Twin Study: Relationship between Birth Weight, Zygosity, Placentation, and Pathologic Placental Changes

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Abstract. We examined the placentas of 182 like-sexed live-born twins: 73 placentas (40.1%) were monochorionic and 109 (59.9%) were dichorionic. All twin pairs with monochorionic placentas were monozygotic (MZ), but 28.9% of pairs with dichorionic placentas were MZ. Analysis of birth weights demonstrated that dichorionic and dizygotic (DZ) twins were heaviest, and suggested that the chorion status is a more important determinant of birth weight than zygosity. Vascular anastomoses were identified only in monochorionic placentas and occurred in 79.5% of cases. All placentas with deep anastomoses had superficial anastomoses. A higher proportion of velamentous and marginal insertions of the umbilical cord in monochorionic placentas (27.4%) compared to dichorionic placentas (13.8%) supports the belief that lateral placental growth is greatest in twin gestations in which the embryos are initially most closely apposed — The theory of trophotropism.

Key words: Twins, Placenta, Zygosity, Chorion, Anastomoses

INTRODUCTION

The criteria used to assess twin zygosity have been continually changing since the two types, monozygotic (MZ) and dizygotic (DZ), were distinguished. Examination of the fetal membranes has been considered an important aid in determining zygosity, although it is known that dichorionic (DC) status cannot be equated with dizygosity [3]. More recently, blood group antigens have provided an accurate tool to determine zygosity, and a better estimate of the relationship between zygosity

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and placentation can now be obtained.

The examination of the twin placenta has also been stressed in recent years as a means to assess prenatal development [8]. It has become apparent that the placental vasculature is an important environmental factor which may influence differential twin development. The studies of Hyrtl [16], Schatz [22] and Benirschke [4] showed the importance of two types of vascular anastomoses in monochorionic (MC) placentas: 1) superficial communications joining artery to artery or vein to vein, and 2) deep vascular anastomoses where a cotyledon is supplied by an artery from one fetus but drained by a vein to the other. A disequilibrium between the deep and superficial anastomoses may be responsible for the differences in birth weight observed between MC twin pairs and result in the twin transfusion syndrome in the most severe cases [17]. On the other hand, Corey et al [14] suggested that birth-weight differences in twin pairs which varied with chorionic status may be largely attributable to the degree of proximity of the placental implantation sites, so that twin pairs with close implantation sites compete for nutrients in utero to a greater extent than those with more distant implantation sites.

Finally, the study of other morphologic features of the placenta, such as infection of the placenta, as well as the insertion and number of cord vessels, may give us a better understanding of the role of the placenta as an environmental factor in determining fetal growth [20].

With these concepts in mind we undertook to examine in a careful fashion 182 twin placentas from liveborn pairs of known zygosity as determined by multiple genetic marker analysis. We were particularly interested in looking at the relationship between birth weights, vascular patterns, cord anomalies, infections, zygosity and membrane status.

MATERIALS AND METHODS

The placentas and cord blood samples from 182 pairs of like-sexed twins were collected at the time of delivery. The births occurred at the hospitals of the Indiana University Medical Center and at six outlying hospitals over an interval from September 1982 through December 1984. The unfixed placentas were examined grossly by two of us (MAR and TMU) and microscopically by one of us (TMU). The gross examination included the size and weight of the placentas, the length and position of the insertions of the cords, the number of cord vessels, the type of fetal membranes, and the presence and nature of any demonstrable vascular anastomoses.

Vascular anastomoses were determined by injecting vessels on the fetal surface of the placenta with a food-dye-saline mixture and observing the vessels corresponding to the noninjected twin for reflux. In addition to permitting the evaluation of superficial anastomoses, this technique also allowed the occasional determination of deep placental anastomoses by visualizing discoloration within the cotyledons of the noninjected twin. If a superficial vascular anastomosis was visualized, the
nature of the two anastomosing vessels was determined by tracing back to the um- 
bilical cords, and the diameter of the vessels at the site of the anastomosis was 
measured.

Pathologic placental changes, such as infection of the villi (villitis), infection 
of the fetal membranes (chorioamnionitis), and infection of the cords (funisitis), 
were demonstrated by microscopic examination of the placentas, which included: 
blocks through the proximal and distal portions of the umbilical cords, one block of 
a roll of fetal membranes separating the two amniotic cavities, and one block each 
of the peripheral and central portions of the placenta corresponding to each twin. 
The fetal membranes were classified as monoamnionic-monochorionic, diamnionic-
monochorionic, and diamnionic-dichorionic. Two types of diamnionic-dichorionic 
placentas were distinguished: fused placentas (DC-fused) and separate placentas 
(DC-separate).

