Placentation in Multiple Births

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Outcomes of multifetal pregnancy in prenatal life are markedly affected by chorionicity. Several disease processes are found in monochorionic (MC) twins that do not occur in dichorionic (DC) twins. Improvements in prenatal outcomes will depend on reliable first trimester diagnosis of chorionicity, allowing early monitoring for complications of MC placentation. Particular structures and functions of MC twin placentas affect outcomes and can be targeted for specific treatments, especially in twin-twin transfusion. The causes of severe DC twin fetal growth discordance are clarified. In post-natal life, zygosity is a determining effect in genetic predisposition to many chronic diseases, including neoplasia. Few MC twins know that they are monozygotic (MZ). Few twin researchers realize that MZ twins may be genetically discordant. Abandonment of the word "identical" for MZ twins would assist in clarifying these issues of zygosity, concordance and discordance.

Chorionicity (C) and zygosity (Z) are closely related. For optimal prenatal care of twins, knowledge of C is paramount. Unfortunately, it is not universally available. In addition, secure knowledge of Z is important in several aspects of life-long health care in twins. Finally, information on C and Z is important to twin researchers, but often is unavailable or incorrect. Matters are not infrequently compounded by the fact that most people (including twin researchers) fail to realize that MZ twins are not necessarily (or possibly ever) genetically identical (Keith & Machin, 1997). Similarly, the fact that they have occupied the same womb does not guarantee that they have experienced the same environmental events (Machin & Keith, 1999). Whereas this paper is primarily about the subject of placentation, the issue of Z must always remain in the background. To the extent that there are any simple rules about Z and C, they are as follows: 1) unlike-sexed twins are usually DZ (exception: 46,XY/45,X MZ twins; Schmid et al., 2000); 2) MC twins are usually MZ (only one known exception; Bieber et al., 1983); 3) Like-sexed (LS) DC twins may be MZ or DZ; 4) Whereas DZ twins are always DC, MZ twins may be DC or MC; however, the majority are MC. MZ twins can have either MC or DC placentation.

The first axiom of understanding placentation in twins is the recognition that the MC placenta, whether DA or MA, mimics the structure of a singleton placenta and was designed to function as such. Because it never was 2 placentas that subsequently fused, it is not surprising that most of the adverse outcomes in MC twins result from the structure of their MC placenta *per se.* Whereas both types of twin pregnancies (MZ and DZ) have risks of "common denominator" disorders such as pre-eclampsia, pre-term labor and PROM, DC twins constitute relatively low-risk pregnancies compared with MC twins. If the diagnosis of MC twins is not made with certainty in the first trimester, most of the special complications of MC placentation will already be at an advanced stage by the time of dating ultrasound at 18 weeks, (by which time it is in any case harder to diagnose C.)

The second axiom is that the biology of MC and DC twins is profoundly different. This summary paper considers the differences between the structure and function of DC and MC twin placentas, and then applies these facts to the need for different pregnancy (and "whole life") management for DC and MC twins in the following clinical situations: Significant growth discordance; impending and actual fetal demise; discordance for major malformation *vis-a-vis* attempted "selective" termination; prolonged delivery interval; prenatal genetic diagnosis; post-natal genetic diagnosis; organ transplantation; special problems of MC twins: TRAP, twin-twin transfusion (TTT), both antenatal and perinatal; special problems in MCMA twins. "Whole life" health issues in twins largely revolve around Z. The designation at birth of MC twins as being MZ is a basic necessity that should be communicated to parents. Likewise, LS DC twins should not *a priori* be designated as being DZ. In this way, later confusion about "identical" twins, etc., can be avoided. Common errors and omissions in early and timely diagnosis of Z frequently result in missed opportunities for early treatment and prevention of disease in a co-twin.

