Ultrasound Obstet. Gynecol. 10 (1997) 79–81

Editorial

Chorionicity determination in twin pregnancies: double trouble?

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Twins have always been a major reason for concern in perinatal medicine; although they occur in about one in every 80 deliveries, twins are responsible for almost 10% of all perinatal morbidity and mortality[1]. Not surprisingly then, the diagnosis of a twin pregnancy is often followed by mixed feelings by both the parents and the obstetrician; prematurity, growth disturbances, transfusional syndromes, single fetal death, and discordant fetal anomaly are some of the most common worries.

Outcome in multiple pregnancies depends on the number of fetuses, the gestational age at diagnosis, and the chorionicity. The number of fetuses is highly relevant in those pregnancies conceived with assisted reproductive techniques, where the prevalence of triplets and other higher-order multiple pregnancies is substantially increased. Progressively earlier diagnosis of twin gestations also has implications for pregnancy outcome, as the earlier the gestational age at diagnosis, the higher the possibility that spontaneous fetal loss will be recognized. The routine prenatal determination of chorionicity, one of the main, if not the most important, determinants of pregnancy outcome in twin gestations, has traditionally been far from optimal and referral centers may be faced with complicated twin pregnancies in which no information on chorionicity is available from previous ultrasound reports.

What are the current obstacles for routine determination of chorionicity in clinical practice? First, accurate determination of chorionicity demands some knowledge of embryology. Briefly, non-identical twins are derived from two fertilized eggs and, by definition, are always dichorionic, since they develop two placentas. Conversely, identical twins are derived from a single fertilized egg, but may not always develop a single placenta. The complex mechanisms underlying identical or monozygotic twinning show that, when the splitting process occurs before the 3rd day after fertilization, identical twins also develop two placentas, one for each twin, which are indistinguishable from the placentation seen in non-identical twins. If this peculiarity of nature did not take place, the confusion over zygosity and chorionicity would never exist! However, if the single fertilized egg splits after the 3rd day, the twins will share a single placenta and, by definition, will be monochorionic, a phenomenon that occurs in approximately two-thirds of monozygotic twin gestations. Depending upon the time when splitting occurs, monochorionic placentation can result in twins in separate amniotic sacs (monochorionic-diamniotic) or in twins sharing a single amniotic sac (monochorionic-monoamniotic).

Second, the impact of chorionicity on the fate of the pregnancy is not fully appreciated. Monochorionic twins are at a much higher risk of poor perinatal outcome than dichorionic twins, which is mainly attributed to the presence of fetoplacental vascular anastomoses almost invariably present in monochorionic but practically never in dichorionic twin pregnancies. Hemodynamic imbalance between the two placental circulations is thought to be responsible for both acute and chronic transfusion syndromes with potentially devastating effects on the pregnancy, including fetal death and neurological sequelae in survivors. Management of a twin pregnancy with one severely compromised fetus is also strongly influenced by chorionicity; in a dichorionic pregnancy there are no consequences for the co-twin if fetal demise occurs, but single intrauterine death in a monochorionic pregnancy can lead to acute hemodynamic imbalance and severe hypotension in the surviving twin, which may result in co-twin death or, if the co-twin survives, in necrotic lesions of the brain.

Therefore, in monochorionic twins, early delivery or separation of circulations may need to be considered, depending on gestational age. Other unique, although extremely rare, complications occurring exclusively in monochorionic twins are cord entanglement in monoamniotic pregnancies, twin reversed arterial perfusion sequence, and conjoining, all invariably associated with high mortality rates.

Prenatal determination of chorionicity by ultrasound is not always easy, even for a skilled operator. Before the advent of ultrasound, chorionicity was traditionally established following delivery by sexing the babies and examining the number of placentas and membranes. Different-sex twins, delivery of two separate placentas, or the presence of more than two layers in the intertwin membrane are each definite proof of dichorionicity. Similarly, the absence of interposing chorion between the two amnion layers or the absence of a separating membrane is certain proof of monochorionicity. Based on these postpartum observations, initial attempts to determine chorionicity *in utero* were based on the ultrasound detection of similar criteria[2–5]. Unfortunately, fetal sex cannot be confidently determined by ultrasound in the first and early second trimesters, and, even after that time, there is a small but definite possibility of mistakes in sex assignment[6]. The main limitation, however, is that, according to the expected sex distribution, all monozygotic and 50% of dizygotic twins are of similar sex and, therefore, chorionicity based on sexing the fetuses remains undetermined in about two-thirds of the cases. Similarly, prenatal determination of the number of placentas may not always be straightforward. Two completely structurally independent but fused placentas and a single monochorionic placenta may have a similar prenatal ultrasound appearance. Close implantation sites and growth of the placental masses as pregnancy progresses in most dichorionic pregnancies mean that most of the placentas will, at some point, appear fused and, therefore, confident identification of structurally separate placentas will be difficult to document. Furthermore, placental shape and position within the uterine cavity can similarly make them appear separate in some views but fused in others.

