



# Effects of Chorionicity and Zygosity on Triplet Birth Weight

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Birth weight in triplets is, on average, lower than in singletons and twins, and more children are classified as having very low or extremely low birth weight. Still, there is limited research on factors that affect triplet birth weight, and samples under study are often small. Chorionicity and zygosity influence triplet birth weight, but it is unknown whether the effect of zygosity can be entirely ascribed to the effect of chorionicity or whether zygosity has an additional effect on triplet birth weight. This question was investigated in 346 triplets (from 116 trios) registered with the Netherlands Twin Register for whom data on chorionicity were available. 'Triplet' refers to one child and the set of three triplets is referred to as 'trio'. Trios and triplets were classified based on zygosity and chorionicity. With regression analysis, the effects of zygosity and chorionicity on triplet birth weight were examined, while controlling for gestational age, sex, and maternal smoking during pregnancy. In addition, within the dizygotic trios a within-family comparison was made between the birth weight of the triplets that were part of a monozygotic pair (with some pairs sharing a chorion), and the birth weight of the dizygotic triplet. Based on the classification on individual level, monozygotic, monochorionic triplets had a lower mean birth weight than dizygotic, dichorionic triplets. Most remarkably, in dizygotic trios, monozygotic pairs only had a lower mean birth weight than their dizygotic sibling triplet when the pair shared a chorion. We conclude that having shared a chorion, rather than being monozygotic, increases the risk of a low birth weight.

■ **Keywords:** triplet, birth weight, birth weight discordance, chorionicity, zygosity

Triplets often have a low birth weight. This is partly due to low gestational age; they are often born premature. However, zygosity and chorionicity also affect birth weight. Triplets, which refers to one child (the set of three triplets is referred to as a 'trio'), can be either monochorionic (MC), dichorionic (DC) or trichorionic (TC). A smaller number of chorions is associated with lower birth weight because sharing a placenta causes competition for nutrients (Adegbite, Ward, & Bajoria, 2005; Bajoria, Ward, & Adegbite, 2006; Geipel et al., 2005). In addition, there is some evidence that when more triplets share a chorion the gestational age of the trio is lower (Spencer et al., 2009).

Zygosity also is an important factor in birth weight, but is not independent from chorionicity. Monozygotic (MZ) triplets can share one chorion, can be DC, or have each their own chorion (i.e. trichorionic, TC); dizygotic (DZ) triplets are DC or TC; trizygotic (TZ) triplets are always

TC (Fox & Sebire, 1997). In an earlier study, we found that, on average, MZ triplets have a lower birth weight than DZ and TZ triplets (Lamb et al., 2011). However, other studies did not find a significant effect of zygosity on triplet birth weight. This could be due to the size of the MZ samples Newman, Jones & Miller, 1991; Orlebeke, Boomsma, & Eriksson, 1993).

Studies combining information on chorionicity, zygosity, and birth weight in triplets are rare. In one study describing

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15 spontaneously conceived trios, no conclusions regarding the effects of chorionicity and zygosity could be made due to the small sample size (Machin & Bamforth, 1996). This study suggested that, compared with the triplets born after assisted reproductive techniques (ART), being spontaneously conceived is associated with a higher proportion of MZ embryos. Many MZ embryos are MC with vascular anastomoses (i.e. connected blood vessels between the circulation of both, or more, embryos). These anastomoses can result in twin-to-twin transfusion syndrome, which causes lower birth weight, and even fetal death in some cases. A second study with data on both zygosity and chorionicity showed that, based on zygosity, MZ triplets are lighter than DZ triplets, and DZ triplets are lighter than TZ triplets. Based on chorionicity, the DC triplets appeared to be the lightest. Zygosity and chorionicity were not combined in this study to predict birth weight, and because of the low sample size none of the effects were significant (Guilherme et al., 2008).

