Zygosity and Chorionicity in the Prognosis of Triplet Pregnancies: Contribution of Microsatellites

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We assess the prognostic values of zygosity and chorionicity in triplet pregnancies (TP) with the assistance of microsatellites analysis. 53 consecutive TP whose prenatal care and delivery occurred in our maternity hospital were included in this prospective study. Zygosity of all sets of triplets (alive or stillbirth, after 22 weeks of gestation) was determined by PCR-amplified microsatellites markers analysis. Chorionicity was determined by placental analysis in our fetopathology referral department and validated by molecular analysis of zygosity. Placental conformations, obstetrical and neonatal outcomes of TP were studied according to their zygosity and chorionicity. Monozygotic and to a greater extent, dizygotic TP were associated with an increase in placental ischemic injuries, velamentous cord insertions, twin-twin transfusion syndromes, fetal anomalies, and perinatal mortality when compared with the trizygotic TP (p <0.05). Monochorionic and more significantly, dichorionic TP presented with the same increases as trichorionic TP (p <0.05). Thus chorionicity easily determined by ultrasound evaluation during the first trimester of the pregnancy must remain the main prognosis criterion in TP. Taking into account the rare indications of prenatal diagnosis of zygosity, it became apparent that chorionicity has a greater impact than zygosity when distinguishing high-risk groups of TP.

Keywords: zygosity, chorionicity, triplets, microsatellites, prognosis

Advanced maternal age and the widespread use of artificial reproductive technology are responsible for the increasing rates of multiple pregnancies over the last three decades (Blickstein & Keith, 2005). It has been demonstrated that triplet pregnancies (TP) have a higher risk of adverse obstetric and perinatal outcomes than single and even twin pregnancies (Ballabh et al., 2003; Wen et al., 2004). The impact of TP on women's health leads to increased rates of preeclampsia, diabetes mellitus, premature rupture of membranes, obstetric haemorrhage, and preterm delivery. Similarly, fetal outcomes among triplets are worse than those of singletons and twins; with increased rates of intrauterine growth retardation (IUGR), twin-twin transfusion syndromes (TTTS) and stillbirths.

Chorionicity refers to the type of placentation unlike zygosity which refers to the type of conception. Zygosity indicates if triplets arose from one fertilized egg (monozygotic) or from two eggs fertilized by two different sperms (dizygotic), or from three eggs fertilized by three different sperms (trizygotic). The type of placentation depends on when the zygote divides into two genetically identical twin embryos. If the split occurs during the first 3 days following fertilization, the pregnancy will be dichorionic with unconnected or fused placentas. If the division occurs 4 to 8 days post-fertilization, the two fetuses share the same placenta but each fetus has its own amniotic fluid cavity. When the division occurs between days 8 and 13 post-fecundation, the result is monochorionic monoamniotic twins with fetuses sharing the same placenta and amniotic fluid cavity. In TP, diagnosis of chorionicity and amnionicity is established by comparing each triplet with one another by sequential analysis: fetus one with fetus two, one with three, and two with three. Therefore one zygote can give two or three embryos by successive division. The number of placentas and amniotic fluid cavities shared depends on the time of the division. Recent studies have demonstrated a poorer maternal and fetal prognosis in cases of dichorionic (DC) and monochorionic (MC) TP when compared

Received 24 June, 2008; accepted 26 September, 2008.

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to trichorionic (TC) pregnancies (Adegbite et al., 2005; Bajoria et al., 2006; Geipel et al., 2005). These results suggested that chorionicity was the most important prognostic factor in TP, particularly when considering that chorionicity can be easily determined by ultrasound evaluation during the first trimester of pregnancy. However, these studies were all retrospective, multicentric and contained no information on the methods used to diagnose the final chorionicities.

Zygosity is a prime factor in the pathophysiology of multiple pregnancies as it correlates with chorionicities. Despite this, the first study to use an accurate molecular biology technique to assess zygosity and to evaluate its impact on twin pregnancy outcomes, was the report of Carroll et al. (2005). Until now, no study comparing the outcomes of TP according to their zygosity has been completed.

