

Complex reciprocal translocations, more complex than initially thought: a case report

Megan Dufton, Ph.D.^{a,b} and Renda Bouzayen, M.D.^{a,b}

^a Atlantic Assisted Reproductive Therapies, Halifax, Nova Scotia, Canada; and ^b Department of Obstetrics and Gynecology, Dalhousie University, Halifax, Nova Scotia, Canada

Objective: To present a case of a couple who experienced spontaneous abortion after the transfer of a preimplantation genetic testing for structural rearrangement (PGT-SR) normal/balanced embryo. The embryo was later determined to have significant paternally inherited chromosome deletion that was not previously identified as part of a complex translocation.

Design: Case report.

Setting: Single infertility practice.

Patient(s): A 35-year-old patient with a history of five spontaneous abortions and her 36-years-old partner, a carrier of a balanced reciprocal translocation.

Intervention(s): In vitro fertilization with PGT-SR and follow-up genetic testing.

Main Outcome Measure(s): Identification of a paternal reciprocal translocation, pregnancy outcome after PGT-SR, and follow-up genetic testing after the spontaneous abortion of a PGT-SR normal/balanced embryo.

Result(s): Karyotyping for a couple with a history of recurrent pregnancy loss identified a paternal reciprocal translocation between chromosomes 5 and 17 after G-banding analysis. In vitro fertilization with PGT-SR resulted in one normal/balanced embryo. The couple experienced a 9-week spontaneous abortion of the transfer of the embryo. Testing of product of conception identified a 3.2-Mb deletion on chromosome 17 resulting in the loss of 55 known genes and deemed likely pathogenic. Repeat karyotyping using G-banding and metaphase fluorescence in situ hybridization identified an additional chromosomal translocation, a segment of chromosome 17 translocated to chromosome 6, the same segment of deoxyribonucleic acid absent from the fetus.

Conclusion(s): Preimplantation genetic testing for structural rearrangement cases are complex. Genetic testing must be completed with the best available technology by a reliable testing center. We, therefore, recommend that all chromosomal translocations detected by G-banding be further investigated with metaphase fluorescence in situ hybridization. When unexpected results occur in this patient population, testing beyond the standard of care may be required, including advanced molecular testing. (Fertil Steril Rep® 2021;2: 487–92. ©2021 by American Society for Reproductive Medicine.)

Key Words: Reciprocal translocation, PGT-SR, G-banding identification, FISH, pregnancy loss

Discuss: You can discuss this article with its authors and other readers at <https://www.fertsterdialog.com/posts/xfre-d-21-00108>

INTRODUCTION

Chromosomal translocations can have devastating effects on a carrier's fertility (1). Chromosomal translocations or structural rearrangements are the rearrangement of chromosomal segments between nonhomologous chromosomes. The most common

forms of translocations are Robertsonian and reciprocal. Robertsonian translocations involve the breakage and joining of two acrocentric chromosomes, whereas reciprocal translocations involve the exchange of distal segments between two chromosomes. Reciprocal translocations

are the most common type of translocation and are identified in approximately 0.14% of newborns (2). Less common are insertions, which occur when a segment of a chromosome is translocated and inserted into an interstitial region of another chromosome or into a different region of the same chromosome.

Balanced translocation carriers are generally phenotypically normal. During meiosis, balanced translocation carriers are at great risk of creating unbalanced gametes (3). The production of embryos from unbalanced gametes typically results in failed implantation, miscarriage, or, in more rare cases, the birth of an unhealthy child. As a result,

Received June 23, 2021; revised August 16, 2021; accepted August 20, 2021.

M.D. has nothing to disclose. R.B. has nothing to disclose.

The Harold B Atlee Endowment Fund from the Department of Obstetrics and Gynaecology at Dalhousie University.

Reprint requests: Megan Dufton, Ph.D., Atlantic Assisted Reproductive Therapies, 1535 Dresden Row, Halifax, Nova Scotia, Canada B3J 3T1 (E-mail: megan.dufton@dal.ca).

Fertil Steril Rep® Vol. 2, No. 4, December 2021 2666-3341

Crown Copyright © 2021 Published by Elsevier Inc. on behalf of American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

<https://doi.org/10.1016/j.xfre.2021.08.003>

those with balanced translocations are often diagnosed with reduced infertility or recurrent pregnancy loss (1).