The effect of chorionicity and zygosity on birth weight has been examined in twins (Dube, Dodds, & Armonson, 2002; Loos, Derom, Derom, & Vlietinck, 2005; Loos, Derom, Vlietinck, & Derom, 1998; Lee, Oh, Lee, Kim, & Jun, 2010). Twins can be distinguished into three groups: MZ MC, MZ DC and DZ DC. Two studies with, respectively, 1080 and 569 twin pairs reported that MZ DC twins did not differ in birth weight to DZ DC. The MZ MC twins had a lower mean birth weight than DZ DC twins (Dube et al., 2002; Lee et al., 2010). In contrast, a third study in 4735 twins showed that mean birth weight was highest for the DZ DC twins, lower for the MZ DC twins, but even lower for the MZ MC twins (Loos et al., 1998). In a follow-up to this third study, within 4529 pairs with information on placenta fusion and umbilical cord insertion, it was shown that DC twins weighed more than both MZ DC and MZ MC twins, but only when the placentas were fused and the cord insertion was peripheral (Loos et al., 2005). Otherwise, the mean birth weight for all three types of twins was similar. In general, it seems that MZ MC twins have lower birth weights than MZ DC and DZ DC twins, suggesting that chorionicity is more important than zygosity. In addition, when comparing the MZ DC and the DZ DC twins, placenta fusion and cord insertion seems less optimal with respect to nutrient intake for the MZ than for the DZ twins, which suggests a disadvantage for birth weight in MZ compared to DZ twins.

Birth weight discordance is also expected to be influenced by chorionicity as well as zygosity. MC twins were found to be more discordant than the DC (Blickstein & Keith, 2004; Gielen et al., 2008; González-Quintero et al., 2003). Gielen, Derom, Derom, Vlietinck, & Zeegers (2009) hypothesized that MZ DC twins are least discordant because they do not share a placenta but are genetically identical, whereas DZ DC and MZ MC twins are assumed to be more discordant because the first share, on average,

50% of their genetic material and the latter are in competition for nutrients.

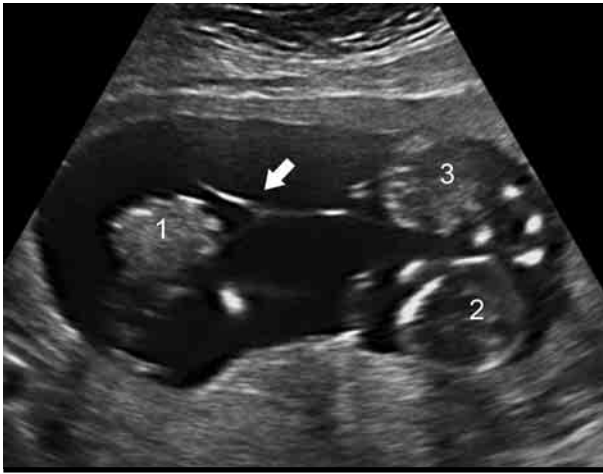
Based on the limited literature, it is expected that zygosity and chorionicity are independently associated with triplet birth weight. In this paper, we explore the effects of chorionicity and zygosity on triplet birth weight using information on 346 triplets registered with the Netherlands Twin Register (NTR). To obtain information on chorionicity, data were linked to PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands (Casparie et al., 2007). PALGA was founded in 1971 and has nationwide coverage since 1991. The registry was established by a number of Dutch pathology laboratories that aimed to promote information exchange between laboratories, in addition to providing useful data to others in the health care field and research. Triplet placentas are sent for histopathological evaluation, including both gross and microscopic evaluation and calling of chorion type, and those data are added to PALGA.

By linking the triplet information from the NTR to chorionicity information from PALGA, we aim to assess whether zygosity has an additional effect on triplet birth weight when chorionicity is accounted for. To investigate this question, trios and individual triplets were classified based on zygosity and chorionicity. With regression analysis, it was investigated if triplet birth weight is predicted by chorionicity and zygosity, while controlling for other factors that are known to affect triplet birth weight (i.e. gestational age, sex, maternal smoking during the pregnancy). Triplet birth weight was also classified as normal, low, very low, or extremely low, and percentages of each birth weight classification were given as a function of triplet and trio zygosity and chorionicity. In addition, groups based on triplet and trio zygosity and chorionicity were compared on birth weight discordance and on gestational age, because it has been suggested that with less chorions in a triplet pregnancy, gestational age is lower (Spencer et al., 2009). Finally, within the DZ trios, which provided a within-family comparison between an MZ pair and a DZ triplet, the effect of zygosity and chorionicity was examined.