DC and MC Twin Placentas

MZ twins that split within 2 days post-conception (PC) have one placenta each, and are thus DC whether the placentas are fused or separate. All DZ twins are DC. Only one paper has ever documented inter-twin vascular anastomoses in DC placentas (Robertson & Neer, 1983), and most experienced investigators, including myself, have never seen one. In this regard, I have observed an MZ twin placenta that was half DC and half MC, presumably because the twinning event happened at the cusp of 2 days pc, forming a "compound" DC/MC placenta with anastomoses. Reports of TTT and TRAP in apparently DC placentas have appeared (French et al., 1998), but other

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explanations are possible for these complications. Whereas vascular anastomoses between DZ DC placentas could explain twin blood chimerism, it is important to look for alternative mechanisms in the majority of cases, for example, acute mechanical disruption of fetal blood vessels during delivery. For all practical purposes, the diagnosis of DC placentation absolves the twins from the most intractable complications of inter-fetal vascular communications.

The third axiom is that the dangers of MC twin placentation largely relate to the structure of fetal blood vessels joining the twin umbilical cords across the single placenta. Two prime factors are operational. The first is that the location of each cord insertion determines the proportions of placental parenchyma allocated to each twin when their embryonic umbilical circulations competitively colonize the chorionic plate (Machin & Keith, 1999). Sharing is by no means always equal, and a peripheral (marginal or velamentous) cord insertion is no match for a paracentrally (eccentric or central) inserted cord. In its extreme form, this disparity results in severe IUGR and the small twin develops oligohydramnios. However, this phenomenon of unequal sharing should not be confused with antenatal TTT, in which simultaneous oligo- and polyhydramnios are present, at least in MC, DA twins. Unequal sharing and TTT may occur in the same pregnancy. Since the advent of high resolution sonography, cord insertion points can be documented by ultrasound in all MC twins. Velamentous cords are more common in twin than singleton placentas, and are very frequent in MC placentas (Machin, 1997). The reason for this is that DC placentas may arrive separately at the endometrium, even if they implant close together. Therefore, they have a good chance of orientating themselves correctly as they implant. However, because the MC placenta is a truly single placenta, it follows that if one cord insertion is orientated towards an optimal implantation, the other may not be, but their relationships are fixed, and the single placenta cannot "spin round" to get both cords to point of optimal nourishment.

The second is that 80% of MC twin placentas have inter-fetal vascular connections. This fact is the key to understanding the development of TTT, TRAP and the complications of fetal demise (Bajoria et al., 1995; Machin et al., 1996). It is also important to understand that each and every MC placenta has a unique connective pattern. Thus, a wide array of types, degrees and speed of onset of clinical problems related to inter-twin vascularity is seen. Detailed post-partum investigation of MC placental vascularity has clarified the main points (Machin & Keith, 1999), and it now remains to apply sophisticated non-invasive diagnostic methods to the placenta *in vivo*. In this way, specific treatments can be tailored for individual cases. It is totally inappropriate to propose that the well-known transfusional diseases of MC placentas constitute homogeneous disorders, each requiring one standard treatment, particularly as some treatments are invasive. Fetoscopic procedures are indicated in some cases; when performed, they confirm the post-partum vascular findings.

It is best to understand the vascular anatomy of MC placentas before using fetoscopic methods. A brief summary follows (Machin & Keith, 1999). The perfusion zones of the MC twin placenta are not isolated from each other, as they are in DC placentas. Rather, there is a "zone of ambiguity" on an equatorial line running at right angles to the line joining the two cord insertions and roughly equidistant between those insertions. In this ambiguous zone, the parenchyma belongs entirely neither to one twin nor to the other. Across this zone there run "flyovers or aqueducts" (as it were), carrying direct, surface arterio-arterial (a-a) and veno-venous (v-v) connections. At equal cardiac outputs and pressures, the net blood transfusion that takes place in these superficial connectors is negligible. However at the surface level in this zone, there also exist arterio->venous anastomoses (a->v an) in which the artery is a branch of the umbilical system of one twin (donor), and the vein is a branch of the umbilical system of the other (recipient). Because of the arterio-venous pressure gradient, a net transfusion always occurs in such a->v ans. Having said this, it is important to note that the transfusion does not occur on the surface (as in a-a and v-v connectors), but rather in an underlying shared cotyledon, the so-called "third circulation" described by Schatz more than 100 years ago. Much confusion exists about the actual whereabouts of the transfusion in a->v ans, the concept being that one would have to ablate the cotyledon itself if performing fetoscopic surgery for TTT. But this is not the case. While it is true that the transfusion occurs at capillary level in the shared cotyledon, the feeder artery and vein on the placental surface have a stereotypic anatomy which is easily recognized by post-partum perfusion, at fetoscopy and by non-invasive ultrasound methods. This anatomy is no different from that of every artery and vein entering a cotyledon in any placenta, be it singleton, twin or quintuplet. Stated another way, the artery leaves the chorionic surface through a foramen that it shares with the emerging vein. Arteries and veins never enter and leave the underlying parenchyma randomly or independently, but always together in pairs. This is true of artery/vein pairs connected to the same cord or to two cords. It means that an a->v an can always be recognized as such at fetoscopy (De Lia et al., 1993; Quintero et al., 1998) or post-partum injection, and this anatomic relationship has also been observed recently by Doppler ultrasound (Machin et al., 2000; Taylor, Farquharson, et al., 2000).