Preimplantation genetic testing for structural rearrangements (PGT-SR) can be used in conjunction with in vitro fertilization (IVF) to dramatically reduce miscarriage rate and improve ongoing pregnancy rate for chromosomal translocation carriers (4). Preimplantation genetic testing for structural rearrangement involves testing the genetic status of embryos generated in an IVF cycle to determine if an embryo has inherited an unbalanced translocation from a carrier parent. Embryos are biopsied to provide material for genetic testing. Those determined to have a normal amount of genetic material or a balanced translocation are selected for transfer to the uterus, whereas those identified as abnormal or unbalanced are not (5). Unfortunately, translocation carriers tend to produce a low percentage of normal or balanced embryos (3). On average, only 27.3% of biopsied embryos of reciprocal translocation carriers will be normal or balanced. As a result of this preimplantation embryo selection, translocation carriers have a significantly reduced chance of miscarriage and increased chance of ongoing pregnancy and live birth compared with natural conception (4, 5).

The accuracy of how a translocation is identified and described can be influenced by the cytogenetic method used to describe it (6, 7). Cytogenetic analysis and diagnosis of translocations in adults and children are historically performed by Giemsa staining (G-banding) and/or fluorescence in situ hybridization (FISH) (6, 7), with FISH providing a higher level of resolution. The identification of translocations present in PGT-SR embryos are normally tested by FISH, array comparative genomic hybridization, quantitative polymerase chain reaction (qPCR), single nucleotide polymorphism array, or next-generation sequencing (NGS) (8, 9). The genetic testing method used for PGT-SR can significantly affect the outcome of the IVF cycle. Next-generation sequencing has been shown to result in superior pregnancy outcomes compared with other methods of testing (9). The pregnancy outcome differences between testing methods again demonstrates the complexity of accurately identifying translocations.

This report examines the case history of a reciprocal translocation-carrying couple who completed the transfer of PGT-SR tested balanced/normal embryo that resulted in the miscarriage of an unbalanced fetus. This case report highlights the complex nature of accurately identifying and describing reciprocal translocations.

CASE REPORT

History

Informed consent was obtained from the patients for the publication of this case report. The 35-year-old patient, gravida 5, with a history of five spontaneous abortions and her partner presented at our IVF clinic. The spontaneous abortions ranged in gestation age from 4 to 12 weeks. The patient had a notable history of ulcerative colitis. She had colectomy and ileostomy followed by the creation of a J-pouch and reversal of the ileostomy. Additionally, she had mild scoliosis. Standard recurrent pregnancy loss workup was completed including

the antiphospholipid antibody test, lupus anticoagulant test, anticardiolipin antibody test, beta-2 glycoprotein 1 antibody test, and sonohysterography on the female patient, and karyotypes were completed on the male and female patients. The antibody testing and sonohysterography had no significant findings. The karyotypes identified a balanced reciprocal translocation in the male patient and nothing else of note.

Karyotyping was performed at a regional cytogenetic testing center. G-banding was performed at a resolution of 400–550 bands, and four cells were karyotyped. The identified balanced reciprocal translocation involved chromosomes 5 and 17. The breakpoints were described as band 15.1 on the short (p) arm of chromosome 5 and band 25.1 on the long (q) arm of chromosome 17.

Treatment

The couple opted to proceed with IVF with PGT-SR. The standard NGS assays with 10-Mb resolution did not provide sufficient coverage of the chromosomal breakpoints, and as a result, qPCR targeted probes were designed to identify the breakpoints to a greater than 98% accuracy.