## Method

### PARTICIPANTS

Triplets were registered at birth with the Netherlands Twin Register (NTR). The NTR was established in 1987 and collects longitudinal data on multiples by mailed surveys and through interviews, home visits, and deep phenotyping (Boomsma et al., 2002; Willemsen et al., 2010). Questionnaire 1 (Q1) was sent to mothers of triplets soon after registration. In 2005, a questionnaire on familial twinning (Qft) was sent to all mothers of multiples, including all triplet mothers (Hoekstra, Willemsen, van Beijsterveldt, Montgomery, & Boomsma, 2008). In 2008, all mothers of triplets received the second NTR survey, Questionnaire 2 (Q2). Q1; Q2 included items about the

**FIGURE 1**

Ultrasound picture of a monochorionic, and therefore monozygotic trio at 12 weeks gestational age. The arrow indicates the meeting pointing point of three amniotic membranes. No lambda sign is visible at the side on which the membranes insert the placenta. Numbers indicate the three fetuses.

**FIGURE 3**

Ultrasound picture of a trichorionic trio at 12 weeks gestational age. The arrowheads indicate lambda signs between each fetus. These three fetuses do not share their placentas. The membranes between the fetuses are thick. This trio can be trizygotic, dizygotic (one identical duo), or monozygotic. Numbers indicate the three fetuses.

**FIGURE 2**

Ultrasound picture of a dichorionic, triamniotic trio at 13 weeks gestational age. The arrowhead indicates the lambda sign, which proves that this fetus does not share its placenta with Fetus 2 or Fetus 3. The arrow indicates the amniotic membranes of fetuses 2 and 3, which are a monozygotic pair. At this time, it is unsure if Fetus 1 shares zygosity with fetuses 2 and 3. Note the difference in thickness of the septum between Fetus 1 and fetuses 2 and 3 versus the thickness between fetuses 2 and 3. Numbers indicate the three fetuses.

triplet pregnancy, birth, and additional triplet characteristics (Lamb et al., 2011).

In Q2 and Qft, mothers were asked for permission to link their data to other registries. For 334 mothers of triplets who gave permission to link data (88% of the mothers who returned Q2 and/or Qft), information from PALGA was requested. Data were linked on last name of the mother (maiden name), date of birth of the mother, and first initial (if available). For a subgroup, a second attempt was done with birth date of the triplets as an extra

identifier, resulting in a total of 234 hits. Of the 100 trios without successful linkage, 23 were born before 1990, 23 maiden names of the mothers were missing, and, for 26 mothers, we were uncertain of their maiden names. In the end, PALGA provided placenta pathology information for 234 sets of triplets (successful linkage of 70%), with the first record dating from 1978 and the last dating from 2006. For 176 trios, information about triplet chorionicity was available (75% of the sample provided by PALGA).

Triplet zygosity in the NTR data was based on DNA for 2.6% of the sample, on chorionicity for 9%, on blood group assessments for 2%, on a series of survey questions for 70.4%, and on a one-question survey item on zygosity for 14.5%. Triplets with inconsistent data on zygosity in the NTR and chorionicity from PALGA were excluded (19 trios). The zygosity of two trios was DZ and TZ (this was confirmed with the presence of an opposite-sex pair within the trio), while PALGA data on chorionicity indicated MC. For 17 trios, information on zygosity indicated TZ while chorionicity indicated DC. In addition, zygosity was changed from DZ and TZ into MZ (two trios) if only one chorion was present according to PALGA information.

Information on triplet birth weight, zygosity, and chorionicity was available for 114 complete and two incomplete trios. All of these trios were born between 1987 and 2005. Age of the mother at the birth of the triplets ranged between 21 and 41 years ( $M = 30.6$ ,  $SD = 3.7$ ).

To investigate the representativeness of the sample, the NTR trios were compared regarding parity, birth weight, gestational age, and the age of the mother at the time the triplets were born with the total Dutch triplet population, based on information from the Netherlands Perinatal Registry (PRN-foundation, 2011). In addition, the analyzed sample was compared to the group without chorionicity

information or without a link to PALGA, on maternal education, age of the mother at birth of the trio, and population density of the area in which the triplets resided.

## MEASURES

Data on triplet birth weight, gestational age, and parity were obtained from two NTR surveys, Q1 and Q2. Information on ART came from the NTR surveys Q1, Q2 and Qft (van Beijsterveldt et al., 2008). ART included in vitro fertilization, intracytoplasmic sperm injection, and ovulation induction with hormone tablets or subcutaneous injections.

Information on maternal smoking (yes or no) during the triplet pregnancy was obtained from Q1 and Q2. Around 90% of the mothers reported that they did not smoke during pregnancy, around 7% smoked 0–5 cigarettes per day, and about 2% smoked 5–10 cigarettes per day.