The aim of this study was to determine the involvement of zygosity and chorionicity on the placental structure and the obstetric, fetal and neonatal outcomes, through the prospective study of 53 consecutive TP. Each determined chorionicity was validated by an anatomopathological analysis and a molecular analysis of the corresponding zygosities by the study of microsatellite polymorphic markers.

Material and Methods

This prospective study was carried out in our tertiary referral center between March 1998 and February 2006. Over this period, 53 consecutive TP that were delivered in our department were followed. TP resulting from embryo reductions of high-order multiple pregnancies and TP artificially reduced to twins were excluded from the final analysis. The TP in which the determination of zygosity was impossible due to technical difficulties or in which the results of the anatomopathological chorionicity disagreed with the molecular zygosity were also excluded from the final analysis.

The gestational age was determined in accordance with the last menstrual period and was confirmed by an early ultrasound evaluation, which was performed on average at 12 weeks of gestation. In our department of obstetrics, all TP were admitted for bed rest after 28 weeks of gestation. If tocolysis was necessary, it was usually stopped at 33 weeks gestation after the agreement of the obstetricians and pediatricians. A course of prenatal corticosteroids by intramuscular dose of 12 mg betamethasone per day for 2 days was received by expectant mothers at risk of preterm delivery before 34 weeks gestation. In our center, TTTS was not managed by invasive procedures. IUGR was defined by a fetal weight estimation below the 10th percentile in accordance with the AUDIPOG (Mamelle et al., 1996) curves at the last ultrasound evaluation before delivery. These growth curves which are well established in the French population are calculated according to the sex and the gestational age of singletons. A stillbirth was defined as fetal death after 22 weeks gestation. We defined neonatal mortality as the death of a newborn delivered after 22 weeks gestation during the 28 days following birth. Global mortality described in our study was defined as the sum of stillbirths and neonatal deaths.

The expectant mothers included in the study had signed an agreement for analyses of the DNA of the newborns at birth, and for the possibility to carry out an autopsy and DNA sampling of stillbirths. Samples (2 ml each) of cord blood were obtained from the live triplet newborns. For the stillbirths, a piece of pulmonary tissue was collected by the Department of Fetopathology. DNA was extracted from the cord blood and from pulmonary tissue, and zygosity was determined by the Department of Genetic Biochemistry. All placentas were identified, in relation to their triplets, in the delivery room and transferred for anatomopathological analysis of chorionicity. Stillbirths were also addressed in our Department of Fetopathology for autopsy and pulmonary tissue takings. Zygosity was determined using Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) amplification of highly polymorphic microsatellite markers (Cirigliano et al., 2003) using the PowerPlex® 16 System (Promega Corporation, Madison, WI, USA). The 16 microsatellite markers that were used comprised 15 autosomal codominant unlinked markers and a gender-determining marker, Amelogenin. Methods for PCR amplification, sample preparation and electrophoresis for the PowerPlex® 16 System were performed according to the manufacturer's instructions. The raw data was collected and analyzed using ABI PRISM® 310 Genetic Analyser and GeneScan® analysis software respectively. These 16 markers presented a power of exclusion of at least 0.9999994 between triplets in our population. Placental examinations were performed according to the methods of Benirschke (Benirschke, 1961) in the Department of Fetopathology. Velamentous cord insertion (VCI) was defined as insertion of the fetal vessels in the amniotic membranes at a distance away from the placental margin. Placental ischemia was defined by the association of pitted and hypovascular villous for the gestational age, increased syncytiotrophoblast knotting and fibrin deposition.

Data were analyzed with Stata 8.0 (Stata Corp, College Station, TX). Data of continuous variables were expressed as means and standard deviations (SD). An analysis of variance was performed with an ANOVA test for these data. For parametric data, chi-squared was used for blocked comparison if the number was greater than 30, and Fisher's exact test was used if the number was less than or equal to 30; p values less than 0.05 were considered significant.