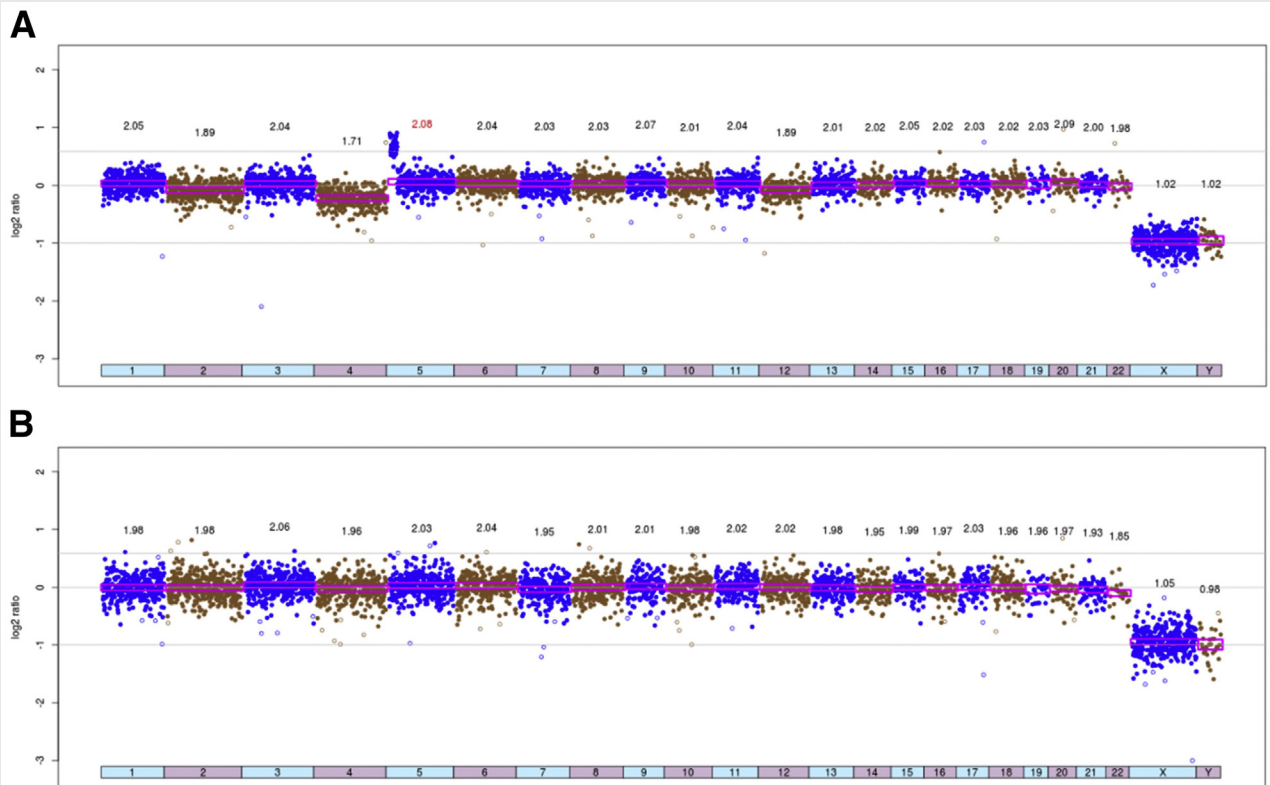
Because of a low antral follicle count and increased follicle-stimulating hormone (FSH) level, the patient was placed on an estrogen-primed antagonist ovarian hyperstimulation treatment plan. Ovarian hyperstimulation was started with the use of 450 IU of recombinant FSH (Gonal-f; Merck Serono, Rome, Italy) and 150 IU of urinary FSH and luteinizing hormone (Menopur; Ferring, Toronto, Canada). Stimulation occurred over 12 days, at which time the leading two follicles reached 17.5 mm in diameter. Ovulation was triggered using 10,000 IU of human chorionic gonadotropin. Thirty-six hours after the trigger injection, six oocytes were retrieved. Four of the oocytes fertilized after intracytoplasmic sperm injection. Two embryos reached an appropriate stage for blastocyst biopsy on day 6 of culture, grading 4BB and 3BB by the Gardner grading scheme. Laser-assisted trophectoderm biopsy was performed to remove approximately five cells from each embryo. After biopsy, the embryos were vitrified. All stages of biopsy, tubing, and vitrification were witnessed by a second embryologist. The biopsied tissue was submitted to the genetic testing center.

Results of Treatment

The PGT-SR testing was performed using NGS and the targeted PCR probes. The PGT-SR testing revealed that the first embryo had the unbalanced derivative of the identified translocation. The second embryo was determined to be euploid, either normal or a balanced derivative of the translocation because the testing could not discern between the two (Fig. 1).

