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ASSISTED REPRODUCTION TECHNOLOGIES

Testicular hypoplasia in monochorionic dizygous twin with confined blood chimerism

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Introduction

Monochorionic diamniotic (MCDA) twins identified by ultrasound in early pregnancy are rarely dizygotic. Since Shouter et al. first reported that MCDA twin pregnancies can occur in dizygous (DZ) twin pregnancies, there have been about ten reports of such cases [1]. MCDZ twins can be easily overlooked except in cases of discordant sex discovered during prenatal care or delivery of MCDA twins. In our case, chimerism of a set of MCDZ twins was confirmed by STR analysis for both parents and the live male twin after the discovery of discordant sex in a pair of MCDA twins at delivery. Testicular atrophy was suspected in a follow-up sonogram of the testes of the live male twin; Testicular hypoplasia was revealed by a subsequent testicular biopsy. We report herein a rare case of testicular hypoplasia in a male infant with chimerism that was observed on follow-up examination after birth.

Capsule Testicular hypoplasia can occur in a chimeric male born with a female co-twin.

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Case report

An exemption from the Institutional Review Board (IRB) approval process was obtained for this study, because the patient signed informed consent about the proposed surgical treatment. All identifying patient information has been excluded from this publication.

A 31-year-old woman (para 0-0-2-0) visited our fertility center for the treatment of secondary infertility. After failing five cycles of timed intercourse (TI) and two intrauterine inseminations (IUI), she conceived via in vitro fertilization and embryo transfer (IVF-ET). The patient's ovaries were stimulated with a combination of recombinant follicle-stimulating hormone (FSH) (150 IU) subcutaneous and human menopausal gonadotrophin (hMG) (150 IU) intramuscular per day. When the leading follicle reached a size of 14 mm, cetrorelix 0.25 mg subcutaneous was administered daily until the day of human chorionic gonadotrophin (hCG) injection. A total of 12 oocytes were retrieved 35 h after hCG administration. Nine oocytes were fertilized normally via intracytoplasmic sperm injection (ICSI). Three embryos were transferred without complications under transabdominal ultrasound guidance on post-oocyte retrieval day 3, after assisted hatching (AH), while the remaining six embryos were cryopreserved. Thirteen days after embryo transfer (ET), the patient's serum β-hCG level was 540 mIU/mL.

At 6.1 weeks gestation, ultrasound examination showed two fetuses and two yolk sacs within a single gestational sac. At 8.8 weeks gestation, a monochorionic diamniotic twin pregnancy was confirmed. Nuchal translucency measurements of both twins were within normal range and both twins had low adjusted risks for trisomies 21 and 18. Ultrasound examinations at 17, 23, 28, and 32 weeks gestation showed consistent growth of both twins. No developmental abnormality was observed in either twin until 33.8 weeks gestation.

At 33.8 weeks gestation, the intrauterine death of one fetus was noted on ultrasound. The patient was admitted to the hospital and delivered by cesarean section. Twin A was a male weighing 2,160 g with Apgar scores of 7 and 8 at 1 and 5 min, respectively. Twin B was a female weighing 2,355 g with Apgar scores of 0 at both 1 and 5 min. The placenta and membrane appeared monochorionic and diamniotic on macroscopic inspection.

Methods

Karyotype studies

Blood samples were collected from both parents and the live male twin, and karyotyping was performed. Skin fibroblasts were obtained from the live male twin, on which karyotyping was performed; karyotype testing was not performed for the dead female fetus.

STR analysis

Multiplex STR amplification method is generally used for individual identification. We applied this STR analysis to determine if the twins were monozygotic or dizygotic. Sixteen genes including 15 STR genes and the amelogenin gene were analyzed. Short tandem repeat (STR) analysis was performed for peripheral blood lymphocytes of both parents. Peripheral blood, hair, and tissue samples were collected from the live male twin, and STR analysis was conducted. The placenta was composed of part of the live male infant, part of the dead fetus, and a mixed portion composed of both the live male infant and dead fetus. STR analysis was performed for each of these three parts of the placenta.

Gonad examination

From birth to 7 months of age, pelvic sonogram was conducted on the bilateral testes periodically. Testicular biopsy was performed together with lingual frenuloplasty under general anesthesia at 8 months of age.

Endocrine studies of the live male twin

FSH, LH, and testosterone levels were measured, and the hCG stimulation test was performed at 3 months of age. An endocrine study was also performed for FSH, LH, and testosterone at 5 months, 7 months, and 39 months of age.