Twin zygosity was determined by typing the following genetic markers on the 
cord blood specimens: ABO, Rh, MNS, Kell, Duffy, Kidd, P, haptoglobin, phospho-
glucomutase, acid phosphatase, adenine deaminase, adenilate kinase, properidine 
factor, group-specific factor, and orosomucoid. Twins were considered to be DZ 
if they differed in one or more of the markers. It was estimated that a pair of 
like-sexed twins who were concordant for all these markers had a probability of 
99.8% of being MZ.

Most of the twins were examined at the hospitals within the first five days 
of life. The diagnosis of the twin transfusion syndrome was based on clinical and 
hematologic data. Information regarding parity, gestational age, preeclampsia or 
eclampsia, and birth weight, was obtained from the hospital records or personal 
interviews with the mothers.

RESULTS

The correlation between zygosity determination from genetic marker analysis and 
type of twin placentation is shown in Table 1. Zygosity could not be determined in 
12 cases (6.6%) because of failure to obtain cord blood. Of the 182 twin placentas, 
73 (40.1%) were MC and 109 (59.9%) were DC. All of the twins with MC placentas 
were MZ, whereas 25.7% of the twins with DC placentas were MZ and 63.3% 
were DZ. The proportion of MZ twins in DC-fused and DC-separate placentas was 
roughly equivalent.

The mean gestational age of the twins was 37 ± 2.5 weeks with a range of 30 
to 42 weeks. The MZ twins had an older mean gestational age than the DZ twins 
(37.6 ± 2.5 vs 36.8 ± 2.4 weeks, respectively). However, this difference was not 
statistically significant.

The mean placental weight (combined weights for separate placentas) was 783 
g. MC placentas were slightly heavier (m = 793 g) than DC placentas (m = 776 
g), and the placentas of male twins (m = 792 g) were heavier than those of female 
twins (m = 773 g), but these differences were not statistically significant.
Table 1 - Twin zygosity and twin placental types

<table>
<thead>
<tr>
<th>Placental type</th>
<th>MZ</th>
<th>DZ</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoamnionic-monochorionic</td>
<td>1 (0.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diamnionic-monochorionic</td>
<td>72 (39.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dichorionic-fused</td>
<td>11 (6.0%)</td>
<td>27 (14.8%)</td>
<td>4 (2.2%)</td>
</tr>
<tr>
<td>Dichorionic-separate</td>
<td>17 (9.3%)</td>
<td>42 (22.1%)</td>
<td>8 (4.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>101 (55.5%)</td>
<td>69 (37.9%)</td>
<td>12 (6.6%)</td>
</tr>
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</table>

The mean birth weights of the twins are compared according to twin and placental type in Table 2. Male twins were significantly heavier than female twins; DZ twins were significantly heavier than MZ twins, and twins with DC placentas were significantly heavier than twins with MC placentas. In order to determine whether it was the zygosity or the number of chorionic membranes which had the stronger influence on twin weight differences, we compared the birth weight of MC-MZ, DC-MZ, and DC-DZ twins (Table 2). There was a significant birth-weight difference between MC-MZ and DC-DZ twins; a suggestive but not significant difference in birth weights between MC-MZ and DC-DZ twins, and no significant difference in birth weights between DC-MZ and DC-DZ twins.

Intrapair birth-weight differences between male and female twins, MZ and DZ twins, MC and DC twins, MZ twins with MC placentas and MZ twins with DC placentas, and twins with DC-fused placentas and twins with DC-separate placentas, were not statistically significant. The same findings were true for the intrapair birth-weight difference between MC twins with and without superficial vascular anastomoses, and MC twins with and without deep anastomoses.

In 12 MC-MZ and 9 DC (2 MZ and 7 DZ) pairs, the birth-weight difference exceeded 20% of the weight of the heaviest twin. However, sufficient data to warrant a diagnosis of twin transfusion syndrome were obtained in only 2 MC-MZ cases, and their corresponding placentas showed a single artery-to-artery anastomosis. We are aware that this is a very conservative figure because our study only included liveborn twins, and complete data were not available in every case. Of the 9 DC placentas associated with a birth-weight difference of over 20%, 6 were DC-fused and 3 were DC-separate placentas.