Educational level of the mother was assessed in Q2 with four categories: *lower education*, *secondary education*, *upper secondary education*, and *higher education including academic education*. As a small percentage of mothers had received only lower education, the first and second categories were merged.

Postal codes of the families were linked to information from Statistics Netherlands to obtain information regarding the population density of the areas where the families resided (Statistics Netherlands, 2011). Population density was classified as more than or less than 1000 persons per square meter.

## DATA ANALYSES

### Classification

Triplet birth weight was classified as normal (NBW) if birth weight was more than 2500 grams, low (LBW) if birth weight was between 1500 grams and 2500 grams, very low (VLBW) if birth weight was between 1000 grams and 1500 grams and extremely low (ELBW) if birth weight was less than 1000 grams.

Two classifications of the sample were made: (1) classification based on *trio* zygosity and chorionicity and (2)

classification based on *triplet* zygosity and chorionicity. The first classification defines the trios with respect to zygosity (MZ, DZ or TZ) and chorionicity (MC, DC or TC), resulting in the following six groups: MZ MC, MZ DC, MZ TC, DZ DC, DZ TC and TZ TC. The second classification defines the zygosity (MZ or DZ) and chorionicity (MC or DC) of two children within a trio (e.g. child 1 with child 2 or with child 3). When a triplet forms an MZ pair with at least one other child, this pair is classified as MZ. In addition, when a chorion is also shared, this pair is classified as MC, otherwise as DC. When a child has a DZ relation with both other triplets, this individual is classified as DZ, who is by definition DC. This classification results in three groups: MZ MC, MZ DC and DZ DC (Table 1).

Birth weight discordance for trios was calculated as the difference between the heaviest and lightest triplet, as a percentage of the heaviest of the trio. This method is often used to quantify triplet birth weight discordance (Bagchi & Salihu, 2006; Blickstein, 2002). Next, birth weight discordance for the triplet classification was calculated as the discordance within the trio (i.e. as described above) for the MZ MC, MZ TC and TZ TC trios. Because not all triplets in the DZ DC and DZ TC trios received the same triplet zygosity and chorionicity classification, their birth weight discordance was calculated as follows. Birth weight discordance was calculated within the pair for the triplets forming an MZ pair. For the triplet that formed a DZ relation with both others in the trio, birth weight discordance was calculated between the birth weight of this triplet and the mean birth weight of the MZ pair.

### Statistical Analyses

**The Effect of Chorionicity and Zygosity on Triplet Birth Weight.** To examine the effect of trio zygosity and chorionicity on triplet birth weight while correcting for the effect of gestational age, sex, and maternal smoking, a multiple regression analysis was performed. The analysis included variables that were specified as follows: Gestational age as a continuous predictor with the actual gestational age minus

**TABLE 1**

Description of Classification Based on Zygosity and Chorionicity

Triplet zygosity	MZ	DZ	TZ
Chorionicity			
MC	N trios = 11 MZ MC, N triplets = 33	—	—
DC	N trios = 5 Excluded *	N trios = 24 MZ pair = MZ MC, N triplets = 48 DZ triplet = DZ DC, N triplets = 24	—
TC	N trios = 2 MZ DC, N triplets = 5	N trios = 18 MZ pair = MZ DC, N triplets = 36 DZ triplet = DZ DC, N triplets = 18	N trios = 56 DZ DC, N triplets = 167

Note: MZ = monozygotic, DZ = dizygotic, TZ = trizygotic, MC = monochorionic, DC = dichorionic, TC = trichorionic; For each type of trio, the number of trios is given (N trios) in addition to the classification of the triplets in the trio (e.g. DZ DC trios, N trios = 24) can be classified into triplets that together form an MZ pair, that is, MZ MC triplets (N individuals/triplets = 48) and one triplet that has a DZ relation with both other members of the trio, that is, a DZ triplet (N triplets = 24)). MZ DC trios were excluded because it is not possible to decide which two triplets share a chorion.

40 (range, -13 to -3); with sex (0 for boys and 1 for girls) and maternal smoking (0 for nonsmoking and 1 for smoking) as dichotomous; and with chorionicity and zygosity as two categorical predictors with both three levels. Dummy coding was used for these last two predictors.