Results

Karyotype studies

The blood karyotypes of the father and mother were 46, XY and 46, XX. The blood karyotype of live male twin was 46, XX [22]/46, XY [14] (Table 1). The skin fibroblast karyotype of live male twin was 46, XY.

STR analysis

STR analysis for blood of both parents showed normal genotypes. Three or four alleles per locus suggesting a definite mixture of two people were observed at the CSF1PO, TPOX, D18SS1, DSS919, and FGA loci in the peripheral blood of the live male twin (Table 1). None of the surplus alleles were

Table 1 Results of short tandem repeats in blood samples

	Locus	Father		Mother		Twin A	
Sample		blood		blood		blood	
Blood karyotype		46, XY		46, XX		46, XY[22]; 46, XX[14]	
		allele 1	allele 2	allele 1	allele 2	allele 1 paternal allele	allele 2 maternal allele
CSF1PO	5q33.1	14	12	11	10	12	11,10
TPOX	2p25.3	11	10	12	8	11, 10	12, 8
D18S51	18q21.33	19	13	15	15	19, 13	15
D5S818	5q23.2	12	12	13	11	12	13.11
FGA	4q31.3	22	18	26	23	22, 18	26

inconsistent with alleles of the father or mother. Besides this, unbalanced peak height suggesting a mixture of two different people was also noted in five additional STR loci, D7S820, D13S317, D16S539, vWA, and amelogenin. Three or four alleles per locus were not found in STR analyses of tissue, hair, or placental samples from live male twin. These results indicate that live male twin had confined blood chimerism, and that the live male infant and dead fetus were dizygous twins.

Gonad examination

Physical examination of the live male infant was performed just after birth, including the genital area. No abnormal findings were observed excepting genital abnormalities and tongue tie. The scrotal size was small, and the right testis was larger and rounder than the left. The right testis was located in the scrotal sac, while the left testis was located in the left inguinal canal. The right testis was hypoechoic with surrounding echogenic scrotal wall in sonographic findings just after birth. A pelvic sonogram of the live male twin was performed 7 months after birth. The size of the right testis was decreased and the left testis was undescended. Eight months after birth, a testicular biopsy was performed together with ligual frenuloplasty. The left testis was found in the spermatic cord of the inguinal canal and was severely atrophied, with only fibrous tissue observed upon biopsy. The right testis was not found in the spermatic cord; peripheral

