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A Case of Monochorionic-Diamniotic Twin Pregnancy with Differing Phenotypic Sex

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Abstract

A 37 year old Asian woman G2P0010 with a known twin gestation at 30 weeks 2 days by in vitro fertilization dating, immigrated to the United States from China to continue her prenatal care. She had three embryos conceived by intracytoplasmic sperm injection (ICSI) that were transferred on day 3. First-trimester ultrasound reports from China revealed monochorionic diamniotic twin pregnancy. On presentation, to our office, an ultrasound showed Twin A with male genitalia and Twin B with female genitalia and a thin dividing membrane which was consistent with monochorionicity. Placental pathology also confirmed monochorionicity. This case explores a potential mechanism for differing fetal sexes in a case of monochorionic-diamniotic twins due to fusion chimera formation. Chimera formation has been thought to occur via disruption of the zona pellucida during the ICSI process and transfer of cells between different embryos. As assisted reproductive technologies become more prevalent, exploring the developmental pathogenesis and genetics of twinning is warranted to better understand discordant findings from prenatal ultrasound and genetics. The interactions of the transferred embryos could lead to sustained stem cell chimerism that is present after birth.

Keywords : Amnion, Chimera, Chorionicity, Embryos, Fetal sex, Intracytoplasmic Sperm Injection, Mosaicism

Introduction

Monochorionic twins are typically the result of the division of one fertilized zygote, thus monozygotic. Twins with differing fetal sex are almost always dizygotic. Rarely, monozygotic twins can result in differing fetal sex due to somatic mutations or chromosome aberrations. Most reported cases are postzygotic loss of the Y chromosome which leads to a female twin with Turner Syndrome. Discordant sex-chromosome chimerism with karyotype (XX/XY) is a rare chromosomal abnormality in humans [1]. This case presents an example of chimeric monochorionic twins leading to discordant phenotypic fetal sexes and explores an embryologic mechanism for chimera formation.

A chimera is the fusion of two different genetic lines into a single embryo via admixture of cells while the embryo becomes a fetus. Different mechanisms of chimeras have been proposed. Fusion chimeras are formed when two zygotes fuse together during the early embryonic stage and "twinning" chimeras occur due to transfusion of blood products between two embryos across a fused placenta. These mechanisms are in contrast to a mitotic error event in a single zygote, which results in mocaism [2].

The first case of XX/XY chimera was first described in 1962 but incidence of this karyotype is not known. The XX/XY chimera can have a spectrum of genital phenotypes, ranging from normal male or female genitalia to different variations of ambiguous genitalia [3]. A few case reports exist about chimeras after IVF and there is potential for damage to the zona pellucida from ICSI leading to mixing of embryos and formation of fusion chimera.

Cases of XX/XY chimeras after IVF have been described in recent literature, but few describe multiple gestations. Theoretically, the risks of "fusion" or "twinning" chimerism may be higher in IVF cycles where two or more embryos are transferred and that implant together during a treatment cycle. Often the karyotype and genetics are unknown until later in pregnancy via sampling of amniotic fluid or a sample of buccal cells, skin and/or blood of the neonate. The diagnosis of

chimera and suspected time period during the intricate process of embryogenesis and fetal development that chimerism occurred can be analyzed if these results are available [4].

Determining a monochorionic placenta requires an assessment of amnionicity and chorionicity is less accurate in the second and third trimesters. (Pretorius, 1993) reports 90% accuracy of dichorionic-diamniotic placentation and 72% of monochorionic-diamnoitic placentation based on membrane thickness alone [5]. It is possible to have an error in chorionicity via ultrasound resulting in misdiagnosis of monochorionicity when ultimately the twins are dichorionic.The final placenta and membrane pathology is also diagnostic and can confirm chronicity as it did for the pregnancy described in this case.

Materials and Methods

This is a case report of one twin pregnancy of advanced maternal age who conceived via in vitro fertilization. The case was analyzed in the setting of current literature on the topic of chimera formation. Literature review, electronic medical record review and patient interview at time of presentation were used to summarize this unique case. A combination of the expertise of Reproductive Endocrinology and Infertility and Maternal Fetal Medicine departments were utilized to analyze paper records from China and United States electronic medical records.