Complications of Twin Pregnancy (Placentation)

Growth Discordance

By extension of the (simplistic) "nature vs nurture" model for classical twin studies, it has long been assumed that growth discordance in anatomically normal MC twins is "acquired" whereas that of DC (not necessarily DZ) twins is "constitutional". Parenthetically, it is hard to imagine how 40% discordance could be explained in this manner, unless one twin had an overgrowth syndrome, such as Beckwith-Wiedemann syndrome. However, it would be possible for one genetic disorder (thrombophilia) to affect one DZ, DC placenta and not the other.

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Table 1

Placental and Cord Findings in Discordant DC Twins

Discordance, %	20–24.9	20–24.9	25–29.9	25–29.9	= >30	= >30
Separate/Fused	Separate *n* = 5	Fused *n* = 5	Separate *n* = 2	Fused *n* = 5	Separate *n* = 2	Fused *n* = 5
Findings	Vel	True knot	Non-coiled	Vel	Over-coiled	Chorangiomatosis
		Marg/sept + under-coiled		Circummarginate + MPVFD	Marg + over-coiled + MFI	Non-coiled
		Non-coiled		Non-coiled		Vel/sept
				Marg/sept + under-coiled		Marg + CVT
				Vel/sept		Over-coiled

Abbreviations: vel: velamentous cord insertion, marg: marginal cord insertion, sept: cord insertion into or at septum, MPVFD: massive peri-villous fibrin deposition, CVT: chorionic plate venous thrombosis, MFI: maternal floor infarction.

It is logical at this point to inquire exactly what is known about growth discordance in DC twins? In a series of 24 DC twin pairs with growth discordance = /> 20% from my laboratory, the following was found (Machin, unpublished):

Table 1 shows that the cause of less severe (20–24.9%) growth discordance may be "constitutional", because separate DC cases have few findings and the findings in fused cases involve the cord and cord insertion. In the intermediate (25–29.9%) group, cord abnormalities predominate, but parenchymal lesions, perhaps representing differential involvement with thrombophilia, are also found. For the severe (> 30%) group, there were often combinations of findings, but cord abnormalities are frequent. Of the 24 discordant twin pairs shown in Table 1, the DC placentas were fused in 15 (62%) and separate in 9 (38%). In contrast, fused DC placentas generally constitute only 44% of all DC placentas (Benirschke & Kaufmann, 1995). Fusion contributes to the more severe degrees of discordance, perhaps by increasing the frequency of abnormal cord insertions. From a large published series (Benirschke & Kaufmann, 1995), velamentous cord insertions, by types of twin placentation are as follows: DC, separate — 3%, DC, fused — 10%, MC, DA — 13%, MC, MA — 16% In the present small series, the frequency of velamentous cord insertion was 11% in separate and 20% in fused DC placentas with restricted fetal growth of one twin. Velamentous cords are associated with significant problems in singletons, including IUGR (Heinonen et al., 1996). These effects may be exacerbated in twin pregnancy.

