A unique cast model of the placenta in a rare case of feto-feto-fetal triplet transfusion syndrome (FFFTTS) allowed the demonstration of why the transfusion syndrome developed in one fetus and not in the other two in that single placenta. The vasculature anatomy of a monochorionic triamniotic triplet placenta with FFFTTS of three healthy infants (one donor, two recipients) born in the 35th week of gestation was cast by means of dental casting materials. After the cast hardened, the tissue was corroded, revealing the cast blood vessels. The diameters and lengths of the chorionic blood and intraplacental vessels of the cast placenta were measured with a digital caliper. The cast revealed two artery to artery (AeA) anastomoses on the chorionic plate between the two recipients and the donor. Seven artery to vein (AeV) deep anastomoses connected only the arteries of the donor and the veins of the two recipients. The blood vessel connections among the fetuses allowed the evaluation of a pathologic case with its own control in a single placenta. From the vascular appearance, we speculate that the AeA anastomoses between the two fetuses protected them from developing blood transfusions, but that the AeV anastomoses contributed to their development.

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INTRODUCTION

One in 10,000 pregnancies is a spontaneously conceived triple (USA). Monozygotic triplet pregnancies account for 4.5% of all triplet pregnancies while monochorionicity occurs in 1.6:100,000 pregnancies [1]. A monozygotic monochorionic triplet occurrence is very rare and its etiology remains unknown. Monochorionic twins have an approximately 25% rate of perinatal morbidity and mortality, which is much higher than singleton and dichorionic twins. This rate increases in triplets because of the high incidence of preterm labor [2–4]. Twin–twin transfusion syndrome (TTTS) complicates 17.6% of dichorionic triplet pregnancies, a figure comparable to TTTS in monochorionic twin pregnancies [5]. In these cases, blood is transfused from one twin (the donor) to the other (the recipient) via anastomoses between an artery of one twin to an artery of the other one (A–A), vein to vein (V–V) or artery to vein (A–V) [6,7]. The donor twin becomes anemic and the amount of its amniotic fluid decreases, while the recipient becomes polycythemic, causing polyhydramnios.

The type of anastomoses accounts for the development of TTTS. Mild TTTS has been associated with deep A–V anastomoses along with superficial A–A and V–V anastomoses, whereas lack of superficial anastomoses is more likely to cause severe TTTS [8–10]. It was postulated that a single, deep A–V anastomosis causes asymmetric blood flow from one twin to the other [8]. The effect of an asymmetric blood flow may be reduced when superficial anastomoses are present together with an A–V anastomosis [11,12]. Superficial anastomoses are clearly observed on the chorionic plate. Deep anastomoses, i.e., the A–V type, represent a shared cotyledon in which the arterial supply is derived from one twin and the venous drainage is derived from one twin and the venous drainage is to the other [9]. Thus, A–V anastomoses may be suspected by an unpaired artery of one twin and an unpaired vein of the other twin being dipped closely into the placenta at a distance <1 cm [13].
The site of umbilical cord insertion into the placenta in monochorionic twins has been documented: there were no significant differences between those who were non-TTTS and those who developed TTTS [6]. The chorionic vessels are distributed across the chorion in two major patterns: (i) dichotomous (also known as “disperse”) in which there is a fine network of vessels that repeatedly branch into two fairly similar daughter vessels, and (ii) monopoidal (also known as “magistral”) in which the main vessel courses across the placental surface nearly to the edge with a fairly constant diameter and with small diameter side branches [14]. The dichotomous pattern has been described in monochorionic twins, but it was not correlated to TTTS [13].

Feto-feto-fetal triplet transfusion syndrome (FFFTTS) in a monochorionic triplet has been reported [15,16]. The distribution pattern of the placental vasculature has not, however, been described before in a triplet pregnancy. We now report the application of a plastination technique to the single placenta of a triplet pregnancy in order to observe the one blood circulation system shared by two affected twins (TTTS) and one control twin. In this unique case, we were able to show why the transfusion syndrome developed in one fetus and not in the other two.

