reproductive medicine

Case Report

A Case of Sex Discordant Dichorionic Diamniotic Twins after Single Embryo Transfer and the Importance of Zygosity Testing

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Abstract: Single embryo transfer (SET) is a technique used in assisted reproductive treatment (ART) that is used to promote singleton pregnancies. To date, there are five reported cases of dizygotic twin pregnancies with mothers who underwent SET. Here, we present a sixth case of a dichorionic, diamniotic twin pregnancy with sex discordance. The patient is a 34-year-old woman with unexplained secondary infertility who underwent in vitro fertilization (IVF) and frozen-thawed embryo transfer from a SET. The ultrasonographic images from the first and second trimester scans identified dichorionic, diamniotic twin gestations. The delivery was full term and postnatal genetic testing confirmed 46, XX, and 46, XY offspring. Pathology reports of the placental and membrane findings reported diamniotic, dichorionic twins. There was no zygosity testing conducted, thus it is unknown if the twins are monozygotic or dizygotic. Two possible etiologies for sex-discordant twins, in this case, are concurrent natural conception via breakthrough ovulation at the time of SET, or discordant postzygotic nondisjunction of a single embryo. Multiple gestations may still occur in the setting of SET and zygosity testing in these instances would better elucidate our understanding of this occurrence. Moreover, improved data on the zygosity of multiple gestations following SET may enhance patient counseling.

Keywords: single embryo transfer; zygosity testing; assisted reproductive treatment; sex-discordant twins; in vitro fertilization; breakthrough ovulation

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1. Introduction

Single embryo transfer (SET) is a technique used as a method to avoid multiple pregnancies in those who undergo assisted reproductive treatment (ART) [1]. SET is recommended as multifetal pregnancies are often associated with higher rates of complications for both mother and children [1,2]. Twin pregnancies may result from one embryo (monozygotic) or two embryos (dizygotic). The rate of monozygotic twins in ART is around 1–2%, twice that of spontaneous monozygotic twins [3,4]. A study conducted by Vega et al. analyzed 32,600 SETs and found a 2% risk of multiple pregnancies, of which up to 18% can be dizygotic [5].

With sex discordance among multifetal pregnancies, zygosity testing is indicated; however, many same-sex twin pregnancies can also be dizygotic and tend to be underexamined and thus underreported. To date, there are five reported cases of dizygotic twin pregnancies with mothers who underwent SET [6–9]. Here, we present a case of a dichorionic diamniotic twin pregnancy with sex discordance resulting from a SET.

2. Case Presentation

A 34-year-old female with an AMH of 2.0 ng/mL, BMI of 21.3 kg/m^2, and unexplained secondary infertility underwent in vitro fertilization (IVF) after several failed intrauterine inseminations (IUIs). Informed consent was obtained from the patient for the publication of this case report. Additionally, she had a history of endometriosis and subclinical hypothyroidism. She had one prior elective termination and no history of ectopic pregnancy or miscarriage. Her initial evaluation included hysterosalpingography, which showed a normal uterine contour and bilaterally patent fallopian tubes. Her husband's semen analysis indicated normozoospermia based on World Health Organization criteria [10].