Results

The total cohort of the study included 53 TP, namely 159 triplets, for whom molecular, anatomopathological, ultrasonographic, obstetric and neonatal data were collected. Four TP were excluded from the analysis.

https://doi.org/10.1375/twin.11.6.648 Published online by Cambridge University Press

Table 1

Cross Distribution of Zygosities and Chorionicities Among the 147 Studied Triplets

	MZ	DZ	TZ	Total
MC	9	0	0	9 (6.1%)
DC	9	36	0	45 (30.6%)
TC	0	6	87	93 (63.3%)
Total	18 (12.24%)	42 (28.6%)	87 (59.2%)	147 (100%)

Note: MZ: monozygotic; DZ : dizygotic;TZ : trizygotic; MC : monochorionic; DC : dichorionic; TC: trichorionic.

The first was a quadruplet pregnancy reduced to a TP. Two other TP were excluded due to failure of the DNA extraction procedure. A fourth TP was excluded because of a discordance in placental chorionicity and molecular zygosity. A dichorionic pregnancy presented with a molecular profile of trizygotic (TZ) pregnancy. In this case, the anatomopathological record reported a highly damaged placental mass involving a difficult diagnosis of chorionicity. We decided to exclude this pregnancy from our study due to the fact that a control of placental chorionicity would not be possible after the results of the zygosity molecular analysis (all placentas are destroyed after their first examination). The cross distribution of chorionicity and zygosity of our 49 TP are presented in Table 1.

Maternal, obstetric and neonatal characteristics according to zygosity are presented in Table 2. Concerning obstetric complications, 29% of the dizygotic (DZ) TP presented with preeclampsia during gestation compared with none in the monozygotic (MZ) pregnancies ($p < .05$). Diabetes mellitus presented the same pattern with 21 % of cases in the expectant mothers of DZ triplets ($p < .05$). We noticed a significant increase of the global mortality (stillbirths + neonatal mortality) in the groups of MZ (11%) and DZ (14%) when compared with TZ (2%, $p < .05$).

We have evaluated these same maternal, obstetric and neonatal features according to the type of chorionicity (Table 3). In the field of obstetric complications, we emphasized a significant increased rate of premature rupture of membranes in TC pregnancies (42/93, 45%, $p < .05$). We did not, however, notice any difference in the rates of haemorrhage, diabetes mellitus and preeclampsia according to the type of chorionicity. We recorded a significant increase in stillbirths, neonatal and global mortalities in the DC TP groups ($p < .05$).

The main ultrasonographic, placental and umbilical characteristics of our triplets are displayed in tables 4 and 5 in relation to zygosity and chorionicity respectively.

Regarding zygosity, prenatal diagnosis of placenta praevia increased statistically in the cases of TZ TP (9/87, 10%, $p < .05$). TTTS appeared more frequently

Table 2

Maternal, Obstetric and Neonatal Characteristics of the 147 Studied Triplets in Relation to the Type of Zygosity

	MZ *n* = 18 triplets	DZ *n* = 42 triplets	TZ *n* = 87 triplets	*p*
Maternal characteristics				
Maternal age (y)	33 (25–43)	32 (25–41)	31 (21–41)	NS
Nulliparity (n)	3 (17%)	27 (64%)	63 (72%)	<.001
Spontaneously conceived pregnancies (n)	18 (100%)	24 (57%)	9 (11%)	<.001
Pregnancies resulting from ART	0	18 (43%)	78 (89%)	<.001
Obstetric complications (n)				
Preeclampsia	0	12 (29%)	15 (17%)	<.05
Antepartum haemorrhage	3 (16%)	0	9 (10%)	<.05
Premature rupture of membranes	3 (16%)	15 (36%)	36 (41%)	NS
Diabetes mellitus	0	9 (21%)	6 (7%)	<.05
Term of delivery (weeks gestation)	32.3 (28–35)	32.9 (28–37)	33.3 (27–37)	NS
Mode of delivery (n)				
Caesarean section	6 (33%)	24 (57%)	45 (52%)	NS
Vaginal delivery	12 (67%)	18 (43%)	42 (48%)	NS
Birth weight (g)	1500 (600–2420)	1700 (650–2440)	1760 (600–3070)	NS
Fetal sex (n)				
Male	12 (67%)	20 (48%)	33 (38%)	NS
Female	6 (33%)	22 (52%)	54 (62%)	NS
Neonatal characteristics				
APGAR score	9.3 (4–10)	9.1 (3–10)	9.5 (6–10)	NS
Respiratory distress syndrome (n)	9 (53%)	22 (54%)	32 (37%)	NS
NICU admissions (n)	5 (28%)	14 (33%)	23 (26%)	NS
Mortality (n)				
Stillbirths (n)	1 (6%)	2 (5%)	0	NS
Neonatal mortality (n)	1 (6%)	4 (6%)	2 (2%)	NS
Global mortality (n)	2 (11%)	6 (14%)	2 (2%)	< .05

