Chapter 27

New Directions: What is New in Placental Studies?

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The answer to the question in the chapter subtitle depends very much on the knowledge of the examiner of placentas. From my perspective, perhaps the most interesting aspects have been the placental changes that are now being observed in multiple gestations and that result from the different practices of assisted reproductive technology (ART), in-vitro fertilization (IVF), and, especially, intracytoplasmic sperm injection (ICSI). The increased frequency with which excessive numbers of multiples are produced has been widely commented upon, and in recent publications it has been recommended that only single ovum transfers be practiced in ART, rather than the more frequent method of multiple blastocyst transfer. The attending prematurity rate and the frequently serious sequelae of marked prematurity have been the principal reasons for this recommendation. Additionally, because of uterine space limitations when multiple blastocysts compete for implantation sites, some placentas may become "squeezed" by the other placenta(s) and thus acquire less room for their expansion. This may then lead to fetal growth restriction and the more frequent and abnormal (velamentous) cord insertion seen in the placentas of multiples. We recently studied 127 sets of triplet placentas that had more single umbilical arteries (SUAs), more circumvallation, more chorangiomas, and, interestingly, more placentas with increased syncytial knots. More recently we saw a normal-appearing newborn with SUA who had a major contribution of cells that were mosaic for an 18q deletion. Thus one may ask, How normal are children with SUA?

Even more interesting, however, is the increased frequency of monochorionic twin placentas developing with these ART practices. Thus, when three blastocysts were transferred, four embryos may develop. Apparently, one of the embryos had split, to result in a set of monozygotic twins. We also found that when three embryos were implanted, sometimes we ended up with two of the triplets being monochorionic,

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probably due to the death of one embryo with splitting of another and a former quadruplet gestation. The reason for this apparent induced "splitting" has been difficult to explain, but Steinman (2003) has suggested a model that needs future investigation. He proposed that adhesion molecules that normally keep the blastomeres together are weakened in their effectiveness. He believes that this is dependent on the calcium concentration in the culture media that are being used and that contain the blastocysts before their transfer; he incriminates the anticoagulant ethylene diamine tetraacetic acid (EDTA) used for the prevention of coagulation as a possible culprit. Since we still do not have any reliable cause for the process of monozygotic twinning in general, this suggestion needs serious consideration by the community of physicians employing ART. Other suggestions are that there may be a discrepancy of the timing of the embryonic development and the uterine readiness, or even that the handling and potential disruption of the zona pellucida are at fault. It has also been suggested that the splitting of embryonic cells into equal numbers of cells during the MZ twinning event may not be as equal as is usually assumed. This may explain the cause of discordant MZ twin fetal anomalies, and specifically it may be the reason for the development of the twin–twin transfusion syndrome (TTTS) and acardiac twins (Benirschke 2009).

Another development recently discovered in several placental studies is dizygotic twins (DZ) with monochorionic placentation (DiMo) that also may then possess vascular anastomoses (Souter et al. 2003). The finding of DiMo placentas has, in the past, been held to be diagnostic of MZ twinning. That notion now needs revision. Moreover, the cases of blood chimerism, starting with Mrs. McKay many years ago, need reexamination and new special care needs to be exercised by the placental pathologist. Mrs. McKay was found to be a blood chimera while she was pregnant, and when asked about possibly having had a twin, she recalled that her male co-twin had died while very young (Dunsford et al. 1953). His blood group could then still be ascertained and, presumably, she would also still have had a chimeric XY lymphocyte cell line. Similar cases have since come to light, and indicate that this can also happen spontaneously. Thus, truly competent placental studies to demonstrate possible anastomoses are needed; on the other hand, these may also lead to the twin-twin transfusion syndrome (Assaf et al, 2010).