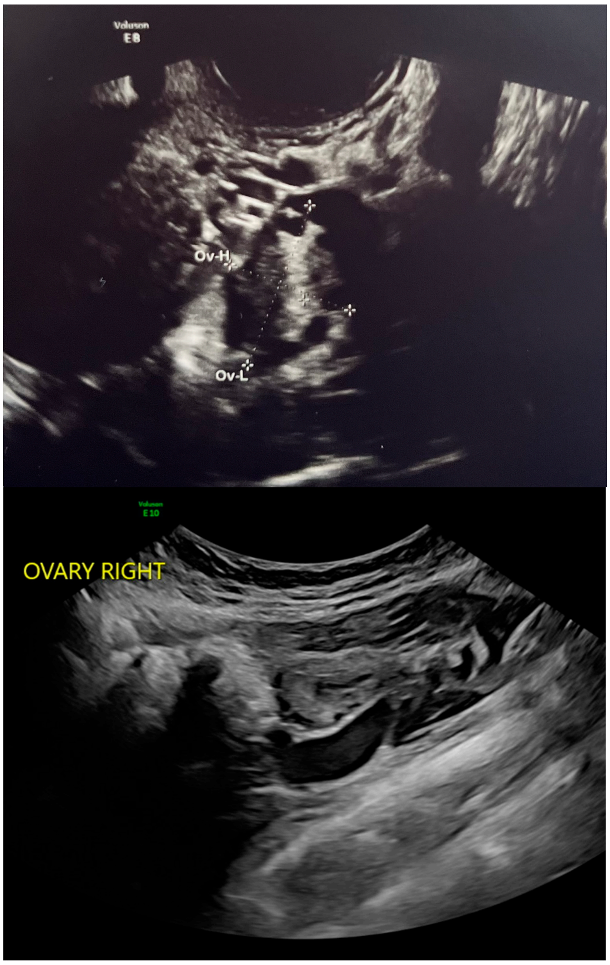
She underwent ovarian stimulation with gonadotropin-releasing hormone (GnRH) agonist suppression (total gonadotropin dose was 2125 IUs) and oocyte maturation triggered with hCG (7500 units) prior to oocyte retrieval. Her oral dose of estradiol was 6 mg daily and her vaginal dose was 2 mg twice daily. Estradiol level on the day of the trigger was 1869 pg/mL. A total of 17 oocytes were retrieved, 13 of which were mature and fertilized via intracytoplasmic sperm injection (ICSI). Three blastocysts developed and were vitrified on embryo culture days 5 (Figure 1) and 6. PGT-A was not performed on the blastocyst prior to transfer. She then proceeded with a programmed frozen embryo transfer cycle with a combination of oral and vaginal estradiol tablets, achieving an endometrial thickness of 9.7 mm on cycle day 17. The ultrasound study on cycle day 17 also showed a 13 mm follicle on the right ovary with a luteinizing hormone (LH) level of 13.4 IU/L and progesterone level of 0.8 ng/mL. The patient then initiated intramuscular progesterone 50 mg on cycle day 17. A single day 5, grade 3AA blastocyst was warmed for transfer and assisted hatching was performed by laser. The frozen embryo transfer occurred without difficulty on cycle day 22, which resulted in positive and rising beta hCG levels. It is unknown if the couple had unprotected intercourse at the time of ovulation.

Figure 1. Image of the single day 5, grade 3AA blastocyst post-thaw (**a**) and pre-transfer (**b**).

At 6 weeks 5 days gestation, two gestational sacs were identified via ultrasound. The ovaries were evaluated and no definitive corpus luteum was noted (Figure 2a). The patient underwent genetic counseling and agreed to initial noninvasive prenatal screening (NIPS) which suggested 46, XX and 46, XY fetuses. The ultrasonographic images from the fetal anatomic survey identified dichorionic, diamniotic twin gestations and again demonstrated no definitive corpus luteum cyst on either ovary (Figure 2b).

The delivery occurred at 38 weeks and 2 days gestation. The patient delivered a 2.74 kg male newborn via vacuum-assisted vaginal delivery and a 2.57 kg female newborn via normal spontaneous vaginal delivery. Postnatal genetic testing confirmed 46, XX and 46, XY offspring. Pathology reports of the placental and membrane findings reported diamniotic, dichorionic twins. The patient and her husband were counseled on zygosity testing to determine if the twins resulted from one embryo or two, but they declined this testing. The conditions for zygosity testing should be for any multifetal pregnancies resulting from a single embryo transfer. The necessity to conduct zygosity testing in sex discordant 46, XX and 46, XY twins is important to determine the pathological mechanism by which this has occurred. For 47, XXY and 46, XO mosaic twins, zygosity testing may also be useful to confirm zygosity as there may be different medical implications if the twins were monozygotic versus dizygotic.

