General Considerations

There is no doubt that the complexity of human twinning cannot be understood without knowledge of placentation, and placental examination is vital to this endeavor. Examination of the placenta also contributes to the determination of zygosity as well as an understanding of abnormal events. With the advent of assisted reproductive technology, twins and higher multiple gestations are becoming more common. Because multiple gestations represent a disproportionate portion of complications with higher rates of prematurity, perinatal morbidity, perinatal mortality, and malformations than singletons, placental examination has become especially important.

Zygosity

With regard to zygosity, there are generally two types of twins, “fraternal” or dizygotic (DZ) twins and “identical” or monozygotic (MZ) twins. In higher multiple births, these types are often admixed. MZ twins arise from fertilization of one ovum by one sperm with subsequent division of the zygote into two genetically identical individuals. DZ twins arise from fertilization of two ova by two sperm, resulting in two nonidentical individuals. MZ twins by definition are identical genotypically and phenotypically and are of like sex. DZ twins often have significant phenotypic
similarity due to their relationship as siblings and may be of different or like sex. An interesting, but as yet unexplained, observation is that among DZ twins there is an excess of like-sex pairs.

**Incidence**

The overall twinning rate has, for many years, been constant at a rate of 1 in 80 births. However, with the advent of assisted reproductive technology, the twinning rate has increased dramatically and is now approximately 1 in 30 births. The occurrence of higher multiple births, such as triplets, quadruplets, etc., has commonly been estimated by the “Hellin–Zeleny” hypothesis. This useful approximation says that if twins occur with a frequency of 1/N, then triplets have a frequency of (1/N)^2, quadruplets have a frequency of (1/N)^3, and so on. The DZ twinning rate varies with ethnicity, race, and geographic location and is estimated to be 1.4 to 49.0 per 1000 births. This, of course, only applies to spontaneous conceptions. In contrast, the MZ twinning rate is nearly constant at about 3.5 per 1000 births. The sex proportion of all MZ twins is 0.487 while DZ twins have a proportion of 0.518. In other words, MZ twins are more commonly female. Conjoined twins and acardiac twins (see Chapter 10) are also more commonly female, while abortuses are more often male. Theories abound to explain these phenomena, but at present the cause is unknown.

**Twin Placentation**

There are three types of twin placentation: diamnionic-dichorionic (DiDi), diamnionic-monochorionic (DiMo), and monoamnionic-monochorionic (MoMo). This nomenclature is based on which of the fetal membranes the twins share (Figure 9.1). In DiDi placentas, each twin has its own chorion and amnion; in DiMo placentas, twins share the chorion but have separate amnions; and in MoMo placentas, both amnion and chorion are shared and the twins reside in the same amniotic cavity. Monochorionic placentas always come from MZ (identical) twins. Dichorionic placentas, however, may occur with both DZ and MZ twins. About 20% of all twin placentas will be DiMo (and thus MZ), 35% will be DiDi twins of different sex and thus DZ, and 45% will be DiDi of the same sex. Of the latter, only 18% will be MZ. Therefore, overall, 72% of twins are DZ and 28% are MZ.

In MZ twins, different types of placentation and twinning occur depending on when the split occurs. They may be DiDi, DiMo, or MoMo, and the later the split, the more structures they share (Figure 9.2). If the split occurs before the formation of the blastocyst in the first 5 to 6 days after fertilization, two separate chorions will develop and the placentation will be DiDi. Even if they are dichorionic, the disks are almost always fused. This occurs in approximately 25% of MZ twins. If the split occurs after formation of the chorion but before formation of the amnion, 7 to 8 days after fertilization, the placenta will be DiMo. This is the most common type of MZ twins, occurring in approximately
Figure 9.1. Diagram of types of placentation in dizygotic and monozygotic twins. Dizygotic twinning results in two zygotes. If these implant close together, a fused placenta results, and if they implant far apart, separate placentas result. Depending on which the embryonic split occurs, monozygotic twinning may result in fused or separate diamnionic-dichorionic placentation (DiDi), diamnionic-monochorionic placentation (DiMo) or monoamnionic-monochorionic placentation (MoMo).
75%. If the split occurs after formation of the amnion, but before the formation of the embryonic axis, from 9 to 15 days after fertilization, there will be one amnion and one chorion and the placenta will be MoMo. Later splitting will result in varying degrees of conjoined twins. The MoMo placenta is rare, occurring in less than 1% of MZ twins. It is also associated with the highest perinatal morbidity and mortality in twins.