Results

The pregnant patient was a 37-year-old G2P0010 at 30 weeks and 2 days gestation with a twin pregnancy. Gestational age dating was performed based on day 3 embryo transfer. She transferred care to our center from a medical institution in China. She had three embryos transferred on her 7th cycle of in vitro fertilization. One was a 5 cell frozen embryo and the other two were fresh day 3 transfers. During work-up for infertility, the father of the pregnancy was found to have a Y chromosome microdeletion (YCM) known as AZF. It is not recorded in the medical record if this was AZFa, AZFb or AZFc. The AZF mutation in general is linked to reduced sperm count and oligozoospermia and azoospermia often requiring ICSI to be performed. Maternal medical history was significant for mild anemia. In China, the pregnant patient was told that she had monochorionic-diamniotic twins which was determined by a first trimester ultrasound.

The ultrasound performed in China at 12 weeks and 5 days reported monochorionic-diamniotic twins, however, did not give a membrane thickness or description of placentation. An ultrasound at 10 weeks had been performed and only commented on two separate amniotic sacs. Non-invasive prenatal testing was low risk for Trisomies 13, 18 and 21 but did not report on sex chromosomes. Nuchal translucency measurements were low risk at 1.3 mm and 1.1 mm. Fetal echocardiograms were normal. Prior to presenting at our institution Twin B which was female by ultrasound was noted to have fetal growth restriction in early third trimester but normal fluid and only a 23% discordance from Twin A. Twin B had elevated umbilical artery Doppler systolic to diastolic ratio and normal middle cerebral artery Doppler measurements.

Once transferred to our Maternal Fetal Medicine clinic, the patient was counseled on the rarity of differing sex in monochorionic-diamniotic twins and about antenatal surveillance for fetal growth restriction for Twin B. She had an ultrasound on this initial prenatal visit at 30 weeks and 2 days and Figure 1 shows the membrane thickness measured 0.27cm on this exam. Serial ultrasounds (Figure 2) and Dopplers were performed on the twins to monitor fetal growth restriction of Twin B. She was induced at 37 weeks and 5 days and had a spontaneous vaginal twin delivery with cephalic Twin A and breech extraction of Twin B. Placental pathology (Figure 3) confirmed monochorionic membranes.

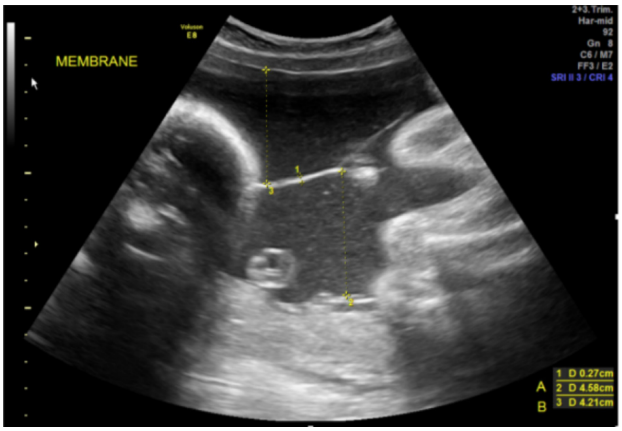


Figure 1: 30w2d ultrasound showing membrane thickness of 0.27cm

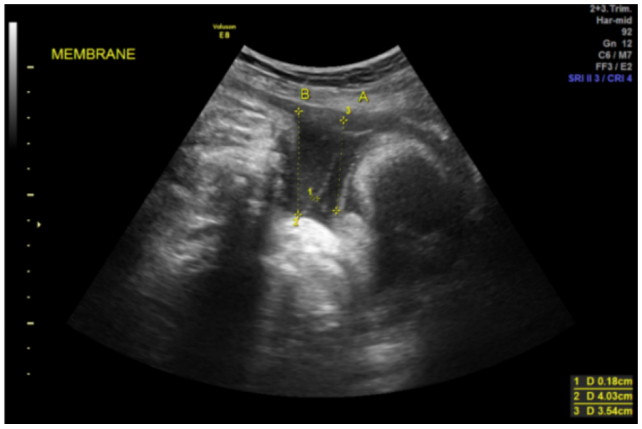


Figure 2: 33w2d ultrasound showing membrane thickness of 0.18cm.