It is not clear whether and to what extent fetal growth normally drives placental growth. It is equally unclear whether restricted placental mass can limit fetal growth. However, because the placenta is a fetal organ, it is reasonable to presume that placental mass in ordinary conditions reflects fetal nutrition and growth. Despite this, significant cord abnormalities, as well as villous lesions such as maternal placental floor infarction on the visible surface and massive peri-villous fibrin deposition (both secondary to thrombophilia) may limit fetal growth.

In discordant MC twins, the situation is simpler. Unequal parenchymal sharing is present in all cases, usually with peripheral cord insertion of the smaller twin. However, it is also true that some cases of severe discordance in MC twins may be caused by unequal splitting of the inner cell mass, with fewer founder cells in the smaller twin (Nance, 1990). It is not yet clear whether antenatal TTT causes growth discordance. Many cases of TTT have unequal sharing and some degree of early discordance, but biometric assessment of further fetal growth may merely identify congested liver causing increased abdominal circumference out of proportion with other measurements, but obviously affecting the calculation of the estimated fetal weight.

Impending and Actual Fetal Demise

Because the fetal circulations of DC twins do not meet, there is no potential for vasculogenic damage to the co-twin when one fetus dies. This is one of the most convincing reasons for ascertaining C in the first trimester. It may be necessary to diagnose Z in the second and third trimesters by fast-track DNA analysis of both amniotic fluids, but this methodology will not distinguish between DC and MC MZ twins. Alternatively, Doppler diagnosis of an a-a anastomosis always indicates MC twins. The death of one DC twin fetus is without affect on the survival of the other DC twin.

The situation is totally different for MC twins, in which the danger of single fetal demise is that the survivor will rapidly exsanguinate via inter-fetal connections into the body and placental portion of the dead fetus, causing hypoxic/ischemic brain damage (Saito et al., 1999). Management of impending fetal demise is therefore aimed to separate the two circulations before the demise of one fetus. If gestation is sufficiently advanced, immediate delivery can be affected. There is no indication for any treatment after fetal demise. The most common cause of fetal demise in MC twins is TTT, for which specific interventions are available (see below), but MC twins are frequently discordant for a range of major anomalies, a few of which may be lethal in fetal life, e.g. various causes of hydrops fetalis. Most commonly, the cord of the dying twin is ablated, thus preventing any back flow of blood into the surviving fetal body tissues. However, the portion of placenta perfused by the dying twin will also act as a blood sump, unless there is unequal sharing and the smaller twin is dying. Therefore, it may be necessary in addition to ablate any connecting vessels on the chorionic plate in order to prevent exsanguination into placental tissue. It is inappropriate to terminate the dying fetus by intra-cor-

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poreal vessel ablation, because this leaves the umbilical cord vessels patent. Likewise, intra-cardiac injection of substances such as digoxin or KCl will almost inevitably result in the death of both fetuses via interfetal connections (Golbus et al., 1988).

Selective Termination (ST)

Similar considerations apply. ST by standard methods is simple and safe in DC twins and fraught with great danger in MC twins. In fact, it may be necessary to adopt a more conservative attitude in MC twins concerning the range of anomalies that can be managed with ST. The only absolute indications for ST would be those anomalies known to have a high risk of fetal demise. For other malformations (including chromosome abnormalities), the risks of ST have to be balanced against the decisions for neonatal management. It is unfortunate that cases are still being reported in which attempted ST by standard methods inevitably results in the death of both MC twins. On the other hand, termination of both MC twins by one procedure is an efficient method for fetal reduction in cases of higher order multiple pregnancies resulting from artificial reproductive technology (ART). However, the methods used for ST in MC twins must involve the separation of the twin circulations — see above. Most of the large publications of selective termination/reduction make the assumption that they are dealing with DC twins (Evans et al., 2001; although MC twins occur in ART pregnancies). This does not help to prevent the use of standard ST methods in MC pregnancies.

Prolonged Delivery Interval

Prolonged delivery interval is out of the question in MC twins, and probably only yields optimal results in DC twins with separate placentas.