Note: NS: not significant ; ART: artificial reproductive technologies ; NICU: Neonatal Intensive Care Unit; MZ: monozygotic ; DZ: dizygotic; TZ: trizygotic

https://doi.org/10.1375/twin.11.6.648 Published online by Cambridge University Press

Table 3

Maternal, Obstetric and Neonatal Characteristics of the 147 Studied Triplets in Relation to the Type of Chorionicity

	MC *n* = 9 triplets	DC *n* = 45 triplets	TC *n* = 93 triplets	*p*
Maternal characteristics				
Maternal age (y)	30 (25–33)	32 (25 – 43)	31 (21 – 41)	NS
Nulliparity (n)	0	27 (60%)	66 (71%)	< .001
Spontaneously conceived pregnancies (n)	9 (100%)	30 (67%)	15 (17%)	< .001
Pregnancies resulting from ART	0	15 (33%)	78 (83%)	< .001
Obstetric complications (n)				
Preeclampsia	0	12 (27%)	15 (16%)	NS
Antepartum haemorrhage	0	3 (7%)	9 (10%)	NS
Premature rupture of membranes	3 (33%)	9 (20%)	42 (45%)	< .05
Diabetes mellitus	0	6 (13%)	9 (10%)	NS
Term of delivery (weeks gestation)	32.8 (31–35)	32.7 (28–37)	33.4 (27–37)	NS
Mode of delivery (n)				
Caesarean section	3 (33%)	27 (60%)	45 (48%)	NS
Vaginal delivery	6 (67%)	18 (40%)	48 (52%)	NS
Birth weight (g)	1660 (1200–2420)	1580 (600–2440)	1770 (600–3070)	NS
Fetal sex (n)				
Male	6 (67%)	16 (36%)	39 (42%)	NS
Female	3 (33%)	29 (64%)	54 (58%)	NS
Neonatal characteristics				
APGAR score	9.1 (4–10)	9 (3–10)	9.6 (6–10)	NS
Respiratory distress syndrome (n)	4 (44%)	25 (58%)	34 (37%)	NS
NICU admissions (n)	2 (22%)	17 (38%)	23 (25%)	NS
Mortality (n)				
Stillbirths (n)	0	3 (7%)	0	< .05
Neonatal mortality (n)	0	5 (12%)	2 (2%)	< .05
Global mortality (n)	0	8 (18%)	2 (2%)	< .01

Note: NS: not significant; ART: artificial reproductive technologies; NICU: Neonatal Intensive Care Unit; MC: monochorionic; DC: dichorionic; TC: trichorionic.

in the DZ group of triplets (4/45, 9%, $p < .05$). Regarding chorionicity, we noticed a significant increase in triplet fetal anomalies in the MC and DC groups ($p < .05$). Diagnosed fetal anomalies comprised six limb anomalies, two cases of cerebral ventriculomegaly, one complex cardiopathy, one digestive atresia, one multicystic dysplastic kidney, and one polymalformative syndrome. TTTS was increased in the cases of DC TP ($p < .05$); the placenta praevia rate increased in the case of TC TP ($p < .05$).

Regarding anatomological examinations, placental ischemic injuries appeared more frequently in placentas stemming from MZ (7/18, 39%) and DZ (14/42, 33%) TP with a p value less than .05. Furthermore, velamentous cord insertions were more numerous in the cases of MZ and DZ TP ($p < .01$). Regarding chorionicity, placental ischemic injuries ($p < .01$) and velamentous cord insertions ($p < .05$) increased significantly in the cases of MC and DC TP.