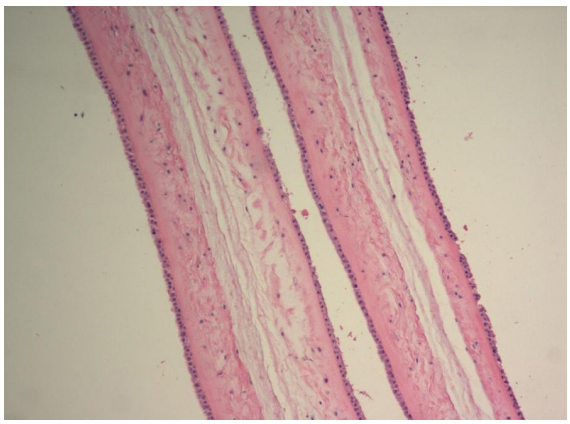


Figure 3: Final placenta pathology with H&E stain showing monochorionic membranes.

Both neonates were followed by medical genetics after delivery. Twin A was born phenotypically male with normal male genitalia, palpable testes with mild hydroceles. The chromosome microarray analysis was a normal male. The fetal karyotype for Twin A showed genetic chimerism 46, XY [17]/ 46, XX [3] as seen in Figure 4. The fluorescence in situ hybridization (FISH) taken from neonatal blood for sex chromosomes confirmed the genetic chimerism. There were 200 cells of Twin A analyzed in FISH evaluation. FISH showed that 184 (92%) were XY and 16 (8%) were XX. The SRY gene was present on all Y chromosomes and was absent on the X chromosomes.

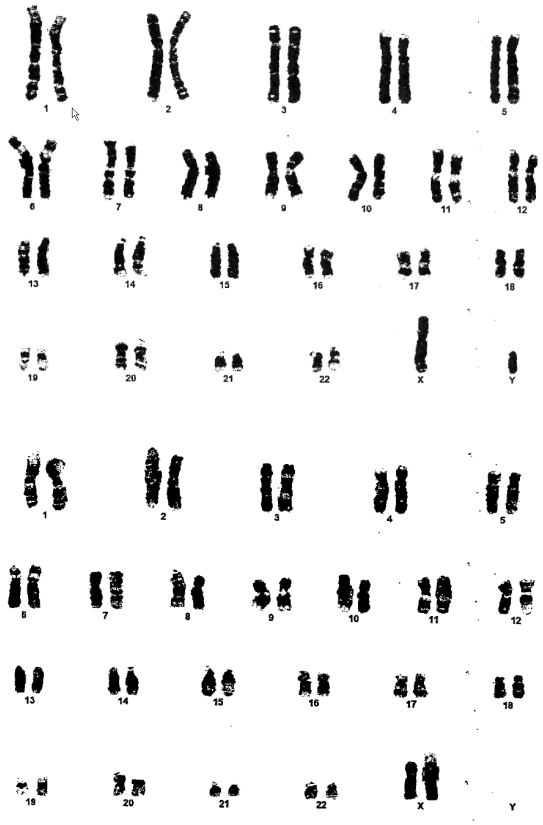


Figure 4: Twin A karyotype: 46,XY [17]/ 46,XX [3]

Twin B was born phenotypically female with anatomically female genitalia without clitoromegaly nor labioscrotal rugation or pigmentation. The neonate did not appear to have a Turner's phenotype on the physical exam. The chromosome microarray analysis on the blood sample was a normal male. The karyotype for Baby B showed genetic chimerism with 46,XY [95] /46XX [5] as seen in Figure 5. FISH performed on blood sample for sex chromosomes showed of the 40 cells analyzed 38 (95%) were XY and 2 (5%) were XX. The SRY gene was present on all Y chromosomes and was absent on the X chromosomes.

The uterus and vagina of Twin B were viewed on pelvic ultrasound of the neonate shortly after birth, however the gonads were not visualized. This ultrasound is shown in Figure 6. Medical genetic specialists recommended an ultrasound to better characterize the internal anatomy. Pediatric urology consult recommended possible laparoscopy and laparoscopic gonadal biopsy after further endocrine testing. Twin B was seen by a pediatric endocrinologist who obtained Mullerian-inhibiting hormone (0.143ng/mL) and Inhibin B (12.1 pg/mL) which were found to be low. The pediatric endocrinologist reported the low levels were expected in the presence of a uterus and a predominantly XY karyotype. When Twin B was four months old, pelvic ultrasound showed a normal-appearing uterus and ovaries. Due to the normal-appearing ovaries on ultrasound, the pediatric urologist recommended deferring laparoscopic inspection or biopsy of the gonads unless there was evidence of virilization. At one year old, pelvic ultrasound showed a uterus smaller in size compared to prior ultrasound and left adnexa with homogenous internal architecture. The right adnexa was not visualized.

