# Initially categorized 46,XY embryo transfer ending with 45,X products of conception—a case report and a review of discordant result management

Prapti Singh, D.O., M.S.,[a] Alyssa Snider, M.S., Ph.D.,[b] Refik Kayali, Ph.D.,[b] and Abigail Mancuso, M.D.[a]

[a] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Iowa, Iowa City, Iowa; and [b] Igenomix, Torrance, California

**Objective:** To report a case of an initially categorized euploid male embryo screened using preimplantation genetic testing (PGT) resulting in miscarriage and testing of products of conception consistent with Turner syndrome, and to discuss additional workup and considerations in cases of discrepancy.
**Design:** Case report.
**Setting:** University fertility clinic.
**Intervention:** Frozen single embryo transfer of a euploid male embryo.
**Patient(s):** A couple seeking procreative management for a female partner having a balanced translocation 46,XX,t(14;16)(q21;q21) diagnosed after the couple's previous child passed because of segmental duplication in chromosomes 14 and 16 and pursued in vitro fertilization treatment for PGT for structural rearrangements.
**Main Outcome Measure(s):** Miscarriage with discordant chromosomal microarray result.
**Result(s):** Couple conceived with the transfer of a euploid male embryo. After the initial confirmation of pregnancy, repeat imaging indicated a missed abortion. Dilation and curettage were performed, and the products of conception were sent for chromosomal microarray. Results indicated Turner syndrome (45,X). Follow-up short tandem repeat analysis confirmed the products of conception were from the tested embryo. After reevaluation of the data, copy number variations below the reporting threshold for the sex chromosomes were observable and compatible with mosaic 45,X/46,XY.
**Conclusion(s):** The limitations of PGT should be kept in mind when counseling patients because of both the sample provided by biopsy, the sequencing platforms and the laboratory pipeline for diagnosis. We recommend that patients be counseled about these limitations and offered antenatal and postnatal testing as indicated. When discrepancies are seen after PGT, collaboration with the reference laboratory and additional testing with short tandem repeat analysis should be considered when possible. (F S Rep® 2024;5:328–32. ©2024 by American Society for Reproductive Medicine.)
**Key Words:** PGT, mosaicism, aneuploidy, euploid, case report

Preimplantation genetic testing (PGT) is used in conjunction with in vitro fertilization (IVF) for various indications, including optimizing the chance of ongoing pregnancy and preventing the recurrence of genetic conditions because of chromosomal abnormalities and/or monogenic conditions. Typically, different types of PGT are used on the basis of indications for aneuploidy, monogenic disease, and structural rearrangements, using PGT for aneuploidy (PGT-A), PGT for monogenic disease (PGT-M), and PGT for structural rearrangements (PGT-SR), respectively.

Although PGT platforms have been developed to be highly accurate, as with any screening tool, there is a chance of both false positive and false negative results. The recent rate of mosaicism varies between laboratories, ranging from 3% to 30% (1–3), suggesting that analytical variability accounts for at least a portion of mosaic reporting. Many studies suggest that PGT-A reported mosaicism is associated with reduced reproductive potential, but the number of instances in which

Received March 7, 2024; revised May 16, 2024; accepted May 17, 2024.
Correspondence: Prapti Singh, D.O., M.S., Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Iowa, 200 Hawkins Drive, 31141 PFP, Iowa City, Iowa, 52240 (E-mail: prapsingh@gmail.com).

F S Rep® Vol. 5, No. 3, September 2024 2666-3341

https://doi.org/10.1016/j.xfre.2024.05.006

mosaicism persisted through the pregnancy is few (4–6), and most mosaic embryo transfers (ETs) result in seemingly healthy pregnancies. A nonselection study in which embryos with copy number variations (CNVs) of 50% or below were blindly transferred showed no significant differences in reproductive outcomes compared with the control euploid (<20%) group (7). Whether mosaicism detected using PGT-A represented an isolated finding in the biopsy, was biologically corrected in the embryo, or was because of an analytical false positive cannot be determined. Skeptics of PGT-A testing are concerned that overcalling abnormalities result in unnecessary embryo exclusion and wastage, even potentially reducing the chance of reproductive success for a patient (8, 9). As such, it remains crucial for PGT-A screening not to cast the net too wide and overcall positive results. Similarly, undercalling can result in adverse outcomes, including failed implantation, miscarriage, or an ongoing aneuploid or mosaic aneuploid pregnancy.