Prenatal Diagnosis in Twins

No standard practices for prenatal diagnosis in twins take C and Z into account (van den Berg et al., 1999). In general, CVS and amniocentesis are performed on both twins if they have been ascertained to be DC (although some of these are MZ). For MC twins, opinions vary as to whether both sacs should be sampled and by what route, and CVS is bound to be somewhat haphazard. Because heterokaryotypia occurs in MC twins, and it may be inappropriate to sample one sac only. When one twin is malformed, both twins should be sampled. The most efficient method to pick up all cell lines in MC heterokaryotypia would be by fetal blood sampling from one cord only. Amniocentesis in monoamniotic twins should detect all cell lines.

Post-natal Genetic Diagnosis

Post-natal genetic diagnosis in MC twins is complicated by inter-twin vascular connections. Because bone marrow stem cells are usually transfused between MC twins in fetal life, "blood twin mosaicism" may be found for a variety of chromosomal and non-chromosomal genetic factors for which the fixed somatic cells of the twins may be 100% discordant (Kaplowitz et al., 1991). Therefore, fixed cells, eg fibroblasts, buccal cells, should be analyzed when anomalous results are found in blood lymphocytes of MC twins. Further, MC vascular connections may yield false-positive neonatal thyroid screening tests when one MC twin is actually hypothyroid (de Zegher & Vanderschueren-Lodeweyckx, 1989). Vascular instability may cause cerebral pathology in both MC fetuses, leading to the false assumption that such disease is genetic rather than acquired. None of these considerations applies to DC twins.

Solid Organ Transplants

MZ twins, whether MC or DC, make good donors/recipients of solid organ transplants (St Clair et al., 1998), with no risk of rejection. However, MC twins should not be used for bone marrow transplantation for childhood leukemias, because most of these disorders undergo pre-mutations in fetal life, with mutual transfusion of pre-malignant stem cells across MC vascular connections. Bone marrow transplantation from an apparently unaffected MC twin usually results in leukemia recurrence in the recipient at roughly the same time as the donor manifests the disease for the first time (Najfeld et al., 1997).

Special Considerations in MC Twins

Special considerations in MC twins concern TRAP and TTT. These disorders have rarely been reported in DC twins and, for practical purposes, they are confined to MC twins. This is the main reason why first trimester diagnosis of C is so desirable. Both disorders are progressive and may present clinically as early as 20 weeks' gestation.

TRAP is the only MC disorder in which a simple procedure to ablate cord or fetal vessels is adequate (Deprest et al., 2000). This is because, by definition, the acardiac twin does not perfuse any of the MC placenta, so there is no danger of the pump twin exsanguinating into a portion of the placental parenchyma that it was not previously perfusing.

TTT is a disorder of variable severity and acuity of onset. Serious disagreement exists about its management, primarily because it is asssumed by two rival groups of practicioners that all cases should be treated in one manner only — either by amnioreduction (AR; Mari et al., 2000) or laser coagulation (LC; Hecher et al., 2000). AR is non-specific at best, but easy to perform. LC is specific (variably so in different hands), but invasive. A sensible middle position would recognize that every MC placenta is characterized by different vascular anatomy than every other (see above), and that this is true in TTT as well. Both groups agree that the diagnosis is made by the concomitant presence of oligohydramnios and polyhydramnios, accompanied by high rates of complications and death (maybe 80%) without treatment, and that the underlying cause is a significant net accumulation of transfused blood in the recipient from the donor via one or more poorly compensated a->v ans or not at all by any co-existing anastomoses or connections (Machin & Keith, 1989). In its simplest form, the TTT MC placenta has a single, causative a->v an. Such cases may have early onset of severe disease, and it is hard to imagine that any treatment other than LC will alleviate the situation. On the other hand, some cases have a causative a->v an but additional connections are also present. These may include a-a and v-v connections, as well as a->v ans back from recipient to donor. For reasons that are not fully understood, TTT sets in despite the presence of these additional

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vascular compensatory structures. It seems likely that AR is effective in some cases by relieving pressure in the recipient sac that affected venous components of v-v and a->v vessels (venous pressure will be high at the recipient end of the causative a->v an). There seem to be good reasons to give AR a try, and then search for evidence that this intervention has been effective (appearance of urine in donor bladder). If there is no benefit, there is little point in continuing with AR, as the case is declaring itself as requiring LC. On the other hand, there is no need to proceed with LC if AR is effective, particularly because the vascular anatomy of such cases is complex and therefore more difficult to delineate at fetoscopy.