Twin placentas from DZ twins are always DiDi but may have different configurations, depending on where the two zygotes implant (see Figure 9.1). If the zygotes implant close together, the placental disks will become fused as they grow, forming a fused DiDi placenta. If they implant further from each other, they will become separate DiDi placentas. At times, the placental disks may be separate but the membranes may be “fused.”

Pathogenesis
A fundamental difference exists in the respective etiologies of DZ and MZ twins. DZ twins (and higher multiples) are the result of polyovulation, a process that is familial, likely hereditary, and related to ethnicity, race, and maternal age. Polyovulation may be related to increased FSH production, increased GnRH production, or greater follicle sensitivity to FSH. Polyovulation can be induced by the administration of
gonadotropins and other hormones, as is evident from their use for stimulation of ovulation in infertility patients. Follicle-stimulating hormone (FSH), and to a lesser extent luteinizing hormone (LH) and estradiol, is elevated in twin-bearing mothers, suggesting that the genesis of DZ twins is at least partially caused by excess production of FSH. Genes responsible for higher FSH levels may explain the why DZ twins run in families and why racial differences in DZ twinning rates exist. There is a steady rise in DZ twinning up to the maternal age of 35 and then after that, a sharp decline.

Although DZ twinning is familial, it is not so for MZ twinning. The incidence of MZ twinning is nearly the same throughout the world and appears to be a sporadic event, unrelated to heredity. The actual cause of MZ twinning is not fully understood, but it appears that MZ twins originate from the spontaneous separation of the blastomeres occurring at random during the early embryonic period. Interestingly, although multiple births after gonadotropin stimulation are generally multizygotic, there is often an admixture of DZ and MZ infants. In vitro fertilization has 12 times the expected MZ twinning rate compared to single sperm injection fertilization.

**Clinical Features and Implications**

The incidence of congenital anomalies in twins is higher than in singleton gestations. Anomalies occur with a frequency of approximately 10% in twins, 3 times the singleton rate. Discordance for anomalies between twins is common, and the discordance is higher in MZ compared to DZ twins, reaching 80%. Triplets and higher multiples share this discordance as well. Certain anomalies are much more common in twins; for instance, sirenomelia is increased 100 fold in twins versus singletons. Some anomalies, such as anencephaly, occur in DZ twins with the same frequency as in singletons but are increased in MZ twins. Other anomalies such as porencephaly and visceral ischemic lesions are much more commonly seen in MZ as they are related to the vascular anastomoses in the placentas of these twins (see Chapter 10). Most anomalies in twins have no evidence of a genetic component even though anomalies with a strong genetic etiology, such as cleft lip and cleft palate, are frequently discordant in MZ twins. Possible explanations for the increased incidence of anomalies and the discordance include adverse placentation [e.g., velamentous insertion of cord or single umbilical artery (SUA)] or unequal splitting.

Umbilical cord abnormalities are much more common in multiple gestations, and this includes velamentous cord insertion, marginal cord insertion, SUA, and hypocoiled umbilical cords (see Chapter 15). Velamentous insertion is nine times more common in twins than in singletons. Marginal insertions are found twice as often in twins, and both velamentous and marginal insertions are found twice as frequently in monochorionic placentas compared to dichorionic placentas. Membranous umbilical vessels are susceptible to compression, thrombosis, and rupture, and if membranous vessels are present over the cervical os (vasa previa), they may rupture during delivery and lead to fetal exsanguinations. Cord prolapse may also occur. Abnormal cord insertions
and SUA in turn are often associated with *preterm delivery, premature rupture of membranes, fetal anomalies, and fetal growth restriction*. Growth discrepancies are seen quite commonly in monochorionic twins with velamentous insertions.