The effects of triplet zygosity and chorionicity on birth weight were analyzed with multiple regression analysis, with gestational age as a continuous predictor, sex and maternal smoking as dichotomous, and triplet zygosity and chorionicity as a categorical predictor with three levels (i.e. MZ MC, MZ DC and DZ DC, using dummy coding). All regression analyses were performed in Stata with correction for within-cluster (i.e. family) correlation (StataCorp, 2005).

**Comparing Gestational Age and Birth Weight Discordance Between the Groups.** First, with an analysis of variance (ANOVA), it was determined if gestational age and birth weight discordance differed between the groups classified by trio and by triplet zygosity and chorionicity. The ANOVA was carried out when the dependent variable (i.e. gestational age, birth weight discordance) was the same for all triplets in a trio; therefore, analyses could be performed at a family level in SPSS (SPSS Inc., 2008).

**DZ DC and DZ TC Trios, Comparing the MZ Pair With the DZ Triplet.** Two regression analyses were performed; first, a multiple regression with gestational age as a continuous predictor; and sex, maternal smoking, being part of the MZ pair, and sharing a chorion as dichotomous predictors. Second, an interaction term between being part of the MZ pair and sharing a placenta was calculated, resulting in '1' for the MZ pairs that shared the chorion and '0' for all others, that is, the DZ triplets and the MZ pairs that did not share a chorion.

## Results

### Representativeness of the NTR Sample with PALGA Information on Chorionicity

Information on chorionicity in the NTR sample was compared to two representative Dutch samples from the PRN-foundation (Table 2). The PRN-foundation has gathered information on birth characteristics in the Netherlands since 2000. The first sample contains data on triplet birth weight, gestational age, and parity and age of the mother at the triplet birth for all triplets born in the Netherlands between 2000 and 2006, including stillbirths and triplets who died soon after birth. The second sample is a sub-sample of the first sample and consists of triplets that were still

**TABLE 2**

Descriptive Statistics for a Complete Group of Triplets Born in The Netherlands Between 2000 and 2006, a Subset from this Group Including all Triplets Still Alive after 28 Days, and the NTR Sample

	Dutch triplets (cohort 2000–2006)		Dutch triplets (cohort 2000–2006) alive after 28 days		NTR sample with chorionicity information from PALGA	
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Cohort	2000–2006		2000–2006		1987–2005	
Parity (% primiparous)	54.7		50.9		51.3	
BW	1745 [168, 1809] <sup>a</sup>	1324	1920 [1888, 1952] <sup>a</sup>	1092	1884 [1810, 1958] <sup>a</sup>	346
GA	32.3 (4.1)	1323	33.4 (2.8)	1092	33.6 (2.4)	346
Age mother	31.6 (4.3)	486	31.7 (4.2)	379	30.6 (3.7)	109

Note: BW = birth weight, GA = gestational age, NTR = Netherlands Twin Register, PALGA = Pathological Anatomy National Automated Archive.

<sup>a</sup> For BW, the 95% confidence interval (CI) is given instead of the SD; for the first two groups, the SD of triplet BW was retrieved from the perinatal registry, CI was calculated with an adjusted N size (N of families instead of N of triplets); for the third group, CI was calculated directly from the data with the STATA option robust cluster.

**TABLE 3**

Characteristics of the Analyzed Sample in Comparison to the Sample for Which PALGA did not Provide Information on Chorionicity and the Mothers Who Could Not be Linked to PALGA

	Analyzed sample	No chorionicity info from PALGA	Not linked to PALGA
N trios	116	60	98
Educational level mother: % low; middle; high	38.6%; 40.6%; 20.8%	49.4%; 28.2%; 22.4%	38.5%; 31.7%; 29.8%
Age (years) mother at birth: range, mean (SD)	21–41, 30.5 (3.7)	24–41, 31.5 (3.3)	20–39, 30.8 (3.6)
Population density (% < 1000 persons per m <sup>2</sup> )	52.6%	53.4%	43.9%;
Range birth cohort trios	1987–2005	1978–2006	1948–2006
Percentage of sample born before 1991	23.7%	17.1%	25.6%

Note: PALGA = Pathological Anatomy National Automated Archive.

alive 28 days after birth. It becomes clear from Table 2 that the NTR sample with data available on chorionicity is comparable to the representative Dutch triplet sample that is still alive 28 days after birth with regard to birth weight, gestational age, and parity and age of the mother at birth of the triplets.

