Twin Differences and Similarities of Birthweight and Term in the French Romulus Population

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Abstract. This study was performed to examine the main characteristics of the French Romulus twin population: zygosity, chorionicity, sex, term and birthweight. A sample of 104 pairs of twins was distinguished by zygosity, chorionicity and sex, and divided into concordant and discordant birthweight groups. Fifty-three % (n = 55) of the fetus pairs studied were born at “ideal term” (35-38 weeks), and 25% (n = 26) were delivered at “preterm” (28-34 weeks). The very preterm (< 28 weeks), and “postterm” (> 38 weeks) represented altogether 22% (n = 23) of the sample. Of the 104 twin pairs, 68% (n = 71) differ by less than 15% in birthweight, and 32% (n = 33) have a birthweight difference higher than 15%. In dizygotic (DZ) pairs females had more tendency to be in the discordant group (p = 0.01) while in monochorionic-monozygotic (MC-MZ) pairs males were more discordant (p = 0.07). We found a significant interaction between sex and zygosity type (p = 0.02). Males had a birthweight difference significantly weaker than that of females in dichorionic-monozygotic (DC-MZ) and DZ twins whereas it was higher than that of females in MC-MZ twins. There were no MZ twin pairs with DC placentaion over than 15% birthweight difference. Log linear analysis demonstrated a three-way interaction (p < 0.05) between term type, zygosity type and hypotrophy. Our data indicate that in the group of twins born between 35 and 38 weeks’ gestation the crucial question still remains unsettled on how term and birthweight are related to zygosity and/or chorionicity.

Key words: Zygosity, Chorionicity, Birthweight, Concordance, Discordance, “Ideal term”, Prematurity, Hypotrophy
INTRODUCTION

The phenomenon of twin births has occupied humankind’s attention throughout history and still there is an important debate on the methodology and theory in twin studies [5, 25, 38]. Fetal growth in twins represents the endpoints of genetic and environmental influences illustrating the interplay between nature and nurture [27]. However, as Price in 1950 [42] stated “the twin method, as ordinarily applied, is too crude for purposes of modern nature-nurture studies”. Indeed, most of the specific characteristics of twins are lost using conventional methods [9, 13, 52].

Controversy exists whether the fetal growth patterns of singleton pregnancies might be applicable to the follow-up of multiple gestations, or whether accurate growth parameters for multifetal gestations are necessary [1, 2, 18]. Although Crane et al. (1980) [10] reported no difference in fetal growth between singletons and twins even in the third trimester, other studies have shown decreased fetal growth in normal twins as early as the 30th week of pregnancy [27, 28, 46]. However, other studies have raised doubts about these commonly held concepts. Authors [1, 18, 33, 36] have indicated that the intrauterine growth of twins was very different from that of singletons, and do not parallel singleton growth. Other authors have noted that the maturation process is accelerated in twin gestations, resulting in more rapid aging of the twin placentas [30, 35, 37, 40].

Gedda et al. [18] argue that in light of demographic, biological, and developmental considerations, it is concluded that low birth weight in twins is a different condition from low birth weight in singletons and should be dealt with independently, especially in view of the different implications for child growth and survival. For example, approximately half of all twins are hypotrophic when compared to singletons in birthweight [6]. An additional problem is the incidence of discordancy on fetal growth of twins. A significant difference in birthweight of twins is termed growth discordance [16, 48, 49] or – more accurately – weight discordance [3]. Blickstein [4] suggests that the data indirectly supported a two-grade definition, namely, mild (> 15% and < 25% birthweight disparity) and severe (> 25%) growth discordant. Discordant fetuses can also be divided into symmetric (30%) and asymmetric (70%) intrauterine growth retardation or small for gestational age [21, 32].

The gestational age denoting “term” in twin pregnancies is still unsettled [3]. Some studies have argued that singleton standards cannot be applied to evaluate the term of twin pregnancies. The term of twin is very different from that of singletons, for this reason some authors suggested that the “ideal twin term” should be that period associated with the best intrauterine growth for gestational age and the lowest morbidity, which is between 37 and 38 for Papiernik and Richard [37], and 35 and 38 for Luke et al. [30]. Luke et al. [30] demonstrated that, using length of stay and growth retardation criteria, nearly 70% of “ideal” twin pregnancies were between 35 and 38 weeks.