In the recent past, much progress has also been made in the therapy of TTTS. It has emerged that serial amniocentesis for the relief of hydramnios is not truly efficacious in the treatment of the "disease," and more centers now have gone over to the more effective laser coagulation of the causative arteriovenous (A-V) shunts in the placenta. The anastomoses are not always easy to identify through the small optics used during fetoscopy, and to select the right ones is a challenge (Chmait et al. 2010). It is therefore strongly recommended that future fetoscopic surgeons familiarize themselves with the vascular anatomy of the placental surface before attempting this challenging procedure. It has also emerged that artery-to-artery anastomoses are largely protective against the development of TTTS. What is less well appreciated, however, is that the neonatal diagnosis of TTTS may be difficult, as blood may shift rapidly during delivery between the twins' circulations.

Thus, false hematologic values can emerge that may not betray the prenatal, in utero, circumstances. This has legal ramifications in particular, and we have repeatedly suggested that it is imperative to adjudicate the cases through an examination of the twins' heart sizes (Benirschke and Masliah 2001). In the true TTTS, there is a major discrepancy of the heart sizes beginning already prior to the emerging hydramnios or other symptoms of TTTS, especially when they are adjusted for gestational age. Thus cardiac size discrepancy, rather than hematologic values, is a more reliable measure of the existence of the TTTS, and this relates to the lower blood pressure of the donor twin. Also, it has now become well established that the prenatal central nervous system (CNS) damage that may occur in TTTS, especially when one twin dies in utero, is the result of a transient severe hypotension because transplacental "hemorrhage" into the dead twin occurs from the living twin; that twin may recover, albeit with CNS damage. This CNS damage is not the result of a coagulation syndrome, as was formerly suspected. It has even been witnessed sonographically as a reversal of flow in such cases. Indeed, prenatal sonography has become an essential tool also in the assessment of the "dividing membranes" of twins, with the twin delta peak sign in DiDi twins (T-sign in DiMo twins) and the ascertainment of possible disruption of the amnionic membranes. Attempts at defining heart sizes of twins should be the future goal.

While speaking of chimerism, the other notable development is the recognition of several maternal autoimmune diseases as being the result of maternal blood chimerism by fetal cells that was initiated during the woman's prior pregnancy. It is envisaged that some fetal lymphocytes (perhaps even stem cells) transfer to the mother; this occurs most likely regularly during all gestations, in small numbers. When significant human leukocyte antigen (HLA) diversity is present, perhaps these transferred white blood cells later direct their attention against the maternal antigens in her future life and "reject" the carrier. Indeed, an important case reported by Srivatsa et al. (2001) indicates that, in a case of postpartum thyroiditis, even the maternal thyroid epithelium may be replaced by fetal cells. The magnitude of this process is under active investigation, but a correlation with the type and possible complications of prior gestations has rarely been possible. Thus, in view of the occasionally massive fetal hemorrhage (into maternal blood with Kleihauer-positive maternal blood that results) that we occasionally witness in the perinatal period, it might be asked whether the resultant induced chimerism is perhaps more often responsible for maternal autoimmune diseases in the future of those particular mothers (Johnson et al. 2001). Therefore, attention might be paid more specifically to the future diseases that might affect the mothers with major fetal blood transfer. It has since also been found that during pregnancy the maternal serum carries a substantial amount of cell-free DNA (Lo et al. 1997). It presumably arises from deported syncytial emboli that occur regularly during gestation and then undergo apoptosis in the lung. It is also the reason why that DNA disappears within 2 days after placental delivery. Cell-free DNA may also be useful for prenatal diagnosis, and is often used for genotyping the fetus in Rh-negative women.