MATERIAL AND METHODS

A triplet gestation was conceived naturally by a 32-year-old healthy woman. A monochorionic triamniotic pregnancy with three viable fetuses was diagnosed at 12 weeks’ gestation by ultrasound (US) scan. All three fetuses had normal nuchal translucency values and appropriate crown-rump lengths for gestational age. Early US screening for malformations (15 weeks’ gestation) revealed 3 appropriate for gestational age (AGA) female fetuses. The triplets were followed-up for fetal growth and cervical length by US examinations every 2 weeks for up to 33 weeks of gestation when there was a size discordancy of > 800 g between one fetus and the other two. It was suspected that the triplets shared the same blood circulatory system and that a feto-feto-fetal transfusion between the fetuses had developed. The triplets were delivered by caesarean section at 35 weeks of gestation due to a 2-week arrest of growth of the small fetus. The infants weighed 2060 g (Fetus I), 2070 g (Fetus II) and 1280 g (Fetus III) and their respective hematocrit values were 52.4, 57.9 and 42.7. The placenta was recruited within 5 min after delivery (Figure 1). The procedure to cast a model of the placenta had been approved by the ethical committee of the medical center and a signed informed consent had been obtained.

Gross pathological examination at birth revealed a monochorionic triamniotic placenta, supporting the diagnosis of FFFTTS. A genetic evaluation that was performed by peripheral blood samples from each of the triplets at the age of 8 weeks and buccal smear samples at 3 months showed monozygosity.

Placental vasculature casting

Plastination of the placental vasculature was performed by means of dental casting materials in order to obtain the real geometries of the placental vessels. The casting material was a mixture of dental powder and liquid (Unifast Trad, GC

![Figure 1. The monochorionic placenta of the triplets 5 min after delivery.](image)
Dental products Corp., Kasugai, Aichi, Japan) at a ratio of 1:4 and colored ink. All three umbilical cords were trimmed to 10 cm from insertion into the placenta, and the vessels were catheterized. The placental vascular bed was rinsed with a solution of 1 L saline and 5000 units/ml of heparin sulfate via the arteries and veins of the umbilical cords to drain all the residual blood. The umbilical arteries and vein were connected to separated syringes, each filled with casting material (25 ml in the vein and 20 ml in each artery). The arteries were red in color and the veins were blue. The casting material was injected first into the umbilical cord of the smallest fetus because our experience has shown that this would ensure that the smallest vessels are adequately filled before going on to the larger ones. When the placenta had been filled with the casting material, it was stored in a refrigerator for four days to allow the material to harden. The biological tissues that surrounded the hardened vasculature cast were then eroded in a solution of 60% KOH and distilled water. The diameters and lengths of the chorionic blood vessels and intraplacental (IP) vessels of the cast placenta were measured with a digital caliper.

RESULTS

The cast model of the monozygotic monochorionic triamniotic FFFTTS placenta succeeded in demonstrating its vascular anatomy, including the chorionic blood vessels, the IP blood vessels and the capillary system. FFFTTS was verified by the weights and hematocrit values of the fetuses, which indicated that Fetus III (the smallest one) was the donor and the two others were the recipients. The cast showed that the insertion of the umbilical cords into the placenta was central for the high-weight fetuses and marginal for the smallest fetus (Figure 2). The arteries of all three fetuses overlaid the veins.

The chorionic vasculature of the larger fetuses (I and II) revealed a dichotomous pattern (Figure 2). The arterial and venous blood vessels were distributed evenly around cord insertion with slightly decreasing diameters (from 2.8 mm to 2.1 mm). After 3–4 generations, the blood vessels continued in a monopoidal pattern. The arteries of Fetus I were connected by a Hyrtl anastomosis [17]. The umbilical arteries of Fetus II could not be measured since the cord had been cut very close to the placental insertion during the exsanguination process in preparation of casting. Some of the IP blood vessels of Fetus II, however, were fairly bare and visible, and they measured about 0.5–1 mm. The ratio between the diameter of the vessels in the chorionic plate and the IP varied from 3.5- to 6-fold.

The chorionic blood vessels of the smallest fetus (III) showed a monopoidal pattern (Figure 2). The vessels were developed in the margins, along the circumference of the

![Figure 2](image_url)

Figure 2. The placental cast model in which arteries and veins are shown in red and blue, respectively. A and B depict the asymmetry in the development of the blood vessels (thick and thin) of Fetus III. C and D denote A–A anastomosis between Fetus I and Fetus II and between Fetus I and Fetus III, respectively. E indicates A–V anastomosis between Fetus I and Fetus III.
placenta. The distribution of the chorionic blood vessels was asymmetric: the diameters of the vessels that had developed along one side of the placental circumference (A in Figure 2) ranged between 2.71 and 4.75 mm, compared to the 1- to 2-mm diameters of the vessels that had developed along the other side (B in Figure 2). The two umbilical arteries merged into one wide vessel, creating the Hyrtl anastomosis [17], then, split again into two vessels, giving rise to a vascular tree.