Doppler ultrasound methods allow detailed, non-invasive diagnosis of MC vascular structure in management of TTT. First, the presence of a-a connections (bidirectional pulsatile flow) indicates a low risk for TTT (Denbow et al., 1998), and a better response to AR if TTT develops (Taylor, Denbow, et al., 2000). Second, a->v ans can also be localized (Machin et al., 2000; Taylor, Farquharson, et al., 2000). When these are present but a-a connections are not found, it is likely that LC will be needed. In that event, the position of the a->v an will already be known when planning the entry path for fetoscopy.

Important differences of opinion about the number and type of vessels to be coagulated are present in recent literature. These differences arise partly through failure to visualize the simple fetoscopic features of a->v ans, and partly because of the risks that one or other fetus will later die in spite of LC. If more early cases proceeded directly to LC (because of failure of the first AR), fewer fetuses might die during or after LC, and a more discriminating approach could be taken to coagulation. The earliest method of ensuring complete separation of the twin circulations was to coagulate any and all vessels travelling to and from the donor under the inter-twin membranous septum (Ville et al., 1995). The rationale for this approach was that the vascular equator does not correspond with the base of the septum, and that not all connecting vessels could therefore be recognized as such from the viewpoint available in the recipient sac. The disadvantage of the method is that the donor (who may already be relatively deprived of parenchyma) loses several "innocent" or "appropriate" a-v vessel pairs that are connected to the donor and thus not in any way involved in the transfusion. At worst, it would only be necessary to coagulate in this manner in areas of uncertainty, and not across the whole of the septal base. The more selective approach is able to identify a->v ans, a-a and v-v connections, and coagulates them only, in order to separate the circulations (Hecher et al., 1999; Quintero et al., 1998). This is accomplished at the vascular equator rather than at the septal base. The advantages to this approach are that the transfusion ceases and that each fetus is protected in the event of demise of the other.

However, the selective procedure is still relatively nonspecific in that it does not relieve the overload/hyperviscosity status of the recipient who is at risk for cerebral, renal and myocardial complications that may be permanent and structural. Whereas a-a and v-v vessels should be coagulated (why would they be present in AR-resistant cases?), bi-directional a->v ans could be left in situ to allow the recipient to download to the donor with mutual benefit (Feldstein et al., 2000). Time will tell whether early diagnosis, early referral of AR-resistant cases for LC, detailed Doppler studies and excellent fetoscopic visualization will allow more sophisticated and selective approaches to proceed a better outcome. It is probably a mistake to design trials directly randomizing cases to AR or LC without an initial trial of AR.

Special Problems in MC, MA Twins

These are well known. Pseudo-MA status can be induced by deliberate and/or accidental septal puncture in MC, DA twins (Feldman et al., 1998). Braiding of the cords is almost universal, and can cause asphyxia and/or death of one or both fetuses. Cords are more likely to be wound around body parts of the other twin, including nuchal cords. Therefore, Cesarean delivery is preferred by many because the cord round the neck of the presenting twin may belong to the other twin. Induction of relative oligohydramnios may prevent some cord complications (Overton et al., 1999). TRAP and TTT both occur (rarely) in MA twins, and diagnosis and treatment is correspondingly more difficult.

Whole Life Issues in Twins

Most MC twins do not know that they were MC and therefore must be MZ. A uniform policy is desirable and should be implemented at all maternity units to ensure the C and Z results are given to parents after twin births as soon as available. Implications for zygosity are immense in many chronic disorders, including neoplasia (Peto & Mack, 2000), allergy and mental illnesses. It is a lost opportunity not to make use of MZ status when it might allow prevention or early diagnosis and treatment in a clinically unaffected co-twin.

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