There has been a remarkable reduction of perinatal mortality since the development of surfactant therapy for prematurely born infants. The old "hyaline membrane disease" has virtually disappeared from our perinatal autopsies as a consequence. Neonatal autopsies are now largely limited to significant structural abnormalities, chromosomal errors, and severely premature infants, those weighing less than 1,000 g. The placental pathologist notices that the latter infants are almost always associated with chorioamnionitis, or the placental sections show at least significant deciduitis. Remarkable progress has been made clinically in identifying the presence of this ascending infection (e.g., sonographic assessment of the cervix, interferon measurements, and identification of the many interleukins present in the cervical os), but few advances have been made in the precise identification of the responsible organisms, let alone in the therapy for these frequently recurrent infections. It has been my contention that the process is initiated by the existence of chronic endocervicitis and that this is then followed by deciduitis of the "forelying" membranes. Subsequently, local phospholipid production leads to cervical dilatation, to foreshortening of the cervix, and to premature labor. Cerclage, occasionally practiced in this clientele, has been only minimally effective, nor has the initiation of antibiotic therapy at the time of cervical "incompetence" been helpful. This disease process has not diminished in frequency and is now the most important cause of significant prematurity. It needs additional attention by the reproductive specialists. Because of the frequent recurrence of this cause of significant prematurity, these patients may benefit from antibiotic therapy in between pregnancies, and this probably should include the husbands, since inapparent nongonorrheal urethritis may accompany these gestations. At least it is worthy of a major clinical trial. We also need to make much greater efforts in identifying the organisms that cause the inflammation.

Much progress is being made in the identification of genes that regulate placental development – in the mouse, that is (Cross et al. 2003). Despite all the publications on putative causes of pregnancy-induced hypertension (PIH; preeclampsia), we are not truly closer to understanding its etiology. It is often speculated that PIH relates to the immunologic disparity of placental and maternal genotypes, but this has not been borne out with sufficient clarity, despite the occasional mutations occurring in the HLA-G complex. Hope exists that, once genetic regulation of murine placentation is better understood, we can decipher human placental development also. But we are not there yet. Microarrays are beginning to be employed without yet defining genes for specific diseases. This regulation is of more than casual interest, as it also involves the determinants of paternal placental "imprinting" and may be of concern to those readers with an interest in cloning and interspecific embryo transfers and in hopes of understanding the complex mechanisms that must underlie the evolution of the many different types of placentas. Aspects of comparative placentation and their evolution have long been hotly debated without clear resolution, and they are currently mostly descriptive (http://medicine.ucsd.edu/cpa). Greater attention might profitably be paid to the often deeply invasive

trophoblast in some of the South American rodents and their possible immunologic consequences. The genetic disparity of these litters from the dams should be more fully investigated because they have claimed little attention to date.

We have made remarkably little progress concerning the etiology of chromosomal errors and their correlation with placental phenotype. For one thing, the high frequency of spontaneous abortions due to aneuploidies in the human population is not mirrored in other species that have been studied. In fact, it seems to be low in primate research centers where breeding is supervised and conceptions are known (Small and Smith 1983), and this is also true from my observations with wild animals at the San Diego Zoo. Abortions occur extremely rarely, and chromosomal errors as their cause are practically unknown or they can be enumerated easily. The reasons for these discrepancies are of potential importance to human reproductive performance and need better explanations than they now receive. But it is also true that we lack an understanding of why major trisomies cause often specific congenital anomalies in the embryo, while they appear to cause few if any characteristic placental changes. To be sure, the "partial mole" due to triploidy can be cited as an exception; but other major chromosomal errors are not known to be reflected in specific placental phenotypes. Perhaps we need to make a greater effort in their recognition and must more carefully compare cytogenetic findings with placental characteristics. Other than sporadic villous changes (edema, abnormal shape, vascular prominence) or the presence of a single umbilical artery, not much is known, not even a correlation with placental weights exists, and the same applies to "confined placental mosaicism," for which no good histology supports the abnormal placental regions.

When unusual moles have been characterized cytogenetically, a number of mosaic or chimeric genotypes have been identified. A beginning is made by the methods of differentiating partial moles from complete moles through the employment of chromosomal in situ hybridization (Lai et al. 2004). And while it is easy to state that complete hydatidiform moles have an exclusively paternal genome, the reasons for this feature (other than speculations about imprinting) are unresolved. It has become clear now that all complete moles must have had an embryo, at least at one time in early development. Not only have some been demonstrated, but this must also be deduced from the connective tissue these moles contain. But what are the precise reasons (e.g., structural anomalies, imprinting) for the death of these embryos? And why are moles so much more common in Asian populations, and why have they never been seen in any other species? These are questions to which placental pathologists should address some of their thoughts and research efforts.