And what about the intertwin membrane? Histological examination of the intertwin membrane following delivery provides definite and conclusive evidence of chorionicity. The dividing membrane in dichorionic pregnancies is composed of two layers of amnion and two of chorion, whereas in monochorionic-diamniotic pregnancies it contains only two layers of amnion with no interposing chorion. The separating membrane is therefore thicker in dichorionic than in monochorionic twins, a feature that has also been assessed by prenatal ultrasound and indeed widely used for more than a decade for the determination of chorionicity[3–5]. However, membrane thickness relies on subjective rather than on objective criteria, which can explain the suboptimal accuracy of this technique in determining chorionicity. Attempts have been made to establish the measured thickness of the membrane. In the only study to date, a cut-off of 2 mm provided a good, but not optimal, measurement to characterize thin or thick membrane in identifying monochorionic or dichorionic twins, respectively[7]. However, with improved ultrasound equipment, it has become evident that there is not only high inter- and intraobserver variabilities associated with this kind of membrane measurement, but also biological and technical limitations such as differences depending on the sampling site, gestational age at evaluation, and resolution of ultrasound equipment[8]. Similarly, counting the layers of the dividing membrane, another technique widely used following delivery, has also been described using prenatal ultrasound; identification of more than two layers has been suggested as a strong evidence of dichorionicity[9,10]. However, in routine clinical practice, this technique has been largely abandoned because it is highly time-consuming, and, in many dichorionic pregnancies, it may not be possible to identify more than one layer. Nevertheless, as resolution of ultrasound imaging improves, it is possible that this technique may find a place in clinical practice in the future.

Given the above-mentioned limitations, which is then the ideal technique for prenatal determination of chorionicity? Composite ultrasound information on fetal sexing, separate placentas, and membrane characteristics may be highly accurate[11], although undoubtedly time-consuming, cumbersome, and sometimes rather confusing. An alternative approach is to establish a diagnostic cascade, where the most predictive characteristics of dichorionicity should be looked for first. Recently, a growing body of evidence suggests that examining the so-called transition zone at the intertwin membrane–placental junction may indeed be the ideal sign. The presence of an echogenic chorionic tissue projection into the base of the intertwin membrane has been shown to be one of the most specific ultrasound landmarks of dichorionic placentation. This ultrasound feature, originally described in 1981 and termed the *lambda* sign[12], has only recently been brought to clinical attention with a new and appealing name, the *twin peak* sign[13]. Initially described at the level of the fusion zone between two adjacent placentas, recent evidence suggests that it is also possible to identify the lambda sign even at the edge of placentas located on opposite sides of the uterus[14,15]. One of the main advantages of this technique is that the intertwin membrane–placental junction is easily identifiable during the first and second trimesters, when most twin pregnancies undergo ultrasound examination. Recent experience suggests that the optimal gestational age to identify this sign and classify twin gestations into dichorionic and monochorionic is at around 10–14 weeks' gestation, at which time the lambda sign is easily identifiable in all dichorionic pregnancies, including those with both fused and separate placentas, and absent in all monochorionic pregnancies[15]. As pregnancy progresses, however, the lambda sign is progressively more difficult to visualize and indeed it disappears by the 20th week in about 7% of dichorionic pregnancies with fused placentas[16]. Therefore, in those twin pregnancies in which no previous information on chorionicity is available, although its presence is still diagnostic, the absence of the lambda sign after the 20th week should be used with caution, and

other prenatal ultrasound features of chorionicity should then be carefully looked for.

Accurate chorionicity determination is perhaps one of the most important responsibilities during obstetric ultrasound of twins. Ideally, chorionicity should be determined in the first trimester when the number of sacs and live embryos and the presence or absence of the lambda sign can be easily established[15,17]. Early detection of monochorionic twins has major implications for pregnancy management and, therefore, intensive antenatal surveillance for transfusional complications in these cases should be undertaken.

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