The current study sample was also compared to data from the NTR triplet sample whose mothers gave permission for retrieving information from national registries but who were not linked to PALGA, and to data from the group of triplets for whom PALGA did provide some information, but not on chorionicity (Table 3). There are no important differences between the groups that could lead to a bias in the analyzed group. Among the mothers whose data were not linked to PALGA, a somewhat higher percentage gave birth to their triplets before 1991 (25.6% versus 17.1%), which is explained by the fact that PALGA did not have national coverage before 1991.

**Normal, Low, Very Low, and Extremely Low Triplet Birth Weight**

The largest part of the sample had an LBW (NBW = 6.4%, LBW = 71.7%, VLBW = 18.8% and ELBW = 3.2%). Table 4 presents an overview of the percentages of triplets according to their birth weight classification, as a function of the trio and triplet zygosity and chorionicity groups. First, as can be seen in Table 4, percentages presented for the MZ TC group should be viewed with care because these are based on small numbers. Due to these small sample sizes, no statistical testing was performed. Considering the groups classified on

trio zygosity and chorionicity, it seemed that VLBW is more frequently present in the MC and DC groups than in the TC groups. Based on triplet zygosity and chorionicity, VLBW triplets are more prevalent in the MZ MC groups than in the other two groups.

**The Effect of Chorionicity and Zygosity on Triplet Birth Weight**

Triplet birth weight as a function of trio chorionicity and zygosity is presented in Table 5. Differences between the groups could indicate an effect of chorionicity as well as zygosity. But neither chorionicity nor zygosity was significantly associated with birth weight. In Table 6, triplet birth weight is presented as a function of triplet zygosity and chorionicity. MZ MC triplets had a significantly lower mean birth weight than the DZ DC triplets ( $\beta = 86.4, SE = 0.07, t(110) = 0.047$ ).

**Comparing Gestational Age and Birth Weight Discordance Between the Groups**

Differences in gestational age and birth weight discordance between the groups, based on trio zygosity and chorionicity, were not significant:  $F(5, 110) = 1.39, p = .23$  and  $F(5, 105) = 0.85, p = .52$ , respectively (Table 5). In addition, differences in gestational age and birth weight discordance were again not significant when groups were classified on triplet zygosity and chorionicity:  $F(2, 108) = 0.81, p = .45$  and  $F(2, 104) = .71, p = .50$ , respectively (Table 6).

**TABLE 4**

Percentages of Children with Normal, Low, Very Low and Extremely Low Birth Weight as a Function of Trio and Triplet Zygosity and Chorionicity

BW	Trio zygosity & chorionicity						Triplet zygosity & chorionicity		
	MZ MC (%) N = 33	MZ DC (%) N = 15	DZ DC (%) N = 72	MZ TC (%) N = 5	DZ TC (%) N = 54	TZ TC (%) N = 167	MZ MC (%) N = 81	MZ DC (%) N = 41	DZ DC (%) N = 209
NBW: BW > 2500 grams	–	–	8	20	9	6	4	10	7
LBW: 1500 < BW < 2500 grams	67	80	63	20	82	74	63	73	74
VLBW: 1000 < BW < 1500 grams	33	20	24	20	7	17	30	12	16
ELBW: BW < 1000 grams	–	–	6	40	2	2	4	5	3

Note: BW = birth weight, NBW = normal birth weight, LBW = low birth weight, VLBW = very low birth weight, ELBW = extremely low birth weight; MZ = monozygotic, DZ = dizygotic, TZ = trizygotic, MC = monochorionic, DC = dichorionic, TC = trichorionic.

**TABLE 5**

Mean Birth Weight (grams), Birth Weight Discordance, Gestational Age and Assisted Reproductive Techniques as a Function of Chorionicity and Zygosity

Chorionicity	Zygosity	N	Min BW	Max BW	Mean BW	95% CI	Mean BW disc (%) <sup>a</sup>	GA (SD)	ART (%)
MC	MZ	33	1100	2465	1749	[1541, 1956]	17.4	33.1 (1.9)	0
DC	MZ	15	1060	2270	1760	[141, 2109]	22.0	33.4 (2.7)	0
DC	DZ	72	565	3090	1845	[1645, 2046]	24.5	32.9 (3.1)	48
TC	MZ	5	770	2540	1546	[0, 10,532]	35.8	31.0 (2.8)	100
TC	DZ	54	905	2920	2069	[1933, 2205]	19.7	34.3 (2.1)	16
TC	TZ	167	730	3190	1888	[1781, 1995]	20.6	33.6 (2.5)	76

Note: MZ = monozygotic, DZ = dizygotic, TZ = trizygotic, MC = monochorionic, DC = dichorionic, TC = trichorionic; Min = minimum, Max = maximum, BW = birth weight, CI = confidence interval, GA = gestational age, disc = discordance, ART = assisted reproductive techniques.