Demonstrable vascular anastomoses occurred in 79.5% of the MC placentas and were not identified in any DC placentas. The most frequent type of superficial vascular anastomoses occurred between arteries. One or more arterial anastomoses occurred in 40 placentas, whereas venous anastomoses occurred in 7 placentas. During part of our study we were not aware that the relationship between small vessels (but not large vessels) on the surface of the placenta is not a totally reliable method for determining their arterial or venous nature (K. Benirschke, personal
communication, 1985) and thus, we were forced to classify an additional 29 vascular anastomoses, which had been classified by small vessel patterns, as being of indeterminate type. Six placentas showed combinations of artery-to-artery and vein-to-vein anastomoses. The diameter of the vessels at the sites of anastomosis ranged from 1 to 4 mm. Deep placental anastomoses were identified in 12 placentas. All the placentas with deep vascular anastomoses had demonstrable superficial anastomoses.

Table 2 - Mean twin birth weights by sex, zygosity, and placental type

<table>
<thead>
<tr>
<th>Twin and/or placental type</th>
<th>Mean birth weight (g)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>2,521</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Female</td>
<td>2,444</td>
<td></td>
</tr>
<tr>
<td>Monozygotic</td>
<td>2,395</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Dizygotic</td>
<td>2,618</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Monochorionic</td>
<td>2,338</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Dichorionic</td>
<td>2,567</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Monochorionic-monozygotic</td>
<td>2,338</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Dichorionic-monozygotic</td>
<td>2,536</td>
<td>P = 0.5</td>
</tr>
<tr>
<td>Dichorionic-dizygotic</td>
<td>2,618</td>
<td></td>
</tr>
</tbody>
</table>

The following pathologic findings were noted on gross and/or microscopic examination of the placentas (percentages are per placenta): monoarterial umbilical cord (restricted in all cases to involvement of one cord only), 2.2%; velamentous insertion of the umbilical cord, 16.5%; urachal and/or allantoic remnants of the umbilical cord, 26%; excessive subchorionic fibrin deposits, 12.6%; infarcts, 8.2%; meconium staining, 6.6%; subchorionic hematoma, 3.8%; succenturiate lobe, 5.5%; disrupted cotyledons, 22%; and inflammation of the fetal membranes, villi and/or umbilical cord, 13.7%.

Isolated villitis occurred in 5 DC (2 DC-fused and 3 DC-separate) and 3 MC placentas; isolated chorioamnionitis in 3 DC placentas (1 DC-fused and 2 DC-separate); and isolated funisitis in 1 DC-separate and 2 MC placentas. Villitis with chorioamnionitis or funisitis was observed in another 5 DC-fused placentas. The villitis, except in one case of acute villitis with associated chorioamnionitis and funisitis, consisted of focal and slight infiltrates of lymphocytes and plasma cells within the villi. Acute chorioamnionitis consisted of a neutrophilic infiltrate of variable intensity within the chorion, with associated neutrophilic collections in the subchorionic fibrin layer, with or without neutrophilic extension into the amnion.
In the 15 cases with chorioamnionitis, both twins were affected in 8 cases, only the first-born was affected in 5 cases, and only the second-born twin in 2 cases. Funisitis was characterized by neutrophils within the walls of cord vessels with variable extension of inflammation in Wharton’s jelly. Of the 15 cases with grossly apparent infarcts, 3 of the mothers had a history of preeclampsia or eclampsia, 10 did not have such complication, and in 2 cases pregnancy history was unknown.

A single monoarterial umbilical cord occurred in four individual twins, representing an incidence of 2.2% per twin delivery. In one case, only one umbilical artery was present in 77 cm of the cord, followed by a 5 cm distal segment of the cord which contained two arteries. Between these two portions of cord there was an area of transition in which one artery joined the other to become a single vessel. Two of the four twins with monoarterial cords had congenital malformations. One had severe hypotonia, dextrocardia, and imperforate anus, while his MZ twin was phenotypically normal. A second twin with a DC placenta and unknown zygosity had multiple, predominantly skeletal, congenital malformations including severe micrognathia, cryptorchidism, rhizomelic shortening of the right arm, hypoplasia of the right foot, syndactyly of four left toes and hypoplastic nails. The twin of this child was phenotypically normal. The two remaining twins with monoarterial cords were phenotypically normal as were their respective twins. Two of the four monoarterial cords had velamentous insertions. In two of the four placentas with monoarterial cords, the twin corresponding to the abnormal cord was heavier than his cotwin, and in the remaining two cases the twin with the monoarterial cord was lighter than the unaffected twin.

