotypes, either monosomy 15 or trisomy 15. Sperm FISH analysis accurately describes the proportion of normal sperm in male patients with chromosome abnormalities. This analysis also predicts the meiotic behaviour of such anomalies and may also serve to compare abnormalities in different patients. For this particular case, sperm FISH analysis revealed disomic and nullisomic sperm cells which lead to the formation of aneuploid zygotes. Probably, abnormal paternal gametes were responsible for the majority of recurrent abortions. This is a very important point about recurrent abortions as parents should be firstly investigated by chromosome analysis in order to rule out a possible balanced translocation.
Theoretically, there are two possible ways that will permit the achievement of live births in this case. One of them is the fertilization of a disomic sperm cell which is carrying translocated chromosome 15 with a nullisomic oocyte. This is theoretically not acceptable due to the low possibility of formation of the nullisomic oocytes during oogenesis. The zygote resulted from this fertilization will receive both chromosomes 15 from the father and this will result in uniparental disomy. Absence of maternal loci on chromosome 15 causes Angelman syndrome recognized with the hallmarks of severe mental retardation, absent speech, jerky gait, inappropriate outbursts of laughter, microcephaly, flat occiput, wide mouth with protuberant tongue and prognathism. On the other hand, fertilization of a nullisomic sperm cell with a normal monosomic oocyte results a monosomic zygote for chromosome 15. If chromosome 15 undergoes duplication, it causes maternal uniparental disomy and Prader-Willi syndrome which is characterized by severe neonatal hypotonia, hypogonadism, mild to moderate retardation and onset of obesity after approximately two years of age.

In this case, preimplantation genetic diagnosis would not give any benefit to the problem and the only option was to recommend sperm donation or adoption.

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REFERENCES

22. Erics M, Balci S. Can a parent with balanced Robertsonian translocation t(21q;21q) have a non-Down’s offspring? Lancet 1999; 353:751.