a



b

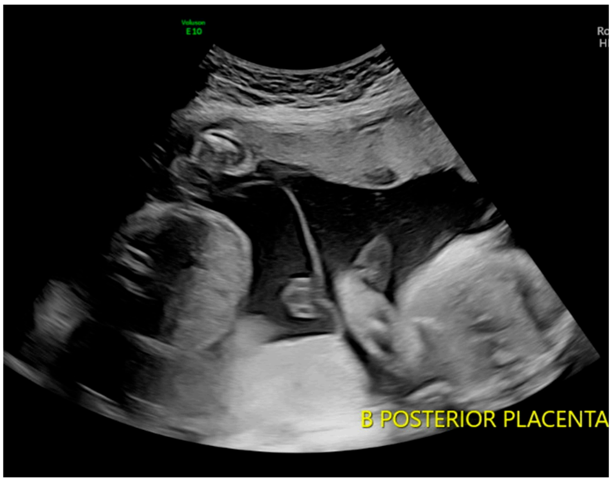


Figure 2. Sonographic evidence of dichorionic, diamniotic pregnancy and no definitive corpus luteum. (**a**). Early pregnancy ultrasound at 6 weeks 5 days gestation. (**b**). Anatomic survey ultrasound at 24 weeks 6 days gestation.

3. Discussion

This report presents a case of SET resulting in twins with sex discordance, indicating possible dizygosity. While a medical error in inadvertently transferring two embryos should always be considered a possibility, it would be nearly impossible due to standard procedural workflows in which a single embryo is directly visualized being drawn into the transfer catheter. The most plausible inference, which is also in agreement with previous case reports, is that the twins are a result of spontaneous conception concurrent with SET. Such occurrences of superfecundation are due to breakthrough ovulation despite ovarian suppression with estradiol during the programmed frozen embryo transfer cycle. Breakthrough, or escape ovulation, is an event that has been reported to occur in 1.9–7.4% of hormonally programmed frozen embryo transfer cycles without pituitary suppression [11,12]. It was noted that there was a 13 mm follicle on the patient's right ovary at the time of her lining check, and this follicle could have ovulated and become fertilized through spontaneous conception around the time of embryo transfer. A dyzygotic result for our patient (if she had pursued zygosity testing) would have confirmed this most plausible hypothesis.

Another possibility, although extremely rare, is that the dichorionic, diamniotic twins reported here resulted from a single embryo. Although monozygotic twins are assumed to be genetically identical, it has been established that monozygotic twins may have phenotypic and genotypic differences [13,14]. Modification of the original zygotic genome may occur via various mechanisms such as blastomere allocation and postzygotic genetic, epigenetic, or environmental events [13,15–17]. These modifications of genetic material may result in two distinct cell populations, and depending on the timing of the event relative to zygotic cleavages, these genetic differences may be seen in multiple somatic tissues or may be mosaic [13,18]. Discordant postzygotic nondisjunction, or crossing over, could result in sex-discordant monozygotic twins. This can be described as confined twin mosaicism, where postzygotic nondisjunction in one twin resulted in heterokaryotypia for the chromosomes involved in sex determination [13].

Sex chromosome discordance is the most common, although still an extremely rare, type of genetic discordance in heterokaryotic monozygotic twins. This is most commonly seen with male twins where one twin's karyotype becomes 45,X (Turner syndrome) from the loss of the Y chromosome by nondisjunction in early development (Figure 3a) [16,19]. This same mechanism can describe the generation of monozygotic 46, XX and 46, XY twins from a 47,XXY zygote, resulting in twins with varying degrees of mosaicism (Figure 3b) [13,20]. A monozygotic result for our patient (if she had pursued zygosity testing) would have confirmed this much rarer scenario.