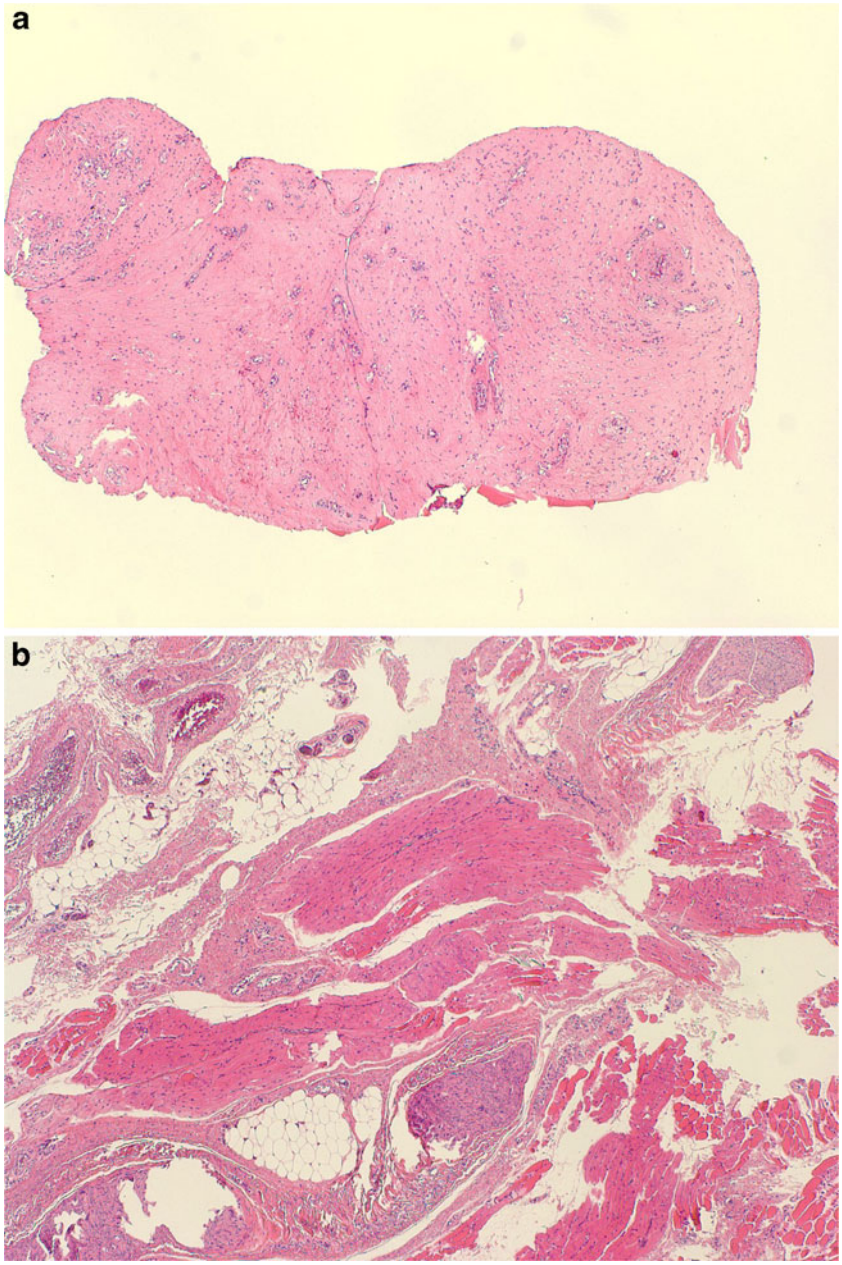

Fig. 1 Pathologic results of testicular biopsy. **a** Left testis. The left testis was found in the spermatic cord of the inguinal canal and was severely atrophied, with only fibrous tissue observed upon biopsy (×40) (panel **a**). **b** Right spermatic cord. The right testis was not found in the spermatic cord. The peripheral nerve and vascular bundle were seen upon biopsy of the right spermatic cord (×40) (panel **b**)

nerve and vascular bundle were seen upon biopsy of the right spermatic cord (Fig. 1).

Endocrine studies of the live male twin

Endocrine evaluation was performed at 3 months of age because of the atrophic appearance of the scrotum and testes, and scrotal sonography revealed small, hypoechoic testes. LH and FSH levels were increased to 44.6 IU/L (reference range 0.02–7.0) and 141.8 IU/L (reference range 0.16–4.1), respectively, and testosterone was decreased to 0.06 ng/mL (reference range 0.6–4.0), which failed to respond after hCG stimulation.

The endocrine evaluation was repeated at 5 months of age because the atrophic change of scrotum and penis progressed, and the testes were not palpable. The parents were very anxious about the progressive atrophic change, and testosterone was replaced to help foster penile growth.

The endocrine tests were repeated after testosterone replacement. Endocrine results obtained at 7 months of age were FSH of 2.49 mIU/ml, LH of 0.24 mIU/ml, within normal range. After that, the patient was lost to follow-up until 39 months of age. On the follow-up visit at 39 months of age, LH was very low (<0.07 mIU/mL); however, FSH was still higher (9.57 mIU/mL) than the reference range at this age (0.26–3.0).

Discussion

In the present case, chimerism was confirmed by observing three or four alleles at the CSF1PO, TPOX, D18SS1, DSS919, and FGA loci and unbalanced peak height at several loci in STR analysis of blood samples of the live male infant. However, other tissues such as hair, skin, and placenta did not exhibit chimerism. Confined blood chimerism (CBC), as observed in this case, is common in MCDZ twins. CBC may result from exchanges of blood stem cells due to placental vascular anastomosis through the common placenta [2]. When information about CBC is lacking, HLA typing, blood typing, and genotyping for disease susceptible genes may be misinterpreted [3].

Due to the reduction in maternal estradiol, after birth gonadotropins typically increase to pubertal levels in normal male infants. Subsequently LH and FSH levels decrease, reaching their nadirs at mid-childhood [4]. Gonadotropin levels in this phenotypic male changed such as that of normal boy. FSH levels decreased until 7 months of age, but had increased slightly at 39 months of age. Although the value of FSH in a normal boy usually decreases to a very low level in this period, FSH increase seems to be related to severe gonadal failure. Low testosterone level before and after the hCG stimulation test indicates that the bilateral testes are not functioning properly. Although a transient increase of testosterone was noted after administration of testosterone for 3 months, the testosterone level measured at 39 months of age was still below the normal range without external administration of testosterone. The low testosterone levels were also related to gonadal failure.