Although at a low error rate of approximately 1%, discordance has been reported in the literature with those that were erroneously diagnosed, resulting in spontaneous miscarriages (10). Additionally, the limitations of the number of cells biopsied, reliance on CNV thresholds for mosaicism designation, and postmitotic changes may lead to such false negative results (2, 11). Our report provides evidence of an initially euploid male embryo that resulted in miscarriage with chromosomal microarray (CMA) consistent with Turner syndrome, and retrospective analysis of the PGT data compatible with low-level mosaicism 45,X/46,XY.

## CASE REPORT

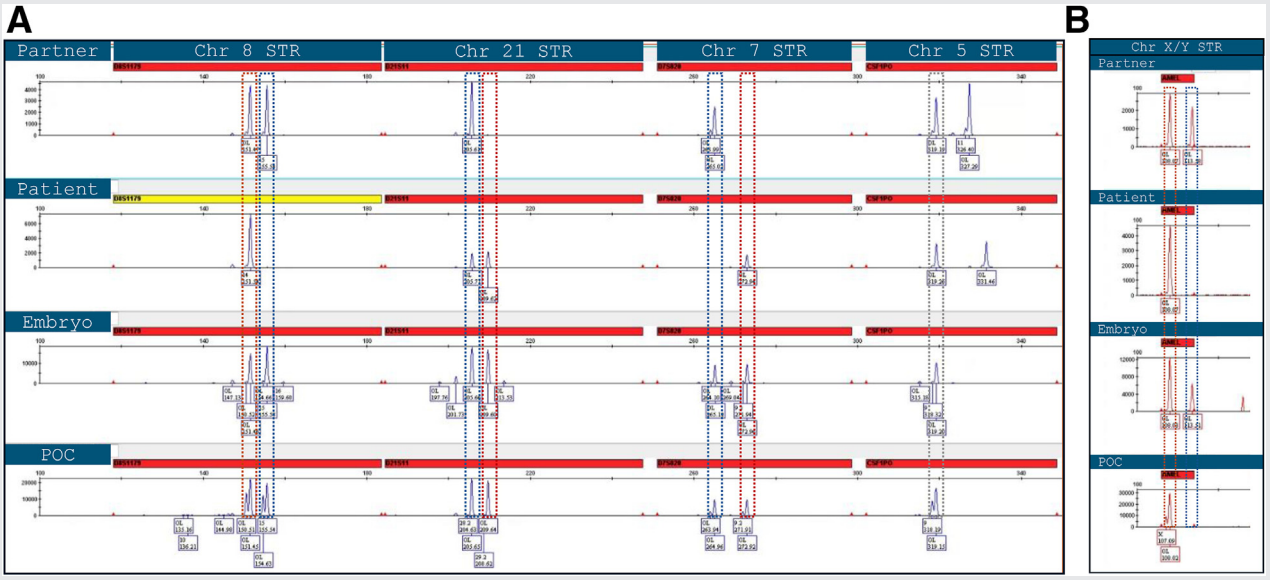
Written consent was obtained from the couple for this case report. A couple presented for preconception counseling after the patient was identified to carry a balanced translocation 46,XX,t(14;16)(q21;q21) with a history of having an affected child with segmental duplications in chromosomes 14 and 16, who passed away. The patient and partner were counseled on the risk of pregnancy loss and recurrence of an affected child with the option of PGT-SR and antenatal testing. The couple decided to attempt spontaneous conception with a plan for antenatal testing. After failed attempts to conceive, the couple presented with secondary infertility and attempted ovulation induction and intrauterine inseminations before pursuing IVF treatment with PGT-SR. At 33 years old, the patient underwent controlled ovarian hyperstimulation with injectable gonadotropins, followed by oocyte retrieval. A total of 14 oocytes were retrieved, of which 13 were metaphase II oocytes. These mature oocytes underwent intracytoplasmic sperm injection insemination, resulting in 11 that were successfully fertilized. A total of 6 blastocysts developed, of which 2 were biopsied on day 5 and 3 were biopsied on day 6. Samples were submitted to the reference laboratory and processed using Thermo Fisher's ReproSeq Whole Genome amplification kit, sequenced using Thermo Fisher's Ion GeneStudio S5 System, and analyzed using the Thermo Fisher algorithm. Copy number variation thresholds used for mosaic calling were >30% for autosomes (30%–50% low mosaic; 50%–70% high mosaic) and >50% for sex chromosomes (50%–70% high mosaic). After PGT-SR, 3 embryos resulted in euploid and balanced results. The patient's first transfer, with the endometrial preparation of oral estradiol and progesterone in oil, resulted in a biochemical pregnancy. Her second transfer, following the same protocol, initially resulted in a viable intrauterine pregnancy at 6 weeks and 2 days. A repeat ultrasound 2 weeks later indicated a missed abortion.