<sup>a</sup>discordance is given between heaviest and lightest triplet from a trio.

**TABLE 6**

Triplet Birth Weight and Gestational Age as a Function of Triplet Chorionicity and Zygosity

Zygosity and chorionicity	N	Min BW	Max BW	Mean BW	95% CI	Mean BW disc (%) <sup>a</sup>	GA (SD)	ART (%)
MZ MC	81	757	3090	1764	[1625, 1902]	16.6	33.0 (2.6)	33.8
MZ DC	41	770	2670	1982	[1770, 2194]	14.3	33.9 (2.4)	15.8
DZ DC	209	565	3190	1920	[1830, 2010]	19.3	33.6 (2.5)	78.8

Note: BW = birth weight, CI = confidence interval; GA = gestational age, ART = assisted reproductive techniques, MZ = monozygotic, DZ = dizygotic, MC = monochorionic, DC = dichorionic.

<sup>a</sup> discordance is given between heaviest and lightest triplet from a trio.

### DZ DC and DZ TC Trios, Comparing the MZ Pair With the DZ Triplet

DZ DC and DZ TC trios provided the opportunity to perform a within-family comparison between an MZ pair and a DZ triplet. In DZ DC trios, the MZ pair shares one chorion; in DZ TC trios, the MZ pair does not share a chorion. In Table 7, the mean birth weights of MZ pairs and DZ triplets are presented as a function of chorionicity. The table suggests that mean birth weight of the MZ pair was lower only when the MZ pair shared a chorion. When the two triplets of the MZ pair each had their own chorion, there was no difference in birth weight between the MZ pair and the DZ triplet. After testing, neither the main effect of being part of an MZ pair within the DZ trio, nor the main effect of sharing a placenta was significant in this group ( $\beta = -124.8$ ,  $SE = 0.07$ ,  $t(40) = -1.68$ ,  $p = .10$ ;  $\beta = 138.8$ ,  $SE = 0.01$ ,  $t(115) = 0.012$ ,  $p = .10$  respectively). However, the interaction between being part of the MZ pair and having shared a placenta was significant ( $\beta = -131.9$ ,  $SE = 0.06$ ,  $t(40) = -2.39$ ,  $p = .02$ ). This indicates that the specific group of MZ triplets that shared a chorion had a lower mean birth weight than those of all other types of triplets within the group of DZ trios.

In addition, in the last column of Table 7, within the trio, mean birth weight of the MZ pair is calculated and subtracted from the birth weight of the DZ triplet. This column shows that, within DZ trios, the mean birth weight of the MZ pair was always lower when the MZ pair had shared a chorion. If they had not, the mean birth weight of the MZ pair was about the same as the birth weight of the DZ triplet.

### Discussion

To the best of our knowledge, this is the first study to examine the combined effect of chorionicity and zygosity on triplet birth weight. It appears that chorionicity is the most important risk factor for low birth weight; no additional effects of zygosity on triplet birth weight were detected. This result was most clearly seen in the DZ trios. A within-family comparison in the DZ trios between a DZ individual and individuals from the MZ pair showed that sharing a chorion explains the main effect on lowering birth weight.

Based on the triplet zygosity and chorionicity, the birth weights of MZ MC triplets were found to be lower than those of the MZ DC and DZ DC triplets. Due to the examination based on triplet zygosity and chorionicity rather than trio zygosity and chorionicity, the results in triplets can be compared to results in twins. Some studies in twins show the same results (Dube et al., 2002), while others indicate that MZ DC twins also have a lower birth weight than DZ DC twins (Lee et al., 2010; Loos et al., 2005). These different findings are explained by differences in study methods. First, when the effect of zygosity on birth weight is small, this effect will only be detected when sample sizes are large. In addition, to find an effect that is caused by zygosity (and not by differences in gestational age between zygosity groups), the effect of gestational age should be corrected for. Not all studies correct for factors affecting birth weight.