The general purpose of this work was to examine the main characteristics of the French Romulus twin population. The objectives were: (1) to examine the population by zygosity (DZ and MZ), chorionicity (MC and DC), sex, term and birthweight; (2) to compare concordant group to discordant group by term, sex and hypotrophy; (3) to study the combination between term and birthweight by twin types.
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METHODS AND MATERIALS

From August 1993 to July 1998, 631 twin pregnancies were delivered at the Maternity of the Antoine Béclère Hospital in Clamart, France. The study sample was limited to cases meeting the following inclusion criteria: (1) at least three routine antenatal care with ultrasound scan at this maternity, and (2) both twins normal and alive at birth. Pregnancies complicated by major congenital malformation (2 cases of trisomy 21), foetal anomalies (8 cases including 3 TTS), and four pregnancies with fetal death of one fetus were excluded. Additionally, one woman with a first-trimester multifetal reduction, eleven women without routine ultrasound scan, unlike sex DZ twin pairs (n = 67), and 16 twin pairs without biological diagnosis of zygosity were excluded. The remaining twin population (n = 104) consisted of 43 MZ pairs (33 MC: 20 females and 13 males, and 10 DC: 6 females and 4 males), and 61 like-sex DZ pairs (32 females and 29 males). Of the 33 MC-MZ pairs, 20 (61%) were female, as were 6 (60%) of the 10 DC. In the 61 DZ twin pairs, 32 (52%) were female, 29 (48%) were male.

The diagnosis of zygosity was established using multiple red blood cell phenotypes (ABO, Rh, K, MN, Ss, Kidd, Duffy); the molecular biology techniques of amplifying DNA polymorphisms at five loci were used in a second step [39]. MC twins were classified as MZ. Among the DC pairs, twins were considered to be DZ if they differed in one or more of the serological markers. They were considered MZ if they were alike for all the markers (serological and DNA) used. All tests were performed in the Center of Perinatal Hemobiology, Saint Antoine Hospital, Paris, France.

The placental chorionicity was determined: (1) by lambda sign examination in ultrasound prediction during twin pregnancy [43, 51] and (2) by placental pathologic assessment at delivery. In case of disagreement between the sonographic and pathological diagnoses, the placental diagnosis analysis was kept.

The sample has been divided by gestational periods and birthweight difference (BWD). BWD was defined as an inter-twin birthweight discordance expressed in percentage calculated as a percent of the larger twin’s birthweight. The cut-off value for discrepancy was set at 15% with inter-twin differences of less than 15% considered concordance. The twin pairs were divided by gestational age as follow: “extremely preterm” (less than 28 weeks), “preterm” (28 up to 34 weeks), “ideal term” (between 35 to 38 weeks) and “postterm” (38 weeks and over). The sample included a total of 104 twin pairs: 2 “extremely preterm”, 26 “preterm”, 55 “ideal term”, and 21 “postterm”.

Statistical analyses

Between-group differences of frequencies were analyzed using the $\chi^2$ test, Yates correction was used when a cell was less than 10. A p-value < 0.05 was considered significant. A multiple regression analysis was performed using sex and term as the independent variables and group I (< 15% BWD), group II (≥ 15% BWD) as the dependent variable which appears to be the best method to explain the concordant and discordant groups. Then, a two-way ANOVA was performed in order to investigate sex and zygosity type as independent variables and BWD as dependent variable. Lastly, the effect of one variable, while controlling the effect of others was calculated using the log linear analysis to determine any association between term type, hypotrophy (weight < 2500 g), and sex.
RESULTS

Population

There were more females than males, which did not differ significantly (56 vs 44%; $\chi^2 = 0.66, P = 0.41$) in our twin sample. In group I (< 15% BWD), 65%, 60%, and 43% were female in MC-MZ, DC-MZ and DZ, respectively. In group II (≥ 15% BWD), 43%, and 65% of MC-MZ and DZ were female, respectively. About the group differentiation between group I and group II distinguished by zygosity, DZ twins were significantly the most discordant for BW ($\chi^2 = 8.08; p = 0.004$) while there was no significant difference regarding chorionicity between concordant and discordant groups ($\chi^2 \text{ cor} = 1.22, p = 0.27$). MZ twins had the lesser tendency to differentiate. There was an effect of zygosity type but neither of sex nor of chorionicity.