The mortality of twins is also much greater than that of singletons, being approximately 10%. Prematurity is one of the most important factors in determining outcome, and this is significantly increased in multiple gestations. In addition, monochorionic twins generally deliver earlier than dichorionic twins. *Monochorionic twins have a higher mortality rate than that of dichorionic twins and the mortality of monoamnionic twins is the highest.* This difference is predominately due to the consequences of vascular anastomoses. Perinatal mortality increases exponentially with each higher multiple offspring, being approximately 16% in triplets, 21% in quadruplets, and 41% in quintuplets. This is one of the prime motivators for the practice of “fetal reduction” during early pregnancy in which triplets are “reduced” to twins.

Numerous maternal complications are associated with multiple gestation as well. These include *preterm delivery, preeclampsia, polyhydramnios, placenta previa, abruptio placentae, uterine inertia, and postpartum hemorrhage*. Placental abnormalities often mirror these events. Hydramnios in twin pregnancies is most commonly due to the transfusion syndrome but may also be secondary to fetal or placental anomalies. Uterine atony and postpartum hemorrhage are most likely caused by increased uterine distension from multiple pregnancy.

**Examination of the Placenta in Multiple Gestation**

**Gross Examination**

Examination of the placenta in multiple gestations involves all the aspects of examination of the singleton placenta. There is then the added complexity of the relationship between the placentas and the fetuses. To derive benefit from the study of twin or multiple births, it is mandatory that the umbilical cords be labeled in the order as they are delivered for identification of the infants with their respective placentas. This of course must be done in the delivery room, and a standard protocol for identification of multiples should be used. For example, ties or clamps may be placed around the placental cut ends of the cords and optimally around individual cord fragments. The first placenta and infant, “A,” should be labeled with one tie or clamp, “B” with two ties or clamps, “C” with three, and so on. The labeling should be explained on the requisition, e.g. A = 1 clamp, etc.

In twins there are several additional features than need to be evaluated:

- The relationship of the placental disks:
  - If separate, the placentation is **DiDi separate**
  - If separate, but connected by membranes, the placentation is **DiDi separate**
  - If fused, the placentation is **DiDi fused** or **DiMo**.
Examination of the nature of the dividing membranes:

- Sections should be taken of the dividing membranes, either by excising and rolling a square of these dividing membranes, or by taking a section from the site where the membranes insert on the surface, the so-called “T zone” or “T section.”

The experienced examiner may make the diagnosis of DiMo versus DiDi twin placentation by macroscopic inspection using the following criteria:

- The dividing membranes of DiMo placentas, with only two amnions, are usually translucent, thin, and contain no blood vessel remnants (Figure 9.3).

Figure 9.3. (A, B) Diamnionic-monochorionic twin placenta. The “dividing membranes” are held up to disclose their transparency. (See Figure 9.4 for contrast with DiDi placenta.)
• DiDi membrane partitions, with two amnions and chorions, are more opaque, containing remnants of vessels that are grossly visible as fine branching streaks (Figure 9.4).

• The fetal surface of DiDi placentas show a white, slightly elevated ridge of fibrin not present in DiMo placentas (Figure 9.4). This is the twin peak sign seen by sonography.

• The area of fusion between the two placentas, the vascular equator, will show an abrupt termination of the surface vessels from each placenta in a DiDi placenta, while in the DiMo placenta, vessels will cross, intermingle, and anastomose (Figures 9.3, 9.4).