Abnormally inserting umbilical cords (either velamentous or marginal insertions) occurred in 27.4% of the MC placentas, 15.5% of the cords of DC-fused placentas, and 12.7% of the cords of DC-separate placentas (Table 3).

### Table 3 - Percentage of umbilical cords with velamentous or marginal insertions according to placental type

<table>
<thead>
<tr>
<th>Placental type</th>
<th>Velamentous (% of cords)</th>
<th>Marginal (% of cords)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monochorionic</td>
<td>9.6</td>
<td>17.8</td>
</tr>
<tr>
<td>Dichorionic-fused</td>
<td>11.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Dichorionic-separate</td>
<td>4.5</td>
<td>8.2</td>
</tr>
</tbody>
</table>

DISCUSSION

There is an increasing body of evidence that numerous characteristics including dermatoglyphics [20], plasma cholesterol [13], cognitive development [21], body size...
[12], and red blood cell mass [11] are influenced by the type of placentation in twins. In this study the placental type and morphology were studied in a consecutive series of liveborn twins so that fetal development could be compared to the prenatal environment as measured by placentral structure. This study demonstrates that MC twin placentas are unequivocal proof of MZ twins. On the other hand, DC twin placentas may be associated with either MZ or DZ twins. In our study, which was restricted to like-sexed twins (and therefore twins in whom a question of zygosity would arise), 26% of the like-sexed twins with DC placentas were MZ, 63% were DZ, and in the remaining 11% of twins with DC placenta the zygosity was unknown. These figures are in good agreement with those of previously reported studies [8,11] and emphasize the need to perform zygosity determinations in like-sexed twins with DC placentas. However, zygosity determinations are not necessary in the 40% of like-sexed twins in whom an adequate examination of the placenta demonstrates MC fetal membranes. As Benirschke [3] indicated, both DC-fused and DC-separate placentas are frequently associated with MZ twins. Other authors [18] suggested that there was a significantly higher incidence of DZ twins in DC-separate placentas compared to DC-fused placentas, but our data (Table 1) indicate that the incidence of dizygosity is almost identical in both placental types (71.1 and 71.2%, respectively).

DZ and DC twins were heavier than MZ and MC twins, respectively, which is in good agreement with previously reported studies [14]. If one examines MZ and DZ twin subsets of DC placentas, and MC and DC placental subsets of MZ twins, the data suggest that both features, in concert, exert a significant influence on the twin birth weight. Our data further suggest that, of the two factors, the nature of the chorionic membranes may be a more important determinant of birth weight than zygosity.

The demonstration of vascular anastomoses between twin circulations is, with rare exceptions, equivalent to a diagnosis of a MC twin placenta and, consequently, to a diagnosis of MZ twins. The incidence of vascular anastomoses in MC twin placentas has varied in different studies. Adler [1] demonstrated artery-to-artery anastomoses in 40% of MC twin placentas, whereas Bleisch [10] observed such anastomoses in all of his MC placentas. More recent studies [2,8], including ours, failed to demonstrate interfetal vascular communications in 10% to 20% of MC placentas. The most common kind of superficial anastomosis was from artery-to-artery, a finding which has also been demonstrated in other studies [2,10]; such anastomoses occurred in 85.1% of the MC placentas with anastomoses. Despite the high incidence of demonstrable superficial vascular anastomoses in MC twin placentas, we were able to visualize, with our technique, deep placental anastomoses in only 17% of the placentas. The actual incidence of such deep anastomoses is probably higher, but would require the laborious technique of latex (or other coaguable substance) injection followed by tissue digestion. Most authorities [4,10,22] believe that the deep anastomoses, which occur between arterial branches of one twin and venous branches of the cotwin within a common placental cotyledon, are responsible for the flow between the twins. Schatz [22] suggested that superficial anastomoses developed as a mode of compensation for the deep placental anastomoses. The
100% incidence of superficial anastomoses in placentas with deep anastomoses in our study adds some support to this theory. According to Schatz's theory, lack of compensatory superficial vascular anastomoses between the circulatory systems of MC twins was postulated to be a contributory factor for the difference in weight between the two members of a twin pair. The extreme case would result in the twin transfusion syndrome in which there are striking differences in birth weights, sizes, and hemoglobin values between the members of the twin pair. Our study shows that the presence or absence of superficial or deep vascular anastomoses does not influence significantly the intrapair birth-weight difference in MC twins (0.605 g vs 0.766 g for MC twins with and without superficial anastomoses; and 0.736 g vs 0.609 g for MC twins with and without deep anastomoses, respectively). On the other hand, one artery-to-artery communication could be demonstrated in each of the placentas corresponding to our two cases with twin transfusion syndrome. Although this has also been shown by other authors [19], a more accurate technique to demonstrate deep anastomoses, and a larger number of cases with twin transfusion syndrome would be necessary to demonstrate the relationship between birth weight in MC twins and interfetal vascular circulation.

