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Development of customized fetal growth charts in twins

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DEVELOPMENT OF CUSTOMIZED FETAL GROWTH CHARTS IN TWINS

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Condensation

The growth of uncomplicated twin fetuses is infuenced by parental variables and fetal gender and it is reduced in comparison with singletons starting from 26-28 weeks onwards. This reduction is more evident in monochorionic twins.

Short versison of the Title

Fetal growth in twin pregnancies

Abstract

Background. Twin gestations are at significantly higher risk of fetal growth restriction in comparison with singletons. Using fetal biometric charts customized for obstetrical and parental characteristics may facilitate accurate assessment of fetal growth.

Objective(s): To construct reference charts for gestation of fetal biometric parameters stratified by chorionicity and customized for obstetrical and parental characteristics.

Study Design: Fetal biometric measurements obtained from serial ultrasound examinations in uncomplicated twin pregnancies delivering after 36 weeks of gestation were collected by 19 Italian fetal medicine units under the auspices of the Società Italiana di Ecografia Ostetrica e Ginecologica. The measurements acquired in each fetus at each examination included biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL). Multilevel linear regression models were used to adjust for the serial ultrasonographic measurements obtained and the clustering of each fetus in twin pregnancy. The impact of maternal and paternal characteristics (height, weight, ethnicity), parity, fetal sex and mode of conception were also considered. Models for each parameter were stratified by fetal chorionicity and compared to our previously constructed growth curves for singletons

Results: The dataset included 1781 twin pregnancies (dichorionic 1289; monochorionic diamniotic 492) with 8923 ultrasonographic examination with a median of 5 (range 2-8) observations per pregnancy in dichorionic and 6 in (range 2-11) monochorionic pregnancies. Growth curves of twin pregnancies differed from those of singletons, and differences were more marked in monochorionic twins and during the third trimester. A significant influence of parental characteristics was found.

Conclusion(s): Curves of fetal biometric measurements in twins are influenced by parental characteristics. There is a reduction in growth rate during the third trimester. The reference limits for gestation constructed in this study may provide an useful tool for a more accurate assessment of fetal growth in twin pregnancies.

Introduction

Twin gestations are at significantly higher risk of fetal growth restriction in comparison with singletons, and this may contribute to their increased incidence of the adverse perinatal outcome. Fetal smallness for gestational age may affect one of both fetuses, with an overall incidence estimated at 5%-10% in dichorionic and 15%-25% in monochorionic pairs [1, 2].

On this basis an accurate sonographic assessment of fetal biometry is warranted with the aim of detecting cases with substantial growth restriction or discordance, and accordingly guiding the antenatal care. In clinical practice, singleton pregnancy reference charts for ultrasound biometry are often applied to multiple gestations, since specific nomograms for intrauterine growth of twins are few and of uncertain clinical validity. In humans this sounds biologically inappropriate as the growth potential of twins might per se be reduced compared to singletons, being limited by the inability of a woman to cope in late pregnancy with two fetuses growing each at the same rate of a singleton.

Most studies have in fact documented a progressive flattening of the fetal growth rate in comparison with singletons starting from 28 to 32 weeks [3-8]. However, some of these studies failed to differentiate between dichorionic and monochorionic pairs or between uneventful and complicated pregnancies. Very recently some Authors [8] have provided ultrasound biometry charts in a large group of normal twin gestations showing a reduced growth rate in monochorionic compared to dichorionic sets. Notably in this study parental factors have not been considered in constructing the nomograms. The use of nomograms customized on the basis of parental factors and fetal sex has been proposed to assess intrauterine fetal growth in singleton gestations. This method compared with standard reference charts has been proven by some to be more efficient in identifying the true small fetuses who are at higher risk of perinatal complications [9-11].

The aim of this study was to produce the first longitudinal charts for fetal ultrasound biometry in uncomplicated twins gestations customized for chorionicity and for parental factors.

Methods

Study Population

This was a retrospective multicentric study performed in 19 Italian units under the auspices of the Società Italiana di Ecografia Ostetrica e Ginecologica (SIEOG, www.sieog.it). All the units had proven expertise in sonographic assessment of fetal growth and were opted in by the steering committee of the study. Data were obtained from the combined ultrasound and delivery databases of each unit for pregnancies delivered between January 2010 and December 2015.

Inclusion criteria were: uncomplicated twin pregnancy of known chorionicity; dating by crown-rump length in the first trimester; known pregnancy outcome; delivery at or beyond 36 weeks of gestation of two live fetuses; birthweight > 5th centile for the national Italian charts [12]; information available on maternal and paternal height and weight, parity and ethnic group. Gestational age was calculated by CRL of the larger twin using the equation of Robinson and Fleming [13]. The diagnosis of chorionicity was based upon the sonographic findings obtained at the first trimester (two placental sites or lambda sign with a single placental site for dichorionic; T sign with a single placental site for monochorionic). At that stage accurate labelling of the twins [14] (twin 1 or A vs twin B or 2) was carried out in accordance with placental site (in case of dichorionic pregnancies with two distinct placental masses), fetal position (up and down; right or left) or cord insertion (monochorionic or dichiorionic pregnancies with a single placental mass). Fetal sex was also noted later in pregnancy to facilitate labelling. The maternal weight recorded during the first trimester at the time of the first antenatal visit was considered.

Exclusion criteria were: conception by heterologous assisted reproductive technology; fetal structural or chromosomal anomalies; uncertain chorionicity; monoamnionicity; spontaneous or iatrogenic reduction from a multifetal gestation; maternal smoking; drug use; occurrence of twin to twin transfusion syndrome (TTTS) or twin anemia-polycytemia sequence (TAPS); pre-existing maternal disease such as hypertension, diabetes, renal and autoimmune disorders; the development of obstetric complications such as pre-eclampsia and gestational diabetes. All the units used the same criteria to define the above mentioned pregnancies complications, according to the guidelines of the Italian National Institute of Health (ISS) for pregnancy care [15].

