Limb-Body Wall Complex in One Fetus of a Dizygotic Twin Pregnancy Conceived by Egg Donation, *In Vitro* Fertilization and Embryo Transfer: Prenatal Diagnosis and Literature Review

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A 45-year-old primigravid woman was referred for genetic counseling at 20 weeks of gestation because of fetal abdominal wall defects, abdominoplacental attachment and lower limb abnormalities. The previous sonographic examination had shown dichorionic diamniotic twins with multiple structural abnormalities in one fetus and normal structure in the other fetus. The twins were conceived after egg donation, and *in vitro* fertilization and embryo transfer (IVF-ET). IVF-ET utilized 39 fresh ova from a healthy 24-year-old donor and fresh sperm from the healthy 46-year-old biological father. Three embryos were implanted and two survived. Level II ultrasound at 20 weeks of gestation revealed a normal co-twin and an abnormal co-twin with marked scoliosis, attachment of the fetal visceral organs to the placenta, multiple adhesive bands, absence of the right lower limb, agenesis of one kidney, abdominal wall defects with eviscerated liver and intestines, and a short umbilical cord and a single umbilical artery, consistent with a diagnosis of limb-body wall complex (LBWC). The woman had refused chorionic villus sampling (CVS) and amniocentesis during the pregnancy. The pregnancy was uneventful until 31 weeks of gestation when premature rupture of the membranes and preterm labor occurred. A 1,474-g normal female co-twin and a 1,168-g abnormal female co-twin were delivered by cesarean section. The abnormal co-twin manifested a ruptured omphalocele with eviscerated liver and intestines, a short umbilical cord, multiple adhesive bands, and absence of the right lower limb, and died soon after birth. The external female genitalia and anus were normal. Postnatal X-ray examination showed eviscerated internal organs, marked scoliosis, and absence of the right lower limb (Figure). Cytogenetic analysis of the cord blood revealed a 46,XX karyotype. A molecular zygosity test of the twins confirmed dizygotic twinning. The normal co-twin was doing well at the age of 2 years.

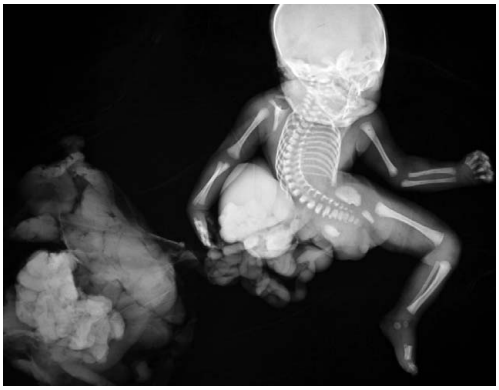

Figure. Postnatal X-ray showing eviscerated internal organs, marked scoliosis and absence of the right lower limb.

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Table. Summary of data for reported cases conceived by *in vitro* fertilization and presenting with severe midline disruption sequence

Case	Pregnancy status	Maternal age (yr)	Assisted reproductive technology	Prenatal invasive procedure	Prenatal ultrasound diagnosis	Postnatal outcome
Litwin et al [6]	Singleton	33	IVF-ET	No	Anencephaly, omphalocele	Termination of pregnancy; LBWC, craniorachischisis, a huge omphalocele containing intestines, shortening of the right tibia, clubfoot, knee ankylosis of the left leg, preaxial polydactyly, complete absence of external genitalia.
Hirokawa et al [7]	Triplet	31	IVF-ET	No	Omphalocele in one fetus	Cesarean delivery at 32 weeks of gestation; first baby: 1,730 g, normal female, survived; second baby: 1,200 g, abnormal female, body stalk anomaly with a shortened umbilical cord, scoliosis, ruptured omphalocele containing liver and small intestines, dead after surgery; third baby: 1,388 g, normal female, survived.
Kähler et al [8]	Twin	33	ICSI-ET	Amniocentesis	Body stalk anomaly in one fetus with intrauterine death	Cesarean delivery at 37 weeks of gestation; normal co-twin: 2,380 g, male, survived; abnormal co-twin: macerated, male; both twins presented with a normal male karyotype.
Shanske et al [9]	Triplet	38	IVF-ET	CVS	Gastroschisis in one fetus	Cesarean delivery at 28 weeks of gestation owing to PROM; triplets A and C: normal, survived; triplet B: 1,320 g, dead after birth, OEIS complex with patent ductus arteriosus, omphalocele, imperforate anus, abnormal left lower extremity, lumbosacral meningocele, 46,XY karyotype.