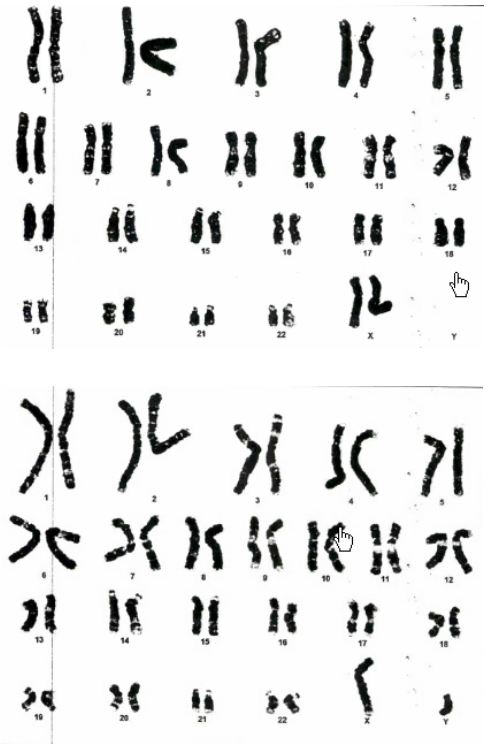


Figure 5: Twin B karyotype: 46,XY [95] /46,XX [5]

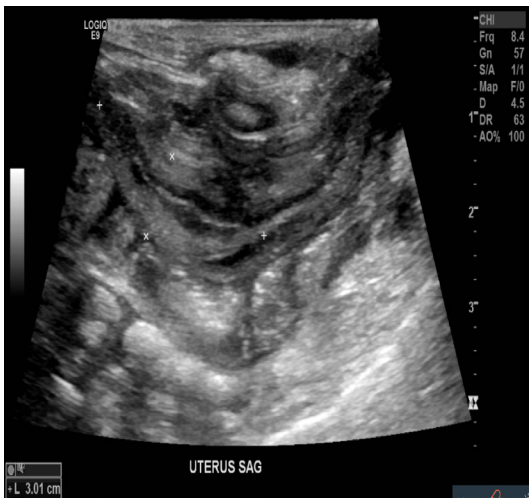
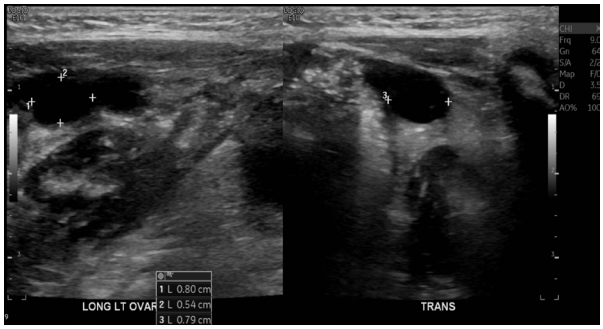


Figure 6: Twin B pelvic ultrasound at 11 days of life with endometrial stripe and uterus.



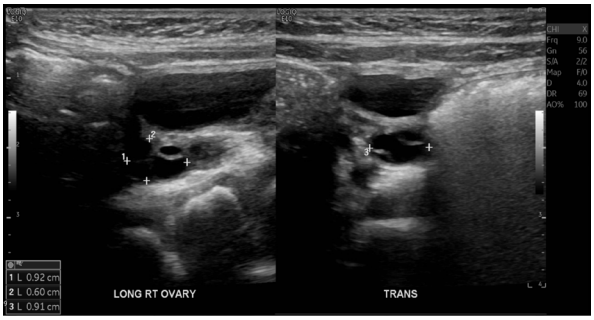


Figure 7: 4 month pelvic ultrasound of Twin B showing bilateral ovaries.

Discussion

Chimerism is the presence of two separate genetic cell lines within a single individual. This occurs with a spectrum of phenotypic consequences particularly with chimerism of the sex chromosomes. This case represents the possibility of an anatomically female phenotype in the setting of a chimeric monozygotic twin pregnancy of XX/XY. How and when the chimerism embryologically occurred is analyzed below.