In this study, groups based on trio and on triplet zygosity and chorionicity were compared on gestational age, as it has been proposed that, with less chorions in a triplet pregnancy, gestational age is lower (Spencer et al., 2009). No evidence was found to support this effect. A lower gestational age would (partly) explain a lower birth weight in

**TABLE 7**

Within-Family Comparison of Dizygotic Trios

	Mean BW MZ pair [95% CI] (N)	Mean BW DZ triplet [95% CI] (N)	% diff BW DZ-MZ
DZ DC	1774 [1573, 1976] (48)	1987 [1763, 2211] (24)	100
DZ TC	2043 [1897, 2188] (36)	2121 [1913, 2329] (18)	61.1

Note: BW = birth weight, MZ = monozygotic, DZ = dizygotic, DC = dichorionic, TC = trichorionic, CI = confidence interval, % diff BW DZ-MZ = the percentage of the trios with a heavier DZ triplet than the mean birth weight of the MZ pair. DZ trios consist of two individuals who form an MZ pair and one DZ sibling. In DZ DC triplets, there were two chorions, indicating that the MZ pair shared a chorion; in DZ TC pairs, all three individuals had their own chorion.

the trios of which more triplets had shared a chorion. Although no significant effects were found for lower gestational age, this characteristic was entered in the regression analysis, to correct for even small effects. Therefore, a lower gestational age could not be the explanation for the lower birth weight, based on triplet chorionicity, in the MZ MC group, and for the lower birth weight in the triplets from an MZ pair that had shared a chorion within the DZ trios.

The percentages of triplets in each group that were born after ART were examined, based on zygosity and chorionicity. Machin & Bamforth (1996) suggested that trios born after ART have a better prognosis than spontaneously conceived triplets, because in the latter group more MZ triplet pairs and MZ trios are born. With respect to birth weight, the current study confirms that, in general, triplets born after ART tend to have a higher birth weight due to the more favorable chorionicity.

Regarding birth weight discordance, it already has been established in twins that the MC are more discordant than the DC twins (Blickstein & Keith, 2004; Gielen et al., 2008; González-Quintero et al., 2003). In addition, one study showed that MZ MC and DZ DC twins did not differ in birth weight discordance even though both had a higher discordance than the MZ DC twins (Gielen et al., 2009). Our findings in triplets suggest that, though the differences were not significant, birth weight discordance was lowest in the MZ DC group, somewhat higher in the MZ MC group, and highest in the DZ DC group. These findings are in line with the hypothesis as proposed by Gielen (Gielen et al., 2009).

A limitation of the current study is that PALGA did not provide chorionicity information for all the families that were linked. An important reason for this deficiency in data was that chorionicity information had to be extracted from the laboratory notes. Recently, PALGA has developed a standardized protocol for reporting on placenta pathology, which will be implemented from January 2012. From that date forwards, new reports on placenta pathology will follow this protocol. Future collaborations with PALGA are therefore likely to result in a higher percentage of successful information gathering.

Not all contrasts that we considered showed significant effects. In part, this might be due to sample size. To date, this study used the largest sample that included both chorionicity and zygosity to examine their additional effects on triplet birth weight. Some findings, though not significant, indicated an interesting trend, which needs to be further explored in future research. If possible, future studies should include information on chorionicity in twin and triplet research. In twins, it has been shown that low birth weight is associated with behavioral and emotional problems, and the data on this association supports a causal one (e.g., Groen-Blokhuis, Middeldorp, Beijsterveldt, & Boomsma, 2011). The effect of low birth weight — and possibly additional effects of chorionicity — therefore also need to be considered in triplets.

In conclusion, our results suggest that chorionicity is a more important factor influencing triplet birth weight than zygosity. Several mechanisms can play a role in this association. In MC twins, fetal growth restriction and discordant birth weight can be caused by placental vascular anastomoses, which can result in acardiac twins, and acute and chronic twin-to-twin transfusion syndrome (Nikkels, Hack, & van Gemert, 2008). In addition, unequal placenta sharing can cause severe growth restriction and birth weight discordance, although only in the presence of arterio-arterial anastomoses (Hack et al., 2008). In contrast, these phenomena are not present in DC twins and discordant growth is often ascribed to differences in placental mass or differences in placental parenchymal lesions (Nikkels et al., 2008).

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