A frozen embryo transfer of the 4BB euploid embryo was performed after a traditional gonadotropin-releasing hormone agonist uterus preparation with estrogen and progesterone suppository supplementation. Fourteen days after the transfer, the patient's serum human chorionic gonadotropin was 536 IU/L. Thirty-three days after the embryo transfer, transvaginal ultrasound was performed. The ultrasound showed a normal-appearing 8-week intrauterine pregnancy with a visible fetal heartbeat with a crown rump length of

FIGURE 1



Copy number variant results of the next-generation sequencing analysis of the two tested embryos: (A) embryo number 1, carrier of the unbalanced translocation, and (B) embryo number 2, diagnosed as euploid.

Duften. Improved detection of complex translocations. *Fertil Steril Rep* 2021.

1.48 cm. After the ultrasound, the patient was referred to obstetrical care. At 9 weeks and 4 days of gestation, the patient experienced spontaneous abortion. The labeling of the remaining frozen embryos was confirmed to ensure the unbalanced embryo was not transferred as a result of human error.

Genetic Testing

Products of conception were collected and submitted for genetic testing. Evaluation for copy number variants (CNVs) by single nucleotide polymorphism microarray (Affymetrix CytoScan HD) was conducted (Fig. 2). The microarray analysis detected two CNVs. The first was a 42-kb loss of the short arm of chromosome 5, position 14.3. This CNV is located within the intron of the CDH18 gene and near the region of the breakpoint on chromosome 5 in the paternal translocation. The second CNV detected was a 3.2-Mb loss of the long arm of chromosome 17, position 23.2 to 24.1. This fragment of chromosome 17 contained 55 genes, 12 of which were OMIM disease genes. This fragment encompassed the breakpoint on chromosome 17 described in the paternal translocation.

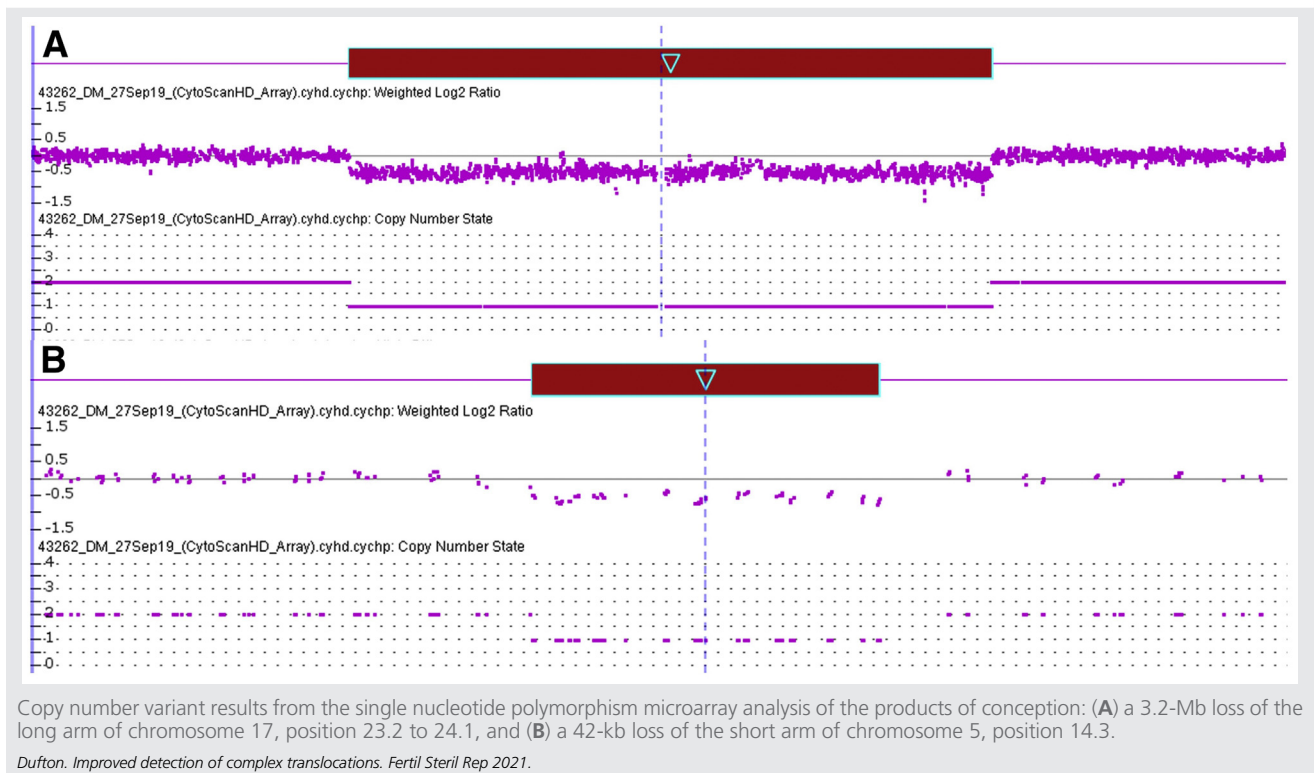
On the basis of these newly identified losses being present in close vicinity to the documented paternal translocation, re-