Gestational age

The gestational age at delivery of all twins in this study was between 26 and 41 weeks. Overall, 53% of the fetuses studied were born at “ideal term” (between 35 to 38 weeks), 25% were delivered at “preterm” (28 up to 34 weeks). Twins born at less than 28 weeks, and “postterm” (> 38 weeks) represented 22% of the twin population. Of the 104 twin pairs, 71 (68%) were in group I (< 15% BWD) and 33 (32%) in group II (≥ 15% BWD). Among group I (< 15% BWD), 47% MC, 60% DC, 40% DZ females, and 67% MC, 60% DC, 65% DZ males were born between 35 and 38 weeks’ gestation. In group II (≥ 15% BWD), 33% MC, 0% DC, 65% DZ females, and 25% MC, 0% DC, 33% DZ males were born at “ideal term” (35-38 weeks). Multiple regression analysis (see methods and material section) showed: (1) in DZ pairs ($F [2, 119] = 4.20, p = 0.02$) with $R = 0.26$, females had more tendency to be in group II (≥ 15% BWD) ($\beta = 0.22; p = 0.01$), (2) in MC-MZ pairs ($F [2, 63] = 2.98, p = 0.058$) with $R = 0.29$, just on line by contrast males had more tendency to be in group II ($\beta 0.23, p = 0.068$), and fetuses born earlier had more tendency to be discordant in both DZ twins ($\beta = -0.13; p = 0.15$) and MC-MZ twins ($\beta = -0.23; p = 0.065$).

Birthweight difference

The birthweight mean for twins born between 35-38 weeks’ gestation in group I (< 15% BWD) was ± 2400 g (range 2384-2428). Of the 104 twin pairs, 68% (n = 71) differ by less than 15% in birthweight, and 32% (n = 33) have a BWD higher than 15%. The twin BWD in group I (< 15% BWD) between twin A and B was between 0-400 g. But the vast majority of twins BWD was between 0-300 g. This was the case for 94% MC, 40% DC, 86% DZ females, and 89% MC, 100% DC, 85% DZ males. In group II (≥ 15% BWD), BWD between twin A and B was between 400-900 g. However, the BWD of the infants ranged from 400 to 700 g for 67% MC, 70% DZ females, and 100% MC, 78% DZ males. Results of a two-way ANOVA showed a significant main effect of zygosity type ($p = 0.02$). There was no main effect of sex, while there was significant interaction between sex and zygosity type ($p = 0.02$). These results confirm the regression analysis done before. Males had a BWD significantly weaker than that
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of females in DC-MZ and DZ twins whereas it is higher than that of females in
MC-MZ twins. There were no MZ twin pairs with DC placentation over than 15% 
BWD in this study group.

Combination between term and birthweight

In our sample, 58% of the twins weighed < 2500 g, if we apply singleton standards to
evaluate hypotrophy to our twin population (Table 1). For twins born between 28-34
weeks'gestation 93% females and 96% males weighed < 2500 g, respectively. In con­
trast, for twins born in the “ideal term pregnancy” (35-38 weeks) 53% females and 48%
males weighed < 2500 g, respectively. It is interesting to note that 47% females and 52%
males weighed more than 2500 g. Among twins born between 35-38 weeks'gestation in
MC group I (< 15% BWD) we observed that 23% of twins weighed < 2500 g, and 27% 
of twins weighed ≥ 2500 g. Twenty percent of DC-MZ twins weighed < 2500 g, 45% of
DC-MZ twins weighed ≥ 2500 g, 33% and 21% of DZ twins weighed ≥ 2500 g and < 2500 g, respectively. In group II (≥ 15% BWD), 23% of DZ twins weighed ≥ 2500 g. 
However, 33% of the same series weighed < 2500 g. In MC twins, there were only 8%
and 21% weighed < 2500 g, and ≥ 2500 g, respectively. A log linear analysis showed a
double interaction between term type and hypotrophy (< 2500g) [p < 0.05]. The more the
term type increased (> 38 weeks'gestation), the lower the percentage of hypotrophy.

There is no interaction between sex. We observed a three-way interaction (p < 0.05)
between term type, zygosity type and hypotrophy which indicated that for fetuses born
between 28-34 weeks' gestation the percentage is high and the same regardless of zygos­
ity type. For twins born in the “ideal twin term” the percentage is weaker but varied dif­
ferently according to chorionicity. MCMZ twins born in the “ideal twin term” had a
lower percentage of hypotrophy (< 2500 g) than DZ twins, in contrast MC-MZ twins had
a score higher than DZ twins when born > 38 weeks' gestation (Figure 1).