Pregnancy was managed with dilation and curettage, and the products of conception (POC) were sent for CMA because the couple wanted to confirm the genetic information of the pregnancy. Chromosomal microarrays reported the POC as arr(X)x1, consistent with monosomy X, or Turner syndrome. Surplus deoxyribonucleic acid (DNA) from the POC and maternal and paternal blood samples were then sent to the PGT laboratory for short tandem repeat (STR) testing. Short tandem repeat analysis was performed after DNA extraction, polymerase chain reaction (PCR) amplification, and fluorescent labeling and size fractionation using capillary electrophoresis producing electropherograms. For STR analysis, both commercial AmpF/STR Identifiler Plus PCR Amplification (Thermo Fisher Scientific) and GeneScan 500 LIZ Dye (Thermo Fisher Scientific) kits were used to process the samples, and PCR products were run on the SeqStudio Genetic Analyzer (Thermo Fisher Scientific). Dominant peaks representing each STR allele were compared between samples for the patient, partner, POC, and embryo. Short tandem repeat analysis confirmed that the POC and the PGT biopsy were of expected maternal and paternal origin, that the pregnancy was the result of the tested, transferred embryo, and that chromosome Y was present in the biopsy sample, consistent with the original next-generation sequencing (NGS) result (Fig. 1). Alternative explanations for the discrepancy were therefore ruled out, including sample contamination, spontaneous conception, sample swapping, or unintended ET. Next-generation sequencing data were reviewed with a focus on the sex chromosomes. Copy number deviations were observable by eye for the Y chromosome; however, the deviations were below the 50% threshold for reporting mosaicism of the sex chromosomes and were determined to be because of common variations in NGS data. The embryo was therefore interpreted as a euploid male embryo (Fig. 2). The couple was able to proceed with a subsequent transfer of a remaining euploid and balanced embryo that resulted in the live birth of a healthy infant.

## DISCUSSION

Our case emphasizes the importance of understanding the limitations of PGT as a screening test and discusses some of the additional testing that can be done to work up discrepancy cases. The American Society of Reproductive Medicine recommends that, before pursuing testing, patients understand the risks, benefits, and limitations of the technology used. This case brings to light the limitation that mosaic calling with PGT utilizes copy number thresholds rather than direct observation of individual cells in a biopsy. The threshold for calling a euploid result varies between <20% and <50% CNV, depending on the reference laboratory and whether the ordering provider elects mosaicism to be reported (7).