investigation of the paternal genetics was performed. Single nucleotide polymorphism microarray analysis did not detect any significant CNVs in the maternal or paternal testing. The paternal testing did not detect the CNV identified in the fetus. Due to fetal CNV being located in close proximity to the paternal translocation, karyotyping was repeated for the male patient. Four paternal cells were karyotyped by G-banding and metaphase FISH with a resolution of 450–550 bands per haploid karyotype. The karyotype confirmed the previous translocation between chromosomes 5 and 17, the breakpoint at 5p15.1 and a slightly different breakpoint at 17q25. Additionally, a fragment of chromosome 17q23–24 was found to be inserted at chromosome 6p23 (Fig. 3). The chromosome 17 fragment was consistent with the 3.2-Mb loss in the fetus.

DISCUSSION

This case demonstrates the complex nature of accurately identifying translocations and structural rearrangements and the potential consequences of misidentification. On the basis of the analysis by the hospital molecular genetics department, it is thought that the 42-kb loss identified in the fetus was likely benign because it only encompassed the intron of one gene (CDH18). However, it is of interest because it was located near the region of the breakpoint on

FIGURE 2



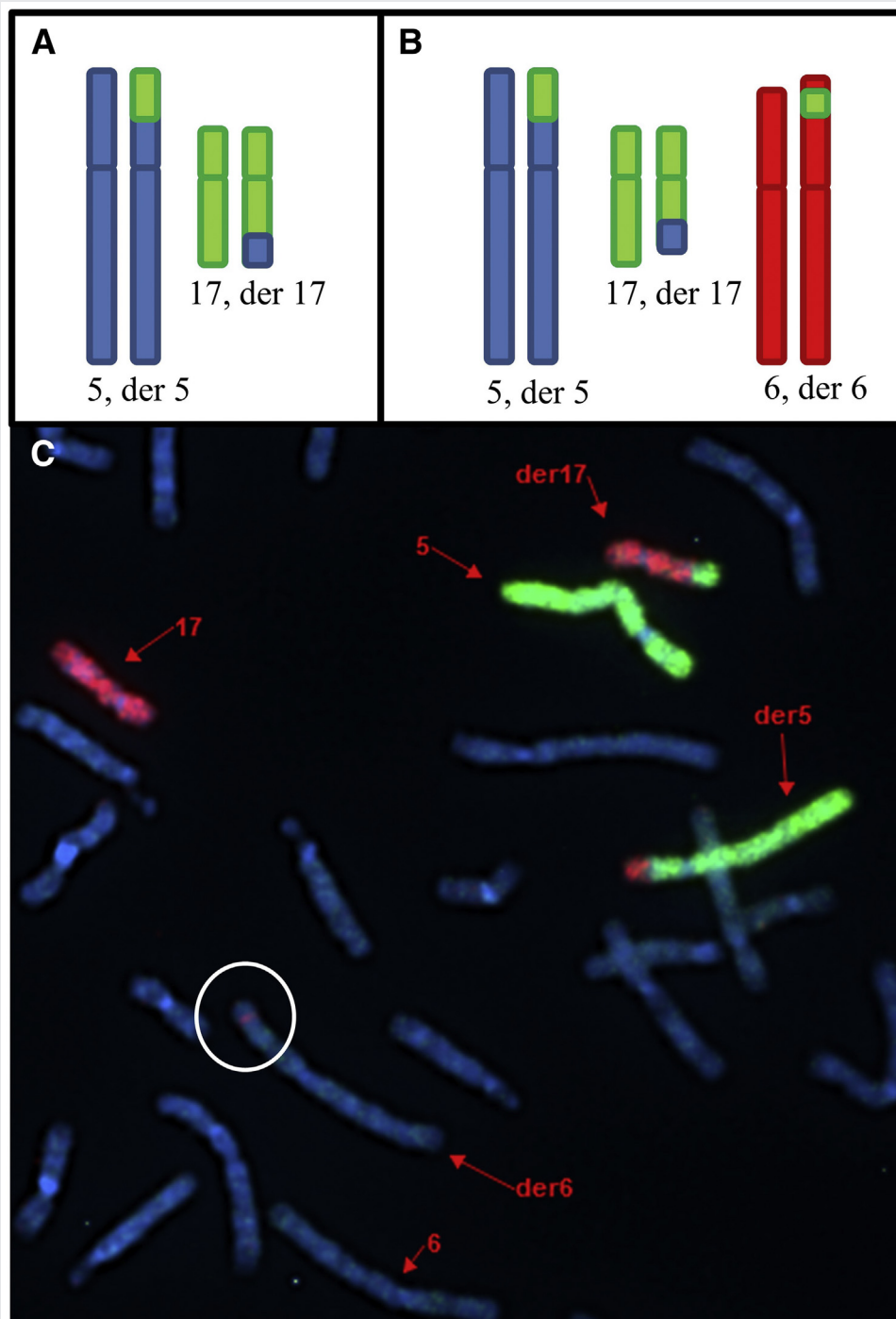
chromosome 5 in the paternal translocation. The second CNV detected in the fetus, a 3.2-Mb loss on chromosome 17, contained 55 genes, 12 of which were known OMIM (an online catalog of known human genes and genetic disorders) disease genes. The DECIPHER database, a database used by clinicians to track and elucidate phenotypic and genotypic data, contains no information on CNVs of similar size and location as described in the fetus. Larger CNVs located in the region of the 3.2-Mb loss recorded in the DECIPHER database were deemed likely pathogenic, with one CNV that overlaps the CNV identified in the fetus being classified as pathogenic. Copy number variants recorded in the Database of Genomic Variants located in this region are much smaller. Most of the genes located in the 3.2-Mb loss are associated with conditions with autosomal recessive inheritance. On the basis of the information currently available in the literature and the available database information, the medical genetics team responsible for interpreting these results deemed the 3.2-Mb loss as unknown significance that was likely pathogenic. The limitations of technology used in the initial genetic karyotype resulted in the transfer of an embryo and subsequent pregnancy loss of an embryo that should have been deemed inappropriate for transfer.