Figure 9.4. (A, B) DiDi fused twin placenta with visible ridge on the fetal surface at the dividing membranes. The latter appear opaque and thicker than those seen in Figure 9.3.
Examination of Vascular Anastomoses

Monochorionic placentas virtually always have blood vessel connections or anastomoses between the two placentas. Vascular anastomoses may be arteriovenous (either from A to B or from B to A), vein to vein, or artery to artery (Figure 9.5). Injecting fluid in one vessel and documenting its appearance in the circulation of the other placenta facilitates examination of the vascular anatomy. General examination of the placenta should be completed first, but samples for histological study should only be removed after injection has been done. Milk, water, any colored liquid, or even air may be used for injection. The following procedure is recommended:

- The umbilical cords should be cut near the placental surface to reduce vascular resistance.
- The amnion should be stripped from the chorionic surface to better expose the vessels.
  - Arteries and veins can be distinguished by the fact that arteries cross over veins. A ratio of 1:1 is usually found in the final vascular ramifications.
- The major vessels may be followed to their ends visually, and it is usually quite clear which vessels are likely to have communications between the two fetal circulations.
- A-A and V-V anastomoses will appear as direct connections between vessels from one placenta to the other. A-A are the most common type and V-V are the least common type. To demonstrate these anastomoses, it is often sufficient to stroke the blood back and forth through the vessels.
- A-V anastomoses are more difficult to demonstrate. Normally, the fetal arteries terminate in the periphery, dip into the villous tissue, and emerge as nearby veins, which then course back toward the

Figure 9.5. Diagram of the vascular equator of a DiMo twin placenta to show A-A, V-V and A-V anastomoses. The normal pairing of artery and vein derived from one twin is also depicted (top). A = artery, V = vein.
umbilical cord. Injection is suggested to conclusively demonstrate these anastomoses (Figures 9.5, 9.6).

- Injection of deep A-V anastomoses:
  - These perfuse a shared cotyledon.
  - A needle is inserted into a vessel near the point of presumed anastomosis and then, holding to the needle to prevent back-pressure, the liquid is gently injected (Figure 9.6). Alternatively, an umbilical vessel may be injected.
  - One cotyledon will distend as the fluid is accumulated. After a short time, the fluid will emerge from a vessel in the other placenta.
- It is suggested that the vascular relationships be recorded in a drawing, if complicated.

Following examination of anastomoses and examination and sectioning of the dividing membranes, the remaining routine sections of each placenta should be taken (see Chapter 3).

**Diamnionic-Dichorionic Twin Placenta**

The DiDi twin placenta is the most common type of twin placenta. Sections of the dividing membranes in fused DiDi placentas will easily demonstrate the presence of two amnions and two chorions (Figures 9.7, 9.8). DiDi placentas share with the other types of twins an increased frequency of marginal and velamentous insertion of the umbilical cord and single umbilical artery. With rare exception, DiDi placentas have no vascular anastomoses. A very common feature of DiDi twin placentas is the phenomenon of irregular chorionic fusion (Figure 9.9). Here, the membranes do not meet over the areas perfused by the individual fetuses, and a portion of one may be covered by the membranes of the
Figure 9.7. (A) Diamnionic (monochorionic) “dividing membranes” of identical (MZ) twins. There is always a space between the two amnions. The amnion usually consists of single layer of cuboidal epithelial cells and scant connective tissue. (B) Diamnionic-dichorionic “dividing membranes.” The right amnion is dislodged from the underlying chorion, a frequent artifact. The trophoblastic remnants in between the membranes have fused. A, amnion; C, chorion; T, trophoblast. H&E. ×100.

Figure 9.8. (A) T section of dividing membranes of monochorionic diamnionic twins. Note the contiguity of the chorion over the surface of the placenta at bottom. (B) T section of diamnionic-dichorionic twin placenta. There are atrophic villi and trophoblastic remnants between the two choronic membranes. Again, the two chorions appear to have fused. A, amnion; C, chorion; T, trophoblast; V, atrophic villi. H&E. ×40.
other. This condition develops when the intraamniotic fluid pressure of one cavity expands its sac and pushes the other away. It is not unlike the process of lifting the marginal chorion in cases of circumvallate placentation (see Chapter 13). As a result, the “vascular equator” is usually not in the same location as the dividing membranes. This is particularly important in the determination of the weights of the respective placentas. One must always separate twin placentas along the vascular equator and not along the dividing membranes. The irregular fusion has no influence on fetal well-being, and no vascular fusion takes place in the areas of overlap.