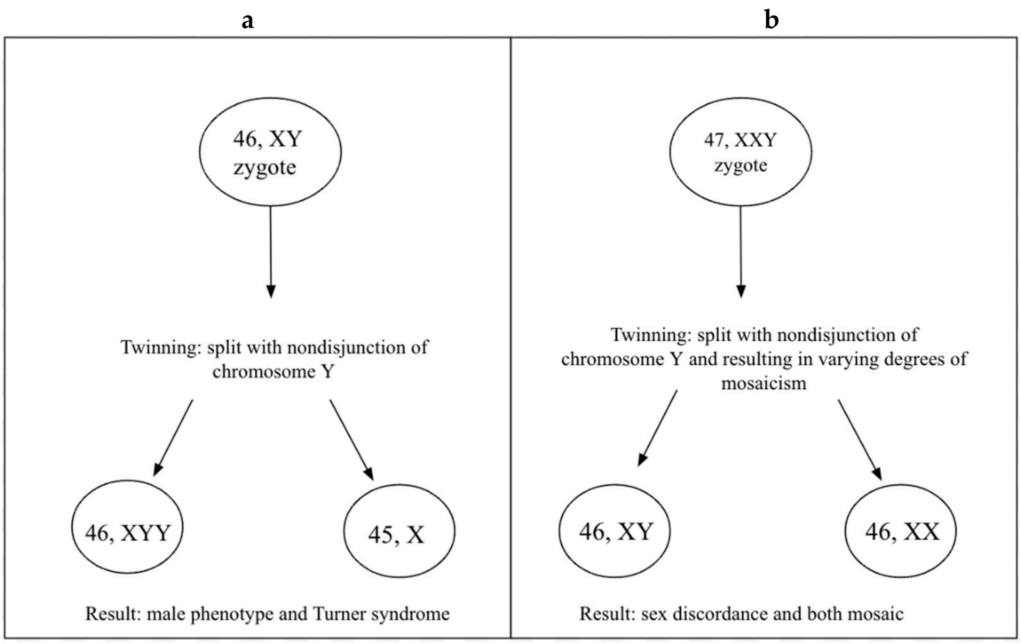


Figure 3. Mechanism of sex chromosome discordance. (**a**) Turner syndrome. (**b**) Monozygotic twinning resulting in 46, XX and 46, XY karyotypes.

Previous studies have shown increased rates of monozygotic twin pregnancies following blastocyst transfer [21,22]. Due to this literature, physicians may assume that twin pregnancies resulting from ART are monozygotic. However, it is important that they assess amnionicity and chorionicity via ultrasonography and supplement that with other methods such as placental and membrane pathology, chromosome findings, infant blood typing, and HLA typing to confirm the zygosity [23]. At our institution, zygosity testing is performed by extracting DNA from the twins and both parents, then identifying specific alleles on a panel of highly polymorphic short tandem repeats (STR) markers to determine STR allele profiles [23]. In recent years, there has been a trend toward conducting zygosity testing due to erroneous assumptions that dichorionic twins are dizygotic or that phenotypic similarity indicates monozygosity [24]. The importance of zygosity determination has been acknowledged in twin populations for medical reasons (risk of disease, organ donor compatibility, etc.) and further financial decisions regarding costs for treatment or management of medical conditions. From the scientific community perspective, twin populations are greatly important to many studies, and inaccuracies in zygosity can negatively impact research findings. As it relates to our field, zygosity testing will improve our understanding of factors impacting multiple gestations in single embryo transfer for IVF and will help guide decisions for patients [24,25].

4. Conclusions

SET is now often recommended to prevent multiple gestations and their associated risks. It is important to recognize that multiple gestations may still occur in the setting of transferring a single embryo, although this is rare. Increased uptake of zygosity testing can provide more insight into natural pregnancy occurrences during ART cycles or may give more credence to the theory of embryonic cleavage forming dichorionic, diamniotic twins. Additionally, with improved data on the zygosity of multiple gestations following SET, the true risk of unprotected intercourse during the embryo transfer cycle can be determined and patients can be better counseled regarding their risk of multiple gestations.

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