As described earlier, there are numerous techniques available to identify translocations. Generally, karyotypes are performed in the case of recurrent pregnancy loss to identify potential translocations. The karyotypes are typically performed by G-banding and/or metaphase FISH (6). Giesma banding does not result in the same level of resolution as

metaphase FISH. However, G-banding has the ability to detect 3–5 Mb changes within the range of the paternal insertion. After G-banding identifies a translocation, FISH is often then performed to better describe the translocation. Unfortunately, FISH was not completed to confirm the paternal translocation, which could have potentially identified the additional insertion. This inaccurate report was then used to design specific qPCR probes used in conjunction with NGS during the PGT-SR testing. As a result, the PGT-SR was successfully able to identify the chromosome 5 and 6 imbalances but not able to detect the chromosome 17 deletion, which was originally undetected in the paternal specimen. Because of the vast investment and importance of PGT-SR results, we must insure the best quality of the information or genetic results used in this testing. On the basis of this, we recommend that metaphase FISH and G-banding be always used in conjunction to describe chromosomal translocations and structural rearrangements before PGT-SR workup, G-banding alone is not sufficient. Translocations and rearrangements can be highly complex, and ensuring that they are properly diagnosed in these cases is the key.

In addition to different methods of karyotyping, there are various genetic testing methods used for PGT-SR. A recently published study by Bartels et al. (9) describes differences in implantation rates and pregnancy outcomes depending on the PGT-SR technology used. The study finds NGS to provide superior outcomes compared with the use of FISH, and array comparative genomic hybridization. These differences are important to consider because they make a

FIGURE 3



Pictorial diagram and metaphase fluorescence in situ hybridization (FISH) results demonstrating the paternal translocation. (A) Reciprocal translocation described by G-banding. (B) Complex translocation described by G-banding and metaphase FISH. (C) Metaphase FISH results, the circle highlights a small amount of genetic material from chromosome 17 located on derivative chromosome 6.