Fig. 1 - Combination between hypotrophy, twin type and term.
Table I - Combination of term and birthweight in the French Romulus Twin Population by zygosity, chorioncity and gender divided into concordant (< 15%) and discordant (≥ 15%) birthweight difference

<table>
<thead>
<tr>
<th>BWD</th>
<th>Group I (&lt; 15% BWD)</th>
<th>Group II (&gt; 15% BWD)</th>
<th>Total</th>
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<td>MC &lt; 15%</td>
<td>DC &lt; 15%</td>
<td>DZ &lt; 15%</td>
<td>MC ≥15%</td>
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<td>Twin Number</td>
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<td>(N = 10)</td>
<td>(N = 35)</td>
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Note: There were no twin pairs over than 15% BWD in the MZ with DC placentation in this study group.
DISCUSSION

Although discordance and concordance have different pathophysiologic and epidemiologic patterns as well as different genetic and biological characteristics only recently studies begun to understand that truly identical twins do not exist, in either biological and behavioral traits. A full discussion on “how identical are MZ twins” is beyond the scope of this paper, and the reader is referred to Keith and Machin [25] and Charlemaine and Pons [9] for more details. In actual fact, the new definition and standard come to show that even MZ twins are not as identical as asserted by most twin studies.

Population distribution by sex

The authors in this work found a slight but no significant excess of females in MC-MZ twins (65%). This excess is in agreement with previous reports [12, 24, 31] which indicate an excess of females among MC-MZ twins. In this series, there was a slight excess (but no significant) of females in DC-MZ (60%) in group I (< 15% BWD), and also in group II (≥ 15% BWD) for DZ (65%) [no statistical significance]. Moreover, it is not clear why there is a slight excess (but no significant) of females among MC-MZ twins in group I (< 15% BWD) [65%], and not in group II (≥ 15% BWD) [43%] in the French Romulus twin population. Yet, we found always no statistically significant more males (57%) than females in group I (< 15% BWD) and not in group II (≥ 15% BWD) [35%) for DZ twins. Finally, the results from this small twin sample did not show significant difference of sex distribution by twin types. Nevertheless, this calls into question the use of not distinguishing female from male in twin studies [19]. It is important to remind here that the purpose of this analysis was not to look at the sex distribution from an epidemiological perspective, but rather to study a possible interaction between the variable sex and the variables birthweight, term and zygosity.

Birthweight

What is the ideal twin birthweight: the exact biologic significance of birthweight in twins is unknown? Gedda et al. [18] have provided biological, etiological, and pathogenic arguments for the distinction of low birth birthweight in twins, suggesting that the definition of low birthweight for singletons is not realistic for twins, that a considerable bias is introduced into population rates of low birthweight among twins and that the etiology of low birth is different from that of singletons. About 50% of twins have a BW lower than 2500 g [6, 18], which means that large numbers of babies are mis-classified as hypotrophic twins when we apply singleton standards. For example, in our study 58% of twins were hypotrophic twins as defined by the singleton live birth values. In addition, many authors [1, 20, 41, 43] have shown that birthweight is affected when factors such as maternal socioeconomic status, medical complications, race, altitude of residence, fetal anomalies and gender are taken into account. There are also significant differences in birthweight by zygosity and chorionicity [11].

Clearly, the use of singleton reference to determine the optimal birthweight and hypotrophy for twins can result in misclassification and confusion. Conceptually, a hypotrophic newborn can be defined as an infant who has not achieved its fetal growth.
potential in utero. But this definition is confusing because approximately half of all twins are hypotrophic when compared to singletons in birthweight [6]. Therefore, the definition and estimation of the optimal birthweight for twins should be based on twin standards [1, 21, 41].

An additional question is the phenomenon of discordancy on fetal growth of twins. About 80% of twin pairs differ by less than 15% in birthweight [7, 14]. Consequently, some authors suggest that BWD exceeding 15% is a biologic and a logical limit [3], which is also associated with twin growth retardation [34]. For other authors, twin pairs are considered discordant if the difference in their birthweight exceeds 20% or more [8, 16, 23, 29, 45].

In our twin sample 68% (n = 71) of twins differ by less than 15% in birthweight, and 32% (n = 33) have a BWD higher than 15%. In our series, the sex distribution in group II (≤ 15 BWD) is different according to the zygosity type. Females had significantly more tendency to be discordant in DZ twins pairs. In contrast to DZ twins, BWD of MC-MZ twin pairs numbered more males. In both zygosity type, fetuses that were born earlier had a greater tendency to be discordant which is a universal statement. DZ twins were significantly the most discordant for birthweight. While MZ twins had a lesser tendency to differentiate. Nevertheless, males had a BWD significantly weaker than that of females in DC-MZ and DZ twins whereas it was higher than that of females in MC-MZ twins. There were no twin pairs over than 15% BWD in the MZ twins with DC placentation in this study group.