Discussion

Our cohort of 49 consecutive TP is the first series to permit a combined assessment of the impact of chorionicity and zygosity with the TP outcome. This was achieved using microsatellite analysis.

The data we collected was in accordance with previous studies regarding TP outcomes (Blondel & Kaminski, 2002). The distribution of the mode of conception of our TP was also in accordance with literature data; 34% were spontaneously conceived, 35% were induced using clomiphene citrate and gonadotrophins, and 30% resulted from artificial reproductive technologies (in vitro fertilization, intracytoplasmic sperm injection). Only our rate of spontaneous pregnancies appeared to have increased slightly when compared to the studies previously cited. This figure is most probably a result of the large proportion of patients from Western Africa used in this study, for whom the rate of spontaneous multiple pregnancies is known to be greater than that of Caucasian patients (Nylander & Corney, 1971). Regarding our TP obstetric complications, we also confirmed the known increase of preeclampsia and premature ruptures of membranes (Malone et al., 1998; Wen et al., 2004). The caesarean section rates we recorded (51%) were far lower than those of other studies; 80 to 100% in Ballabh et al. (2003) and Wen et al. (2004). However, we consider that the policy of vaginal delivery of triplets in an accustomed team like ours does not constitute to an increased risk of adverse outcomes (Alran et al., 2004).

Let us now consider issues pertaining to the fetus. The average term of delivery recorded in our study was 32 weeks gestation; the average birthweight was 1720g, mirroring findings of all recent studies of triplets. Our rate of fetal abnormalities was 8%.

https://doi.org/10.1375/twin.11.6.648 Published online by Cambridge University Press

Table 4

Ultrasonographic, Placental and Umbilical Characteristics of the 147 Studied Triplets in Relation to the Type of Zygosity

	MZ *n* = 18 triplets	DZ *n* = 42 triplets	TZ *n* = 87 triplets	*P*
Ultrasonographic characteristics (n)				
Intra-uterine growth retardation (n)	6 (33%)	6 (14%)	18 (20%)	NS
Triplets with fœtal abnormalities	3 (17%)	4 (10%)	5 (6%)	NS
TTTS	0	4 (9,5%)	0	< .05
Placenta Praevia	0	0	9 (10%)	< .05
Placental characteristics (n)				
Normal conformation	15 (83%)	34 (81%)	63 (72%)	NS
Ischemic injuries	7 (39%)	14 (33%)	15 (17%)	< .05
Cord insertion (n)				
Velamentous	6 (33%)	8 (19%)	9 (10%)	< .01

Note: NS: not significant; TTTS: Twin-Twin transfusion syndrome; MZ: monozygotic; DZ: dizygotic; TZ trizygotic.

Table 5

Ultrasonographic, Placental and Umbilical Characteristics of the 147 Studied Triplets in Relation to the Type of Chorionicity

	MC *n* = 9 triplets	DC *n* = 45 triplets	TC *n* = 93 triplets	*P*
Ultrasonographic characteristics (n)				
Intra-uterine growth retardation (n)	3 (33%)	9 (20%)	18 (19%)	NS
Triplets with fetal abnormalities	3 (33%)	3 (7%)	6 (6%)	< .05
TTTS	0	4 (9%)	0	<.05
Placenta Praevia	0	0	9 (10%)	< .05
Placental characteristics (n)				
Normal conformation	6 (67%)	38 (84%)	68 (73%)	NS
Ischemic injuries	3 (33%)	18 (40%)	15 (16%)	< .01
Cord insertion (n)				
Velamentous	3 (33%)	10 (22%)	11 (10%)	< .05

Note: NS: not significant; TTTS: twin–twin transfusion syndrome; MC: monochorionic; DC: dichorionic; TC: trichorionic.

Although this was higher than that demonstrated in recent triplet studies, we must bring attention to the fact that three of our triplets with fetal abnormalities presented with hexadactyly, a minor abnormality frequently omitted as a fetal abnormality in the majority of reports regarding multiple pregnancies. Finally the perinatal mortality rate of our triplets was 45/1000 (seven triplets died during the perinatal period) which was at the higher end of the perinatal mortality data spectrum when compared to recent studies of triplets (Imaizumi, 2003).