## FIGURE 1



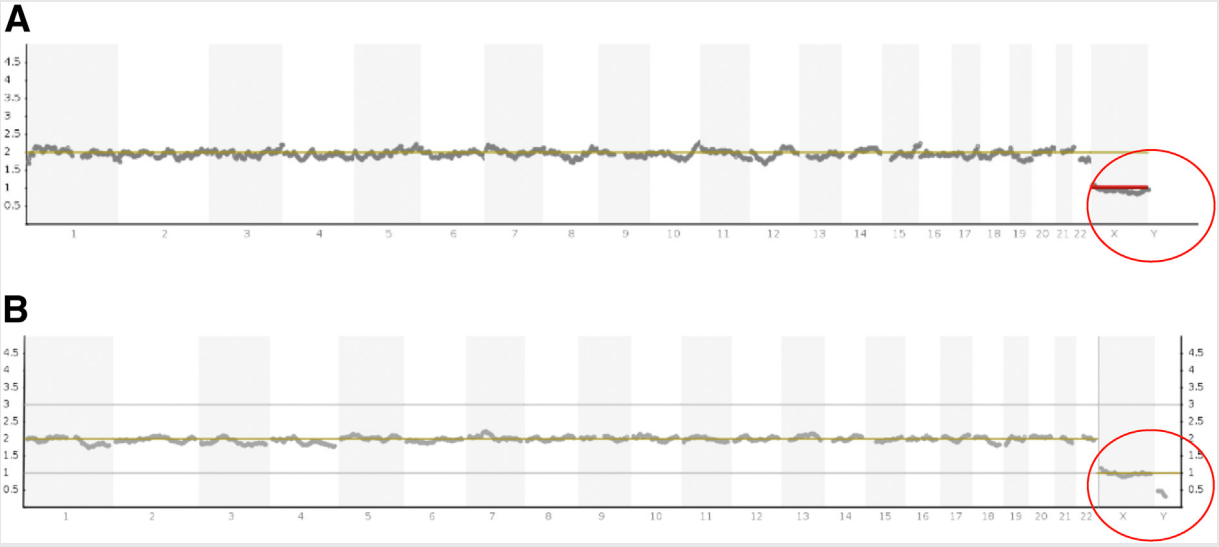

Short tandem repeat (STR) analysis. Each peak represents a unique STR allele. The top row depicts the partner's sample; the second row depicts the patient's sample; the third row depicts the embryo sample; and the fourth row depicts the POC sample. The inherited maternal alleles are outlined in the red-dashed box. The inherited paternal alleles are outlined in the blue-dashed box. The homozygous maternal and paternal allele is outlined in the gray-dashed box. Small peaks represent background noise and may be seen, especially in embryo DNA because of the small quantity of starting material. (**A**) Four representative STR markers are located on autosomal chromosomes: chromosome 8 (D8S1179), chromosome 21 (D21S11), chromosome 7 (D7S820), and chromosome 5 (CSF1PO). The 4 STR alleles shown as well as an additional 11 autosomal STR alleles not shown are identical between the embryo samples and the products of conception (POC) samples and are consistent with maternal and paternal inheritance. (**B**) The STR marker on the pseudoautosomal region of the X and Y chromosomes (AMEL). *Amel STR shows the presence of the Y chromosome in the partner and embryo. Two alleles representing the X and Y chromosomes can be seen in the partner and embryo samples, consistent with the presence of chromosome Y. A single allele is observed for the patient, consistent with homozygosity, and for the POC sample, consistent with monosomy X. The chromosome Y allele is not observable for either the patient or the POC sample.

*Singh. 45, XY embryo resulting in 45, X micarr. F S Rep 2024.*

Many laboratories use a lower threshold of 20% CNV, with intent to a lower chance of false negative results. However, NGS data are prone to analytical noise because of the small amount of genetic material amplified and sequenced. Conversely, low thresholds can reduce the frequency of embryos reported as euploid and result in a high rate of false positive mosaic results. With PGT technology, the frequency of mosaicism reported per trophectoderm biopsy ranges between 3% and 30%, depending in part on the threshold employed by the reference laboratory (3). In cases where mosaicism is reported, most ETs result in seemingly normal newborns with no related aneuploidy in those pursuing additional prenatal testing (11). Only a few postnatal studies show persistent mosaicism or nonmosaic segmental aneuploidy (4–6). The low incidence of PGT-detected mosaicism persisting in ongoing pregnancies could be because of a high degree of ability for embryonic self-correction, a high degree of false-positive mosaic results, or a combination of both.