The right testis was noted on ultrasonogram after birth, but atrophy progressed, and no testicular tissue was found on pathologic examination of a testicular biopsy. The left testis was suggested to be undescended in the inguinal canal and thereafter to be atrophied. There may be a few possible mechanisms for the etiology of testicular hypoplasia like in this case. Testicular torsion has been reported to lead to

Table 2 Previous reports about monochorioic dizygous twins

Case	Conception method	USG finding	Fetal gender	CBC
2003 Shouter	IVF (ICSI)/Blastocyst culture, oocyte donation	MCDA	nl. boy/nl.girl	CBC
2003 Nishio	IVF-ET	MCDA	nl. boy/nl.girl	CBC
2003 Tsuruta	Superovulation and IUI	MCDA	nl. boy/nl.girl	NA
2003 Yamaguchii	TESE-ICSI	MCDA	nl. boy/nl.girl	CBC, triplet→MCDA reduced
2004 Niikawa	IVF-ET	MCDA	nl. boy/nl.girl	NA
2004 Niikawa	IVF-ET	MCDA	nl. boy/nl.girl	CBC, chimeric ABO blood group
2004 Williams	IVF (ICSI)/AH	MCDA	nl. boy/ambiguous genitalia	CBC
2005 Ginsberg	CC-TI	MCDA	nl boy/nl.girl	NA, amniotic fulid STR; dizygocity
2006 Aoki	CC	MCDA	nl.boy/nl.boy	CBC, different ABO typing(AB/B)→DNA polymorphism analysis: CBC
2007 Walker	IVF-ET	MCDA	nl.boy/nl.boy	CBC, subtle phenotypic facial difference between the twins
Current study	IVF (ICSI)/AH	MCDA	Boy with testicular hypoplasia/nl.girl	CBC

TESE testicular sperm extraction; *ICSI* intracytoplasmic sperm injection; *CC* clomiphen citrate; *TI* timed intercourse; *AH* assisted hatching; *MCDA* monochorionic diamniotic; *nl.* normal; *CBC* confined blood chimerism

testicular hypoplasia; however, bilateral testicular torsion is very rare [5]. Although the mechanism of testicular hypoplasia in chimeric males remains unknown, chimerism might have led to the testicular hypoplasia of our patient. Testicular hypoplasia of a chimeric male born with a female co-twin has not previously been reported in humans.

Although most MCDZ twins are conceived via assisted reproductive techniques (ART), they are also infrequently reported to result from conceptions via timed intercourse (TI) or intrauterine insemination (IUI) (Table 2). The reason for the higher frequency of MCDZ twins associated with ART, rather than TI or IUI, is that in natural twin conceptions the embryos are remote, while in ART multiple fertilized eggs are often located in close proximity due to simultaneous ET. In addition, AH increases the possibility of fusion. Tarkowski and Wojewodzka reported that the fusion of two blastocysts can be induced in vitro [6]. As blastocysts are surrounded by tight epithelial cells, blastocyst fusion was previously thought to be impossible. Nevertheless, Redline suggested that there may be a very short period during which two blastocysts may fuse [7]. Thus, AH, simultaneous ET, and ET during the blastocyst stage may create an environment that encourages blastocyst fusion.

Thus, a possible mechanism for MCDZ twinning is the fusion of two fertilized embryos during the blastocyst stage after AH. Another possible mechanism is the fertilization of a binovular follicle, which has two oocytes in a single zona pellucida. van de Leuer and Zielmaked demonstrated that two oocytes in a single zona pellucida can be successfully fertilized [8]. Aoki reported that a pair of MCDZ twins were conceived via ovulation induction by clomiphen citrate [9]. There was no artificial manipulation in Aoki's case.

Twins with CBC are immunotolerant of blood cells from their counterpart twins. Therefore, twins with CBC do not have severe side effects when they receive blood transfusions or tissue transplants from counterpart twins. However, autoimmune diseases may develop in adulthood due to microchimerism [10–15].

As in the present case, dizygosity is usually discovered through analyses of variable number tandem repeats (VNTRs) or STRs after sex discordance is noted during prenatal care or delivery. However, a few MCDZ twin cases have been reported even in sex-concordant twin pairs [9, 16]. In sex-concordant twins, analysis for dizygosity is not usually performed, and therefore the diagnosis of MCDZ twins may be missed. Therefore, when MCDA twins are discovered on ultrasound in early pregnancy, close examination should be performed to distinguish dizygosity. Also, since testicular hypoplasia can occur in chimeric phenotypic males with XX cells, as in the present case, close observation for testicular hypoplasia is recommended in chimeric male infants born with female co-twins.

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