The superficial chorionic A–A anastomoses were already visible during the injection of the cast material since the material came out through the umbilical cord of the closer fetus. The cast model revealed a prominent A–A anastomosis of 1.87 mm between the two larger fetuses (C in Figure 2). Another A–A anastomosis was seen to connect Fetus I and Fetus III via a very small (0.2 mm) vessel (D in Figure 2). Generally, deep A–V anastomoses are identified by a single artery of one twin and a single vein of another twin, which share the same cotyledon. Seven A–V anastomoses were present between the donor fetus (III) and the recipient fetus (II) which shared two cotyledons (Figure 3). A single A–V anastomosis connected Fetus III to Fetus I (E in Figure 2). In both cases, the blood to the cotyledons was supplied via the arteries of the smallest fetus and was drained by the veins of the larger ones. In addition, Fetus II and Fetus III shared another cotyledon, however, each of the fetuses contributed a complete artery-and-vein pair. Another cotyledon was shared by Fetus I and Fetus II: in this case, each fetus contributed two complete artery-and-vein pairs.

DISCUSSION

We constructed a cast to display the placental vasculature from a rare case of FFFTTS of a monozygotic monochorionic triplet pregnancy. The cast demonstrated a single shared blood circulatory system, which we analyzed according to the vascular anatomy observed in placentas with TTTS (Figure 4). It emerged that Fetus II and Fetus III formed pathological twinning TTTS and that Fetus II and Fetus I comprised a “control” pair. The plastination of the placenta was successful since it was delivered at term and the blood vessels were wide enough to allow catheterization of the vessels, smooth injection and fine passage of the cast material via the chorionic vessels into the capillary system. The technique we used is more convenient than other casting techniques due to the casting material which has low viscosity and can be easily prepared. Plastination, although reveals the vascular anatomy, is not applicable in daily practice when pathological examination is required since the tissue is corroded during the procedure.

Four colors were used to identify both blood systems and the anastomoses in most of the studies that demonstrated the vasculature of monochorionic twins [11,13]. Our experience with casting of placentas of singleton pregnancies revealed that when we used different colors for each umbilical artery, the material that had been injected first into one artery was pushed to the adjacent artery via a Hyrtl anastomosis. As a result, the second color that we injected into the other artery was mixed with the first one and it was inconvenient for demonstration. For the current case, we used only two colors, red for arteries and blue for veins, for all three umbilical cords since the anastomoses were highly visible after tissue erosion and it was easy to distinguish between them.

The placenta we cast was diagnosed as being monochorionic in the first trimester, while FFFTTS was suspected on the last trimester, and confirmed after birth by weight discordancy and hematocrit values. It is difficult to infer whether the severity of TTTS is dependent upon vascular anatomy [8]. However, the fact that the triplets were born during the 35th week of gestation implies that the FFFTTS was mild in degree. All the A–V anastomoses involved the arteries of the Fetus III and the veins of Fetus I or Fetus II: this may explain the discordance in weight and hematocrit level of Fetus III compared to the other two. The mild degree of this FFFTTS was probably due to the presence of the A–V anastomoses.

Figure 3. Few A–V anastomoses between Fetus II and Fetus III (circled by dashed line).
(unidirectional flow) along with two A–A anastomoses (bidirectional flow): this setup may improve fetal gross pattern, depending upon oxygenated venous return [6–8,10,11,13,18].

Whether cord insertion in twins is central or marginal is generally thought to be insignificant. The occurrence of marginal cord insertion in TTTS twins, however, is more frequent than in control twins [8]. Central cord insertion in the recipient fetuses (I and II) and marginal cord insertion in the donor fetus (III) were clearly observable in our FFFTTS triplet placenta. It is known that fetal growth in monochorionic twins is proportional to the placent al territory and arteriovenous anastomoses [13]. The vascular cast model of our case of triplets showed that the vascular tree of the larger fetuses occupied fairly similar areas of the placenta, i.e., about four-fifths. The small vascular tree and the A–V shunts to Fetuses I and II inevitably led to the low weight of Fetus III. Central/marginal cord insertion is usually associated with a dichotomous/monopodial pattern, respectively. The larger fetuses had a dichotomous pattern arranged symmetrically around the cord and forming a disc. The marginal cord insertion of the small fetus gave rise to a monopodial pattern in which the vessels were distributed asymmetrically along both sides of the placenta’s circumference.

The rare phenomenon of an FFFTTS-affected triplet pregnancy and the opportunity to make a cast of such a placenta provided us with a unique opportunity to demonstrate the distribution pattern of the triplet placental vasculature, the relations between donor and recipients with both A–V and A–A shunts, and the relations between two fetuses of the same weight with A–A shunts.

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REFERENCES