Madan (2020) has recently categorized chimeras into three subgroups: "microchimeras" due to feto-maternal cell trafficking across the placenta, "fusion chimeras" derived from an admixture of cells from two zygotes, and "twin-chimeras" involving blood transfusions within a fused placenta [1]. For the latter two types, chimeric cells derive from two genetically distinct or tetraparental embryos following some type of "amalgamation event". Theoretically, the risks of this latter type of chimerism may be higher in in vitro fertilization cycles such as the one described when two or more embryos are transferred and that implant together during a treatment cycle. In such cases, close proximity during implantation could facilitate a monochorionic-diamniotic implantation, which may further increase the risk for such inter-embryonic and fetal cell mixing events. However, it remains to elucidate the specific spatio-temporal pathways that result in a sustained mixing between two cell genotypes following embryogenesis.

In the context of in vitro fertilization, there have been at least four such reports of chimerism. The first two reports were in singletons. Strain et al. (1998) examined aborted fetal tissue from both peripheral blood and fibroblasts biopsied from skin which was presumably mesoderm [6]. They found both 46,XX and 46, XY genotypes having tetraparental origins. Simon-Bouey (2003) reported tetraparental 46,XX and 46, XY chimerism in cells taken at amniocentesis in an aborted dysmorphic fetus associated with a multiple cleavage stage embryo transfer [7]. No other fetal cell studies were reported outside of amniotic fluid in that case. Lastly, after a multiple blastocyst transfer leading to a sex-discordant twin delivery at 37 weeks, Souter et al. (2003) reported tetraparental 46, XY [96] and 46, XX [4] chimerism in the anatomic female and 46, XY [46] and 46, XX [4] in the anatomic male. Although skin biopsies at 5 months did not show similar chimerism, placental studies using FISH did show signs of chimerism localized in areas of each fetus's membranes [8]. These authors were the first to suggest that some fusion or amalgamation event between embryonic trophoblasts around the time of implantation could explain their findings without specifying specific cellular details.

More recently, Fumoto et al. (2014) reported results of chimerism in a sex discordant twin delivery after a double blastocyst in vitro fertilization transfer and associated with a monochorionic-diamniotic implantation [9]. While both twins showed chimerism in blood, one of the twins also showed chimerism of endoderm origin in buccal cells when evaluated by microsatellite markers after DNA amplification. This confirmed the report by Strain et al. (1998) of somatic cell chimerism after in vitro fertilization in addition to blood elements, although Fumoto et al did propose that chimerism may have arisen after migration of hematopoietic cells to the buccal membrane secondary to some injury event in that anatomical region [6]. In a case of naturally conceived dizygotic twins rather than via in vitro fertilization, Rodgiguez-Buritica et al. (2015) also reported chimerism of both skin and blood elements in twins having a monochorionic-diamniotic implantation. Male Twin A had chimerism in blood, buccal cells, and "skin" from undescribed region however, Female Twin B had only blood chimerism [10].

The featured case described above shows chimerism with multiple embryo transfers in in vitro fertilization cycles and monochorionic-diamniotic implantations. It raises a central question of, "*How do transferred embryos interact in a*

way that results in stem cell exchanges leading to sustained chimerism after birth?". There have been anecdotal reports that trophectoderm from two blastocysts that are in the process of hatching, or that have hatched from the zona pellucida shell while together in cultured microdrops, can often become strongly adherent [11]. If two blastocysts are transferred together and become situated nearby in the endometrial cavity, such trophectoderm adhesion prior to or at the time of implantation could result in what would develop into a monochorionic-diamniotic placenta later. This could have been the case with our pregnant patient given the earliest ultrasound just commented on "two sacs," but not placenta. This was followed by a 12 week ultrasound report of a monochorionic-diamniotic pregnancy.