**Diamnionic-Monochorionic Twin Placenta**

The DiMo twin placenta is the most common placentation in MZ twins. Both twins reside in the same chorionic sac, but each twin is enclosed in its own amniotic sac. The “dividing membranes” are composed of two amnions only (see Figures 9.7, 9.8). Similar to DiDi twins, the membranes may move over the surface before birth, and the dividing membranes are often at a place that does not correspond with the vascular equator (see above). The cord insertion is often marginal or velamentous, and single umbilical artery in one or both twins is much more common than in singletons.

**Monoamnionic-Monochorionic Twin Placenta**

The MoMo twin placenta is the least common type and occurs only once in 10,000 to 16,000 pregnancies or once in 100 sets of twins. The fetal surface of MoMo twin placentas usually has a continuous sheet
of amnion without ridges or folds between the cord insertions. By definition, there are no dividing membrane. The umbilical cords usually insert close to each other on the placental surface (Figures 9.10, 9.11). There may also be a single fused cord or a forked cord (Figure 9.12). Since the yolk sac develops around day 11, MoMo twin placentas may have a partially divided yolk sac or two yolk sacs. Anastomoses of fetal blood vessels are even more common in MoMo placentas than in DiMo twin placentas and are often large, this perhaps being the reason for the rarity of the twin-to-twin transfusion syndrome in MoMo twins (see Chapter 10).

When examining the apparent MoMo placenta, one must be mindful of artifacts causing a “pseudomonoamnionic” placenta. Disruption of the membranes during delivery may cause the dividing membranes to be pulled away from the fetal surface, giving the erroneous impression of a MoMo placenta. The intertwin membranes may spontaneously rupture prenatally, or there may be intentional rupture of the membrane for therapeutic purposes such as amniocentesis, funipuncture, or treatment for twin-to-twin transfusion syndrome (see Chapter 10). These events may make it impossible to differentiate a DiMo from a MoMo placenta. Clinical history and the position of the cords may be helpful. If the dividing membranes are disrupted before delivery, morbidity and mortality may occur from cord entanglements just as with true MoMo twins.

The perinatal mortality of MoMo twins reaches 40%. Fetal death most commonly occurs due to entanglement of the umbilical cords from fetal movement resulting in venous obstruction (see Figure 9.11). Knotting of the cords is unpredictable and is often found in very young

Figure 9.10. MoMo twin placenta with amnions removed. The cords insert next to each other, adjacent to major anastomoses visible on the surface.
Figure 9.11. MoMo twins at 38 weeks gestation with fetal death of one at 23 weeks. The survivor is alive and well. Note the entangling and knotting of cords, the thin cord of the dead twin, and the extensive infarction of the right placental half.

Figure 9.12. Forked umbilical cord of MoMo twin abortuses at 17 weeks gestation. The smaller twin (right) had a single umbilical artery but no other anomalies. (Courtesy Dr. Marilyn Jones, San Diego, CA.)
pregnancies with early abortion ensuing. Fetal demise usually occurs before 24 weeks, when enough room for fetal motions and entanglement is still possible. After 30 to 32 weeks, few deaths occur.

**Suggestions for Examination and Report: Twin Placenta**

**Gross Examination:** In DiMo twins, vascular anastomoses should be documented. In all twins with fused placental disks, the dividing membranes should be submitted for microscopic examination. The disks should be separated along the vascular equator and then weighed and examined separately. See section above on gross examination for additional details.

**Comment:** Possible diagnoses are diaminic-dichorionic (fused or separate) twin placenta(s), diaminic-monochorionic twin placenta, or monoamnionic-monochorionic twin placenta. A comment on the nature of the vascular anastomoses should also be included. For example, when a single dominant A-V anastomosis is present, the possibility of twin-to-twin transfusion syndrome should be entertained. If multiple anastomoses of various types are present, a comment to that effect should be made. As previously stated, a drawing of the vascular connections can be helpful in many cases.

**Selected References**