Another limitation of PGT is that the 5–10 cells biopsied from the trophectoderm may not be representative of the fetus. Because of company policy, exact CNV could not be provided by the genetic testing company because of concern for inaccurate interpretation. Copy number variation is a bioinformatic extrapolation and not an accurate representation of cellular content; you could have a 42.3% copy number variation for 2 of 6 cells (33%) with an aneuploidy. Thresholds are therefore shared in place of CNV because of this limitation. Of note, the mechanism of Turner syndrome is typically sporadic in approximately 70% of cases attributed to paternal nondisjunction (12). For a mosaic result, this would be because of a postmitotic nondisjunction event in early embryonic development. This indicates limited interpretation because of confined placental mosaicism or fetal mosaicism, with the biopsy not being fully representative of the developing fetus. This can lead to problematic false-positive or false-negative results (13–15). Although NGS has shown an increased ability to categorize PGT biopsies with more precision compared with previously used array comparative genomic hybridization platforms (10, 15), the limitations of mosaic threshold cut-offs, platform sensitivity and specificity, and limited cell biopsies remain. Previous case reports have indicated euploid ETs resulting in pregnancies with discordant results, including molar pregnancies, chromosomal microdeletion, and Turner syndrome (16–19); our case adds to this cohort because the origin of the pregnancy was confirmed to originate from the tested and transferred embryo with STR analysis, excluding alternatives of incorrect ET or spontaneous conception.

## FIGURE 2




Next-generation sequencing (NGS) data show normalized frequencies of sequencing reads aligned to the genome. Autosomal chromosomes are listed sequentially, followed by the sex chromosomes on the x-axis. The y-axis depicts the chromosome copy number. (**A**) NGS data from the products of conception (POC). Surplus deoxyribonucleic acid (DNA) from the POC was submitted to the reference laboratory and processed with NGS. No copy number variations for autosomes or chromosome X were detected. The data show 1 copy of chromosome X and no signal for chromosome Y, which is consistent with 45,X. (**B**) NGS (PGT-SR) data from the embryo initially categorized as euploid male. No copy number variations for autosomes or chromosome X were detected. Data analysis did not detect a copy number variation on chromosome Y, but a small (<50%) copy number depletion on chromosome Y is observable by eye. PGT-SR = preimplantation genetic testing for structural rearrangements.

*Singh. 45, XY embryo resulting in 45, X micarr. F S Rep 2024.*

The Y chromosome, because of long repetitive sequences, makes reference-based methods such as NGS, which are tailored to the diploid genome, more difficult (20). For our case, although the decrease in signal for the Y chromosome was within the threshold for the designation of a euploid male on PGT-A, retrospective analysis suggests it would be compatible with mosaicism of 45,X/46,XY. This indicates placental mosaicism, given that the POC CMA resulted in monosomy X. It has been noted that the rate of mosaicism is twice as high in embryos with pregnancies resulting in miscarriage (21). In addition to mosaicism, other possible explanations for discrepancy cases include spontaneous conception, wrong ET, switching of samples (either of the embryo or POC), maternal cell contamination, or contamination from laboratory personnel. With fingerprinting analysis using STRs, we were able to confirm the biopsy from the tested embryo and POC was genetically identical, differentiating this case from a previously reported Turner syndrome discrepancy case after the transfer of a euploid male embryo, which was secondary to a spontaneous conception at the time of ET (17).

## CONCLUSIONS

Although PGT can be an informative and helpful test, it is a screening test that has limitations related to the biopsy procedure, testing methodology, and the potential for biological self-correction, which should be reinforced at the time of pretest counseling. There is a possibility of both false-positive and false-negative results. Although PGT-A overcalling is a concern, false-negative results can have an impact on patient care and result in adverse outcomes. Chromosome copy number thresholds in PGT-A algorithms must be carefully evaluated to balance the risk of both false-positive and false-negative results. Our case indicated a euploid male embryo that resulted in a miscarriage yielding monosomy X; STR analysis confirmed the pregnancy was from the tested and transferred embryo, and chromosome Y copy number depletion was observable on retrospective analysis, despite being within the euploid range, compatible with mosaicism with a monosomy X cell line. This case report describes a possible etiology of discrepancy cases and some additional testing that should be considered when a discrepancy is noted between PGT results and testing that occurs during or after pregnancy.

## CRediT Authorship Contribution Statement

Prapti Singh: Conceptualization, Investigation, Writing-original draft preparation, Writing—review & editing, Project administration. Alyssa Snider: Investigation, Formal analysis, Writing—review & editing. Refik Kayali: Investigation, Formal analysis, Writing-review & editing. Abigail Mancuso: Conceptualization, Investigation, Writing—original draft preparation, writing-review & editing, Supervision, Project administration.

## Declaration of Interests

P.S. has nothing to disclose. A.S. is a full-time employee of Igenomix. R.K. is a full-time employee of Igenomix. A.M. has nothing to disclose.

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