There are numerous advantages to use highly polymorphic microsatellite markers as a discriminating criterion of zygosity between triplets (Chen et al., 2000); the technique can be easily performed on simple blood samples or samples of placental villous or fetal organs (liver, heart, lungs) in the case of stillbirths. In addition the quantity of biological material necessary to perform microsatellite marker analysis is minimal in comparison with the use of minisatellite probes (Jeffreys et al., 1985). A large part of the analysis and interpretation of our highly polymorphic markers is automated and standardized. Consequently, highly specialised training for good reliability and reproducibility was not required. The efficient nature of the technique (less than 24 hours) enabled us to reach results quickly. Finally, the ability to differentiate the fetuses, stillbirths or alive, with specificity greater than 0.99999 for a minimal cost mean this technique has an exceptional cost effectiveness ratio (He et al., 2001). The assessment of zygosity also holds the advantage of enabling comparison between the results of anatomopathological examination of chorionicities with those of fetal zygosities. The zygosity assessment control meant that by the final analysis we were able to exclude all possible misclassifications of triplet chorionicity. The unique TP that presented a conflict between anatomopathological chorionicity and molecular zygosity was excluded. Thus we could conclude that the rate of error in the classification of chorionicities by our referral department of placental anatomopathology was 2% (1/50). This misclassification rate takes into account the fact that retrospective studies like those of Agdebite et al. (2005), Barjoria et al. (2006) and Geipel et al. (2005), which do not control their chorionicity with zygosity analysis, have at least a two per cent margin of error in their reported chorionicities. Studies conducted in this manner could thus appear partially skewed.

In terms of zygosity, the data we collected allowed us to underline a high-risk group of TP. MZ and to a greater extent DZ TP were associated with an

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increased global mortality in comparison with TZ TP. The placental ischemic injuries and velamentous cord insertions, significantly higher in our study in the cases of MZ and DZ TP could largely account for these results. In addition, the presence of TTTS noted in four triplets of DZ pregnancies might also influence these adverse outcomes. Although we did not record any significant difference in the terms of delivery, birthweights and immediate neonatal complications between MZ, DZ, and TZ TP, we identified a significant increase in obstetric complications (preeclampsia and diabetes mellitus) in the cases of DZ TP when compared to MZ and TZ TP. Our study suffered from a limited statistical power to clearly show significant increases in fetal hypotrophy and prematurity in MZ and DZ TP.

Regarding chorionicity, DC TP constituted a significant high-risk TP group with increased rates of stillbirths (7%), neonatal mortality (11%) and global mortality (18%). Within the framework of chorionicity analysis, TTTS was the most significant feature involved in these adverse outcomes. In addition, placental ischemic injuries and velamentous cord insertions were involved to some extent. 13.5% of our DC TP had been suffering from TTTS. This result was slightly less than data from recent literature suggesting rates between 17 and 28%. Furthermore, 50% of triplets with TTTS died during the perinatal period. This figure is lower than results recorded in recent studies that recommended an active septostomy management, with or without amniodrainage (Adegbite et al., 2005; Bajoria et al., 2006).

A trend in the increase of respiratory distress syndromes and neonatal intensive care unit admissions was observed in the cases of DC TP when compared with MC and TC TP. Adgebite and Bajoria showed the same trends in their recent studies (Adegbite et al., 2005; Bajoria et al., 2006). In our cohort, DC TP seemed to have more precocious premature deliveries and lower triplet birthweight than MC and TC TP. In addition, our TC TP experienced significantly greater rates of premature membrane rupture and placenta praevia. This increase in premature TC TP membrane rupture could be due to a trend toward greater delivery terms compared with those of DC and MC TP. Placenta previa may appear more frequently in TC TP because of a simple surface problem in which a uterus has to support the spread of three different placentas. This view is in complete agreement with the hypotheses of Blickstein regarding the importance of the spatial constraints in the development of complications in TP (Blickstein, 2002).