Moreover, if fusion occurs during the process of hatching, the two blastocoel cavities would lie in close proximity as the primary yolk sacs grow during the second and third weeks of implantation. Each yolk sac sequentially acquires new inward migrating germ layers of extraembryonic endoderm and mesoderm from the embryo during gastrulation during these first few weeks. In fact, fused yolk sacs in twin pregnancies have been reported in cases associated with a forked umbilicus in a monochorionic-diamniotic pregnancy [12]. A close apposition of yolk sacs from closely implanting blastocysts would bring into proximity their developing blood islands. Initially, these islands are not initially enclosed within any vasculature; thus, proximal mixing between these earliest of hematopoietic progenitor stem cells could result in chimeric mixing. This would be followed by secondary co-migration through the developing vitelline vasculature to each embryo's fetal liver and/or other para-aortic sites where they contribute to the next stages of fetal hematopoiesis. These migration of hematopoietic stem cells from the yolk sac to both the liver and para-aortic areas have been well described [13-17]. There remains controversy over the sustained life of these earliest "primitive" erythroid and multipotent hematopoietic stem cells that form the yolk sac. Yet, surface markers associated with some of these cells offer evidence that some migratory cells remain in the adult contributing to sustained somatic chimerism [18].

In addition to hematopoietic elements, it should also be noted that the yolk sac is also the site of formation of the primordial germ cells, which then migrate to the fetal gonad using the hindgut as a migratory scaffolding. A similar mixing of these germ cells between closely implanting embryos, as suggested above for hematopoietic stem cells, could explain the phenomenon of true hermaphroditism, which is characterized by the presence of both XX and XY germ cells in the same gonad. In fact, this was also reported in the chimerism described above by [6]. Although from naturally conceived twins, one case subsequently described a twin with ambiguous genitalia that had true hermaphroditism based upon gonadal touch imprints showing 46, XX and 46, XY [8]. The twins also were chimeric in peripheral blood lymphocytes and skin fibroblasts [19].

The extent to which sustained chimerism could, additionally, arise by the later passage via shared placentas of early differentiating stem cells from the liver or para-aortic areas, still remains unclear. As each fetus becomes enclosed and isolated from body folding during the fourth week, the yolk sacs still remain externalized. The yolk sacs are only incorporated into the midgut later where they eventually even become vestigial as the Meckel's diverticulum. Thus, the structure of the yolk sac remains an appealing site to seriously consider as possibly the first site of cellular chimerism and trafficking that can also involve the embryo's germ layers. The blood elements are mesodermal, the buccal membrane is endodermal and the skin has ectodermal components. Such sustained cellular diversity seems unlikely to occur simply via compartments of a shared placental circulation. These developmental issues might be more appropriately investigated using newly developing ex-vivo mouse embryo cultures, where more targeted cell and tissue transplantation procedures can be designed using defined genetic markers.

Limitations of this particular case report are the lack of investigation into different types of somatic cells and parental DNA comparison. An area of future study in this case report's neonates as well as future similar cases would be to determine the mechanism and temporal-spatial episode of chimerism. Unfortunately, in this case we are presently unable to definitively conclude when embryologically the fusion occurred given genetic samples were analyzed only from blood which is mesodermal. To our knowledge other somatic cells were not similarly analyzed. FISH was performed, but this also only on blood. Thus the extent of somatic cell chimerism found is unknown making it difficult to discern at which point: hatching and trophectoderm implantation, blastocoel fusion, yolk sac rich with primordial germ cells, migration of hematopoietic stem cells or simply placenta transfer. A FISH or microarray evaluation of various germ line tissues of the twins such as buccal for endoderm or skin for ectoderm would be useful but only blood was analyzed [20].

Another area of future study would be comparisons with parental DNA via comparison of each chromosome single nucleotide polymorphisms or other markers such as pericentric markers reflecting crossover to establish conclusively that this case occurred with one egg and one sperm versus two eggs and two sperm. Current literature shows that both scenarios might explain our specific case. Ultimately the proportion of undifferentiated XY gonad cells is important, irrespective of the proportion of XY cells elsewhere in the body and may explain the phenotypic differences given the similar karyotype and FISH results in this case. Implications for the fertility of the phenotypically female neonate are unknown at this time and require further prospective study.

Conclusion

This case explores a potential mechanism for differing fetal sexes in a case of monochorionic-diamniotic twins due to fusion chimera formation. We suspect this was from a disruption of the zona pellucida during the ICSI process and

transfer of cells between different embryos. At this moment in time, phenotypically discordant sex in monochorionic twins is extremely rare and can be difficult to counsel patients on what to expect for their future child or children. It is possible that future similar cases may arise for more in depth study of when and how and when chimerism occurs. Given the unique zygote fusion mechanism required to create a chimera and continued use of ICSI in vitro fertilization, it is possible to see more cases similar to this one.

Acknowledgement

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