Dufton. Improved detection of complex translocations. *Fertil Steril Rep* 2021.

significant impact to our patients' care. When using a third-party genetic testing service, we recommend being familiar with the technology they are offering and the accuracy of their testing.

Preimplantation genetic testing for structural rearrangement is reported to have >98% accuracy, but despite this, errors can occur. The testing is dependent on the accurate detection and description of the translocations during the

workup process. Despite our patients' testing being completed with NGS and qPCR, the testing was not able to detect the undescribed portion of the insertion. Treating patients with translocations and structural rearrangements is technologically complex and is limited by the patients' biology, producing a high proportion of abnormal embryos. As a result, these cases require more attention. Genetic testing of products of conception is not standard of care in our region after first trimester pregnancy loss. The complex history of this couple encouraged us to go beyond our standard of care. If testing of products of conception was not performed, this couple would have continued into another IVF PGT-SR cycle with incorrectly designed probes, which could have resulted in the same outcome. On the basis of our experience with this complex case and being that PGT-SR cases only make up a small proportion of IVF practice, when unexpected events occur in these cases, we recommend the highest level of investigation available be used.

When complex translocations and rearrangements are mistaken for reciprocal translocations the results can be devastating to our patients. The couple has had new qPCR probes created to identify the newly identified complex translocation and hopes to proceed with further treatment.

In conclusion, treating patients with translocations can be extremely complex. The quality of treatment we provide to these patients is highly reliant on the genetic testing we are provided with. Inaccuracy in the initial description of the paternal translocation and insertion likely resulted in this couple experiencing another pregnancy loss. G-banding was not sufficient to accurately describe the paternal translocation, a situation which may occur for several other couples. We, therefore, recommend that in addition to G-banding, metaphase FISH should be performed to more accurately describe a translocation before all PGT-SR workup and treatment. Because of the intricate nature of these cases when

unexpected results occur in these couples, a greater level of investigation is warranted. We recommend testing of products of conception for these patients when unexpected pregnancy losses occur, which should include genetic testing such as microarray.

Acknowledgments: The authors thank Erika Aberg and Jennifer MacGillivray for their review of the article.

REFERENCES

1. Morin SJ, Eccles J, Iturriaga A, Zimmerman RS. Translocations, inversions and other chromosome rearrangements. *Fertil Steril* 2017;107:19–26.
2. Nielsen J, Wohler M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet* 1991;87:81–3.
3. Wang YZ, Ding CH, Wang J, Zeng YH, Zhou W, Li R, et al. Number of blastocysts biopsied as a predictive indicator to obtain at least one normal/balanced embryo following preimplantation genetic diagnosis with single nucleotide polymorphism microarray in translocation cases. *J Assist Reprod Genet* 2017;34:51–9.
4. Fischer J, Colls P, Escudero T, Munne S. Preimplantation genetic diagnosis (PGD) improves pregnancy outcome for translocation carriers with a history of recurrent losses. *Fertil Steril* 2010;94:283–9.
5. Otani T, Roche M, Mizuike M, Colls P, Escudero T, Munne S. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. *Reprod Biomed Online* 2006;13:869–74.
6. Chial H. Cytogenetic methods and disease: flow cytometry, CGH, and FISH. *Nat Sci Educ* 2008;1:76.
7. Switonski M, Andersson M, Nowacka-Woszuk J, Szczerbal I, Sosnowski J, Kopp C, et al. Identification of a new reciprocal translocation in an AI bull by synaptonemal complex analysis, followed by chromosome painting. *Cytogenet Genome Res* 2008;121:245–8.
8. Treff NR, Forman EJ, Katz-Jaffe MG, Schoolcraft WB, Levy B, Scott RT Jr. Incidental identification of balanced translocation carrier patients through comprehensive chromosome screening of IVF-derived blastocysts. *J Assist Reprod Genet* 2013;30:787–91.
9. Bartels C, Makhijani R, Godiwala P, Bartolucci A, Nulsen J, Grow D, et al. In vitro fertilization outcomes after preimplantation genetic testing for chromosomal structural rearrangements comparing fluorescence in-situ hybridization, microarray comparative genomic hybridization, and next-generation sequencing. *F S Rep* 2020;1:249–56.