There are numerous recent studies showing the role of chorionicity in the prognosis of TP (Adegbite et al., 2005; Bajoria et al., 2006; Geipel et al., 2005); however, very few reports have evaluated the role of zygosity (a prime factor in chorionicity) in the prognosis of multiple pregnancies. Dubé et al. (2002) compared the complications of twin pregnancies according to both their chorionicity and their zygosity. This report used the gender of the fetus, chorionicity and blood groups to establish zygosity. Besides a clear bias of connection between the two items studied, the sensitivity of the blood groups in the differentiation of twins is only 83 %. The first and only trial using microsatellite analysis for the assessment of zygosity in this kind of comparative study was published in 2005 (Carroll et al., 2005). The authors of this study report on twins underlined only a significant earlier term of delivery in MZ and MC twin pregnancies. No significant difference was apparent in their study between groups of MZ DC twins and DZ DC twins. Consequently zygosity held no advantage over chorionicity regarding the prognosis of twin pregnancies.

Contrary to results concerning zygosity, the recent literature supports the importance of chorionicity in the prognosis of TP (Adegbite et al., 2005; Bajoria et al., 2006; Geipel et al., 2005). However, these multiple studies showed bias. We noticed that their chorionicity data collection relied on large-scale multicentric registers, and that the diagnosed chorionicity was not controlled by an accurate assessment of zygosity. The authors were all in agreement, however, that the MC and more prominently, the DC TP were markers of adverse prognosis. Their suggested hypotheses were linked to a higher frequency of TTTS in these pregnancies and to an increased rate of premature ruptures of membranes in DC TP. Although these results corroborate our own regarding TTTS, our results differed regarding the rate of premature membrane ruptures. Indeed, our DC pregnancies presented a lesser rate of premature membrane rupture than the MC and to a greater extent, TC TP. For Adegbite et al. (2005), the risk of adverse perinatal outcomes presented a 5.5-fold increase in DC than in TC spontaneously conceived TP. For Bajoria et al. (2006), the risk of perinatal death was eight times higher in DC than in TC pregnancies conceived by artificial reproductive technologies. Our results suggested an even poorer prognosis with a perinatal death rate nine times higher in DC than in TC TP.

The prenatal diagnosis of zygosity may be performed in TP by amniocentesis or chorionic villus sampling (Bajoria & Kingdom, 1997; Cleary-Goldman et al., 2005; Picone & Dommergues, 2004). Faced with the risks of increased rates of miscarriages, stillbirths or premature deliveries in the case of multiple pregnancies(Antsaklis et al., 2002; Appelman & Furman, 2005; Cleary-Goldman et al., 2005), these methods are only performed in parallel with other prenatal investigations in cases of selective fetal termination decisions, stillbirths or imminent fetal death of a triplet in cases where the ultrasonographic diagnosis of chorionicity had not been possible (8 to 10% of cases in theory) (Derom et al., 1991). Therefore these indications are very limited. The ultrasonographic assessment of chorionicity is not only easier and less dangerous, but also less expensive as it is routinely associated with the first

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ultrasonographic evaluation of multiple pregnancies. Considering that the types of zygosity and chorionicity seem to have parallel effects on TP prognosis, it appears evident that the type of chorionicity must remain the main prognostic criterion of TP.

Nevertheless, given the possibility of determining fetal gender with a simple blood sample (Costa et al., 2001) from the expectant mother, molecular biology has already proven its diagnostic strength during the prenatal period without any invasive procedures. Hence it is reasonable to consider that the microsatellite marker analyses of fetuses and subsequently zygosity will be possible in the near future with a simple blood sample from expectant mothers. Thus, the prenatal assessment of zygosity will perhaps become easier and more accurate than the assessment of chorionicity when establishing the prognosis for multiple pregnancies.

Acknowledgment

The authors are greatly indebted to Neha Kaushik, undergraduate medical student at Manchester University, Great Britain, for help with the revision of the manuscript, critically for language and readability.

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https://doi.org/10.1375/twin.11.6.648 Published online by Cambridge University Press