Another important "black box" at present is the etiology of maternal floor infarction (MFI), a condition that can occasionally now be recognized sonographically. In this condition, excessive amounts of fibrinoid are deposited, and frequently MFI is associated with excessive proliferation of extravillous trophoblast. The precise nature of the fibrinoid has not been sufficiently explored, and the reasons for

the frequent recurrences are similarly unknown. It is here where more definitive knowledge of trophoblast regulation that we hope to obtain from the mouse model might be applied. Perhaps the same can be said of villitis of unknown etiology. As with MFI, the condition is occasionally recurrent and leads to growth restriction and may lead to fetal death. Search for an infectious etiology has been negative, and the only detailed study that has identified the population of inflammatory cells within the villi was incomplete (Redline and Patterson 1993) but has more recently been clarified as representing maternal T-lymphocytes (Myerson et al. 2006). These studies suggested that in male births, there was no Y-signal in the affected villous inflammatory cell population. But that was done on histologic sections, not on fresh material. Thus, the exact nature of the infiltrating cell population remained unknown. Clearly, immunologists need to participate in identifying these cells more precisely than we have been able to do so far. A much more intensive study of this problem is now in order.

Placenta accreta was rare many years ago, and placenta percreta was virtually unheard of. Now, both play an important role in gynecologic pathology. We see many cases of placenta percreta annually, and most are identified sonographically long before labor commences. Usually, the uterus is removed in placenta percreta; the placenta is found to lie anteriorly and has dehisced (prolapsed) through a former cesarean section scar. It has not "invaded"; it just sticks out of the uterus because the thin scar tissue has become even thinner as the uterus expands in all directions during the next pregnancy. Most of this is the result of the greater frequency with which cesarean section delivery is done, and the frequently practiced avoidance of a trial of labor after one cesarean section (vaginal birth after cesarean [VBAC]). Are there other causes? Certainly there is no evidence that the trophoblast is truly invasive and destructive at its implantation site; abdominal pregnancies alone support that notion. But perhaps the uteri are less adequately repaired after cesarean section? At least that is what I believe; the rapidity of closure, the single stitch approach, and the nature of the suture material may be responsible for the higher frequency of percretas. One fact is clear, however, the progress made in sonography techniques, the recognition of placental "lakes," apparent hemangiomas, or abnormal-appearing implantation sites by ultrasound has much enhanced early recognition of this potentially serious complication of pregnancy.

Other advances in sonography might be highlighted as they also affect placental pathology studies and give insight into prenatal life. The coiling and entanglement of the umbilical cord (especially in MoMo twins) are now recognized earlier; blood flow can be adjudicated (and reversal can be documented – end-diastolic flow), we will learn to identify more precisely the sites of anastomoses in twin gestations and perhaps, with color power angiography, we will even better assess the development and abnormalities of the villous tree (Konje et al. 2003). The prospects are excellent, and placental pathologists should stay abreast of these developments so as to enable them to make more meaningful studies and diagnoses.

Finally, we have had an interest in understanding the causes that determine the length of the umbilical cord (normal is 55 cm) and its

helical twists. Both aspects warrant more attention than is generally appreciated. Excessively long cords may lead to knots and to encircling around the fetus, and they may cause fetal demise. More often they are also associated with excessive spiraling of the cord; 2.5 twists per 10 cm are the normal, but we have seen many cords with excessively marked twisting associated with intrauterine growth restrictions (IUGR) and fetal demise. What determines the cord's length? We know that immobile fetuses (muscular diseases, osteogenesis imperfecta, etc.) have short umbilical cords, and thus we assume that the length of the umbilical cord may be determined by fetal mobility. But why is the cord occasionally much longer than 55 cm? A gestation that we have observed recently with a long and excessively twisted cord and an IUGR neonate was in a mother who was an avid runner during pregnancy, and we wonder whether the length of the cord may not reflect excessive fetal mobility. Future studies are needed.

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