We noted that chorioamnionitis, when restricted to involvement of only one twin, had a tendency to affect the first-born twin more frequently than the second born. This result supports the belief that most cases of chorioamnionitis, but probably not all cases, are derived from an ascending infection from the mother.

Velamentous insertion of the umbilical cord, with the attendant risk of rupture of vasa previa, occurs more commonly in twin gestations than in singleton gestations. Benirschke and Driscoll [7] cite a figure of 7% for velamentous insertion in twins compared to 1% for singletons. This figure is in good agreement with our experience (8.2% of the twins). In other studies the incidence of velamentous insertion was increased in MC twin placentas compared to DC placentas [5]. Benirschke [5] felt that this finding reflected disturbed placental growth caused by nutrient competition between closely approximated twin embryos. Developing twin embryos which are most closely approximated (ie, MC twins), therefore, are subjected to the greatest peripheral growth of the placental mass, as placental expansion occurs away from a relatively nutrient-deficient, central uterine zone. With this peripheral placental expansion, the body stalks are stretched, and the cords develop a high frequency of velamentous insertion. Developing twin embryos which are initially further apart than MC twins are not subjected to as much peripheral growth and consequently the incidence of velamentous insertion is less. We found a slightly higher incidence of velamentous insertion in MC gestations compared to DC gestations and a significantly (P < 0.05) increased incidence of velamentous insertion in DC-fused placentas compared to DC-separate placentas. These findings are in accordance with this theory of “trophotropism”. The increased proportion of cords with marginal insertions in MC placentas compared to DC placentas also supports this concept, although we did not find an increased incidence of marginal insertion in DC-fused placentas compared to DC-separate placentas.

A monoarterial umbilical cord occurred in the placentas of 4 twins, an incidence of 2.2% of deliveries (1.1% per twin). The incidence of single artery cords
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appears to be higher in twin gestations, varying from 1.8% to 7% [6,9], compared to singleton gestations (estimated incidence, 0.5% to 1.1%) [5]. The results of our series are on the low side probably because of its restriction to live-born twins, and the known association of monoarterial cords with congenital malformations. In twin gestations malformations associated with monoarterial cords occur somewhat more commonly in MZ twins (57%) than in DZ twins (41%) [6]. In Heifetz’s series [15] the single artery cord always corresponded to the lighter of the twin-pair, however, in two of our four cases the heavier of the twin-pair had a monoarterial cord. Occurrence in only one of the cords of pairs of MZ twins emphasizes that monoarterial cords are an acquired, developmental anomaly – a belief which is supported by an increased incidence of other malformations in the placentas which harbor monoarterial cords. Two of the four monoarterial cords in our series had velamentous insertions, and there were anomalous cord insertions in 23.5% of the cases in Heifetz’s series [15] of single umbilical arteries. According to Benirschke [5], who proposed that monoarterial cords result from initially diarterial cords, the association of monoarterial cords with velamentous insertion is a reflection of disturbed placental nutrition. Such disturbed nutrition induces lateral placental growth and therefore velamentous insertion, as well as secondary atrophy of one of the two umbilical cord arteries. The observation of a partially monoarterial and partially diarterial cord in our series supports that monoarterial cords result from secondary atrophy of diarterial cords. Although the congenital malformations (musculoskeletal, genitourinary, and cardiovascular) which occur with increased frequency in infants with monoarterial cords are generally restricted to the twin corresponding to the abnormal cord, it should be born in mind that the twin-mate of such gestations often shows growth retardation [15], supporting the notion of disturbed placental nutrition. Two of the four infants with monoarterial cords in our study had congenital anomalies, whereas their twins were normal. In one autopsy series of 237 cases of monoarterial umbilical cords, malformations occurred in 67% of patients [15].

We conclude that a careful examination of twin placentas, including the routine performance of simple injection studies, may determine the zygosity of the twins and provides insight into such important phenomenon as twin growth, chorioamnionitis, the twin transfusion syndrome, velamentous insertion of the cords, and the development of monoarterial cords.

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