Gestational age

What is the ideal twin term? For Papiernik and Richard [37], the best intrauterine growth and lowest morbidity for twin is between 37 and 38 weeks. Luke et al. [30] found in using length of stay and growth retardation criteria, that nearly 70% of “ideal” twin pregnancies were between 35 and 38 weeks. However, data indicate also that the pattern of twin fetal growth varies by zygosity and chorionicity: MC-MZ twins have, on average, a shorter gestational duration than DZ twins [43]. In the French Romulus population 53% of twins were born between 35 and 38 weeks’ gestation and 25% twin pairs were born between 28 and 34 weeks’ gestation.

Unclear relationship between prematurity and birthweight

First, our findings are in accord with, and complementary to other studies [26, 47] underscoring the combination between birthweight and prematurity for fetuses born “preterm” (< 28-34 weeks’ gestation). Indeed, there was a clear relationship between prematurity and birthweight: 93% (n = 26) females, and 96% (n = 23) males born between 28-34 weeks’ gestation weighed less than 2500 grams. In contrast, this relationship is less clear when we compare twin born at “ideal twin term” window (35-38 weeks’ gestation) [30]. Of 58 females born between 35-38 weeks’ gestation, 53% (n = 31) were hypotrophic (< 2500 g), and 47% (n = 27) weighed more than 2500 g. The ratio for males (n = 52) was nearly similar to females: 48% of males (n = 25) were hypotrophic (< 2500 g), and 52% (n = 27) were above 2500 g (Table 1).

We observed that MZ twin pairs born between 35 to 38 weeks have similar patterns of development (41% and 42% are hypotrophic in MC and DC, respectively) [Figure 1].
We note an effect of zygosity in DZ twin pairs (62% are hypotrophic). Those observations demonstrate that the relationship between term and hypotrophy is not a linear relation. The term had a different effect on hypotrophy according to twin type. However, Table 1 and Figure 1 show that almost the whole sample of twin pairs are hypotrophic (< 2500 g) at < 28 weeks' gestation, regardless of the twin type. Whereas at the “ideal twin term” the rate was higher in DZ and it was higher in MC-MZ at the “postterm” (> 38 weeks' gestation). It is also interesting to note that in our series at the “ideal twin term” the MC-MZ twin pairs did not show more hypotrophy than either DZ and DC twin pairs. Those results show that the crucial question on how term and hypotrophy in twin are related to zygotism and/or chorionicity still remains unsettled [17].

Better understanding twin discordancy

The introduction of new technology is expanding our knowledge of not only the third trimester, but also of the first and second trimester fetus, which has allowed a better understanding of intrauterine growth of fetuses from a very early stage of pregnancy [50]. Those authors suggest that divergent twin growth may develop in the first trimester and persist to late gestation. In summary, preliminary observations suggest that significant first and second trimester inter twin discordance may be the first hint of major biological, environmental, and behavioral differences between and within twin types. It is evident that the onset of discordancy might begin early in twin pregnancy. Thus, there is a need for ultrasonic evaluation early in gestation to document normal growth and to provide baseline data.

CONCLUSION

Human growth can be viewed along a continuum, a process that begins at conception and ends when final adult stature is achieved [22]. Advances in molecular and cytogenetic techniques as well as new developments in biometric measures continue to increase the tools available for understanding intrauterine growth of twin fetuses. Currently, data obtained from singleton measurement charts or tables often are used in twin studies, but it is reasonable to recognize that most of the specific characteristics of twins are lost using conventional methods.

Although there are intrauterine growth charts for twin fetuses [1, 27], there is still no standardized definition of normal intrauterine growth of twins on the basis of twins standards and, consequently, the number of observations using specific criteria for twins have been small. We believe that the foetal growth patterns of singleton pregnancy should not be applied to the follow-up of multiple gestation. Therefore, there is a need of accurate growth parameters for twin gestation. We suggest that the use of criteria generated from uncomplicated twin pregnancies born between 35-38 weeks’ gestation and differing by less than 15% in birthweight is likely to yield to rich rewards in our understanding of fetal growth.

The future of this research is very promising because we know now the main characteristics of the French Romulus twin population by zygotism, chorionicity, term, birthweight and sex. In this context, our results provide important information for our ongo-
ing study on biometric parameters (biparietal diameter, abdominal diameter transverse, femur length, transverse cerebellar diameter) using the same twin population in which we discovered (1) a significant interaction between sex and zygosity, and (2) a three-way interaction between term, birthweight and twin types.

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