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Case	Pregnancy status	Maternal age (yr)	Assisted reproductive technology	Prenatal invasive procedure	Prenatal ultrasound diagnosis	Postnatal outcome
Wood et al [10] Case 1	Twin	37	IVF-ET	No	Omphalocele in one fetus	Cesarean delivery at 37 weeks of gestation; abnormal co-twin: 2,660 g, OEIS complex, cloacal exstrophy, omphalocele, club feet, lipomyelomeningocele, lumbosacral dysraphism, survived after surgery, 46,XY karyotype.
Case 2	Singleton	36	IVF-ET	No	No	Vaginal delivery at 37 weeks of gestation; 2,640 g, cloacal exstrophy, omphalocele, survived after surgery, 46,XY karyotype.
Yokoyama et al [11]	Triplet	43	Egg donation, IVF-ET	No	No	Cesarean delivery at 32 weeks of gestation owing to abruption of placentae; normal co-triplets, female, survived; abnormal co-triplet: 1,180 g, female, OEIS complex, bilateral cleft lip and palate, omphalocele, cloacal exstrophy, imperforate anus, hemivertebrae, 46,XX karyotype, survived after surgery.
Present case	Dizygotic twin	45	Egg donation, IVF-ET	No	LBWC and agenesis of one kidney in one fetus	Cesarean delivery at 31 weeks of gestation owing to PROM and preterm labor; normal co-twin: 1,474 g, female, survived; abnormal co-twin: 1,168 g, female, dead after birth, LBWC with a ruptured omphalocele containing eviscerated liver and intestines, a short umbilical cord, multiple adhesive bands, scoliosis, absence of the right lower limb, 46,XX karyotype.

IVF = in vitro fertilization; ET = embryo transfer; LBWC = limb-body wall complex; ICSI = intra-cytoplasmic sperm injection; CVS = chorionic villus sampling; PROM = premature rupture of the membranes; OEIS = omphalocele-exstrophy-imperforate anus-spinal defects.

Severe midline disruption sequence includes LBWC, omphalocele-exstrophy-imperforate anus-spinal defects (OEIS) complex, pentalogy of Cantrell, omphalocele with cloacal–bladder exstrophy complex, *Disorganization* (*Ds*)-like human malformations, and amniotic band sequence. LBWC or body stalk anomaly describes a heterogenic group of fetal malformations, including lateral body-wall defects and limb reduction anomalies [1,2]. Cases of LBWC with craniofacial defects frequently show severe anomalies of the upper limbs, craniofacial defects, constrictive amniotic bands, and cranioplacental attachment, whereas cases of LBWC without craniofacial defects usually present with major anomalies of the lower limbs, abnormal genitalia, anal atresia, renal defects, abdominoplacental attachment, and umbilical cord abnormalities [3]. OEIS complex (OMIM 258040) arises from a single localized defect in the early development of the mesoderm that will later contribute to the infraumbilical mesenchyme, cloacal septum, and caudal vertebrae. Pentalogy of Cantrell consists of a specific combination of congenital defects with the full pentalogy of a midline supraumbilical abdominal wall defect, a defect of the lower sternum, a defect of the diaphragmatic pericardium, a deficiency of the anterior diaphragm, and congenital cardiac anomalies [4]. Amniotic band sequence consists of a group of sporadic abnormalities characterized by congenital ring constrictions or amputation of digits and limbs, terminal digital fusion (pseudosyndactyly), talipes, and multiple craniofacial, visceral and body wall defects. Birth defects resembling the effects of the mouse mutant gene *Ds*, or *Ds*-like human malformations, include both common (neural tube defects, orofacial clefting, gastroschisis, and limb defects) and rare (anophthalmia and duplicated rectum) human birth defects [5].

To date, only eight cases of severe midline disruption sequence associated with IVF-ET have been reported (Table) [6–11]. Among the eight pregnancies, two were singleton, three were twin, three were triplet, three had OEIS complex, three had LBWC, and one had cloacal exstrophy and omphalocele. One underwent CVS, one underwent amniocentesis, and two were the results of egg donation. The possibility that assisted reproductive technology is associated with an increase in major birth defects cannot be excluded, based on the current evidence [12–15]. Ericson and Källén [16] found about a threefold excess risk of neural tube defects, alimentary atresia, omphalocele and hypospadias in infants born after IVF. Källén et al [14] found an additional increase in risk of neural tube defects, choanal atresia and alimentary tract atresia in infants born after IVF. The present case manifested LBWC. Possible pathogenetic mechanisms for LBWC include early amnion rupture [17], vascular disruption [1,2], and early embryonic maldevelopment [18,19]. Russo et al [3] suggested that LBWC with craniofacial defects is caused by an early vascular disruption, and LBWC without craniofacial defects is related to a defective lateral and caudal folding process of the embryonic disk. Chen et al [20] previously reported LBWC with craniofacial defects after ovarian stimulation with clomiphene. The present case and the case of OEIS complex reported by Yokoyama et al [11] were both the result of IVF with egg donation. Shanske et al [9] suggested that OEIS complex associated with IVF may be caused by multiple factors including IVF, multiple pregnancy, and mechanisms causing uteroplacental vascular insufficiency such as placenta accreta or trauma caused by CVS. The present case did not undergo CVS or have abnormal placentation.

Monozygotic twinning is well known to be associated with major structural midline developmental defects [21]. However, the present case was a dizygotic twin pregnancy. The LBWC in our case was likely to be related to developmental defects of the fetus. This case provides additional evidence for the occurrence of LBWC in multiple pregnancies after IVF-ET with no influence of monozygosity, invasive prenatal diagnostic procedures or ovarian stimulation of the pregnant woman.

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