A gestational age interval between 16 and 36 weeks was considered. Longitudinal measurements were required, with a minimum of two sets of measurements for each twin pregnancy. As this was a retrospective analysis of routinely collected anonymized clinical data, no ethical committee approval was necessary according to national regulations.

We decided to rely on Italian national standard birthweight charts [12] in order to select which twin pregnancies were to exclude due a birthweight below the 5th percentile. In our country we lack customized birthweight charts for twins.

Ultrasound measurements

Fetal measurements were all made in accordance with SIEOG guidelines [16]. The biparietal diameter (BPD) and the fetal head circumference (HC) were measured from a cross-sectional view of the fetal head at the level of the thalami, with an angle of insonation of 90° to the midline echoes, a symmetrical appearance of both hemispheres, a continuous midline echo (falx cerebri) broken in middle by the cavum septum pellucidum and no cerebellum visualized. The BPD was measured at the level of the thalami from the outer to the inner edge of the fetal skull. The HC measurements included the outer edge of the proximal calvarial wall and the outer edge of the distal calvarial wall. The abdominal circumference (AC) was measured on a transverse section of the fetal abdomen, showing the stomach bubble, symmetric lower ribs, and the umbilical vein at the level of the portal sinus. The femur length (FL) was measured in its longest axis perpendicular to the transducer direction, with calipers placed at the ends of the ossified diaphysis without including the distal femoral epiphysis. Estimated fetal weight (EFW) was calculated using the Hadlock III formula, that incorporates HC, AC, and FL [17].

Statistical analysis

Comparison of the characteristics between dichorionic (DC) and monochorionic diamniotic (MCDA) pregnancies was performed using chi square test for categorical variables and t-test or Mann Whitney U test for continuous variables, according to their distribution. For modelling growth curve trajectories of the fetal biometric parameters evaluated we used linear mixed models. The data set considered were hierarchical in nature and a random effect structure that incorporates in the modelling the correlation for both twin-pair and fetus within twin pair was used. The covariates considered in the model as fixed effects were gestational age and other variables potentially influencing the ultrasound measurements as paternal and maternal height (expressed in cm), paternal and maternal weight (expressed in kg), ethnic group (categorized as European, East Asian, Central African and North African) [18], parity (categorized as nulliparous or parous) and gender (categorized as male or female). We performed a logarithmic transformation of gestational age for fitting the models. Using polynomial transformation of different degrees or other method of transformation did not improve the statistical significance. Separate growth curves were built for DC and MCDA twins. These were analyzed in comparison with the growth charts for uncomplicated singleton pregnancies customised for fetal sex, obstetrical and parental characteristics recently developed by SIEOG [19]. Week specific difference in biometric measurements between twins and singleton pregnancies were evaluated by the Wald test. Statistical analysis was performed using SPSS version 20 (SPSS Inc. Chicago, IL, USA) and R software packages (version 3.1.2, http://www.R-project.org).

Results

Complete ultrasound fetal biometric data were obtained from 1781 twin pregnancies including 1289 dichorionic (DC) and 439 monochorionic diamniotic (MCDA) gestations who fulfilled the inclusion criteria. Overall 8923 ultrasonographic examinations were available (6640 in DC and 2463 in MCDA). The median number of observations per twin pregnancy was 5 in DC (range 2-8) and 6 in MCDA (range

2-11). The characteristics of the study population are shown in Table 1. When compared to DC twins, MCDA pregnancies showed a lower incidence of nulliparity ($p<0.001$), of conception by in vitro fertilization ($p<0.001$), an earlier gestational age at delivery ($p<0.001$) and a lower birthweight ($p<0.001$). No significant differences were found for any other feature considered.

Tables 2 to 5 show the fitted regression coefficients and their statistical significance for the biometric variables considered. As expected, gestational age had a significant positive association with all biometric parameters. For BPD, maternal weight ($p=0.003$) and fetal sex ($p<0.0001$) were the other associated covariates in DC twins, while in MCDA only the effect of fetal sex ($p<0.0001$) resulted significant. For HC, maternal weight ($p=0.005$), maternal height ($p=0.004$), paternal height ($p=0.0015$) and fetal sex ($p<0.0001$) had a significant association in DC twins, while maternal height ($p=0.032$), paternal height ($p=0.05$) and fetal sex ($p<0.0001$) were associated in MCDA twins. Maternal weight ($p=0.005$), maternal height ($p=0.0029$) and fetal sex ($p<0.0001$) resulted significantly related to AC measurements in DC twins, while maternal height ($p=0.029$) and fetal sex ($p=0.0027$) showed the same association in MCDA ones. When the FL was analyzed the significant covariates were maternal height ($p<0.0001$) and paternal height ($p<0.0001$) in DC and paternal height ($p=0.001$) in MCDA pregnancies. Since there was a small number of pregnancies in the three non-European groups the data do not allow any comment on the effect of ethnicity on size or growth in twins. The effect size of all the considered covariates in the construction of the mixed regression models are reported in supplemental materials (Supplemental Tables 1-4).

Figures 1 and 2 present the growth curves of the biometric parameters considered in DC and MCDA twins, respectively, compared to singletons. Singleton reference limits were constructed using our national growth charts customized for parent characteristics, obstetrical history and fetal sex [19]. Similarly in Figure 3 the EFW of DC and MCDA twins were compared to singletons. In order to allow a comparison the same covariates were used for singletons and twins (i.e. European ethnicity for both parents, parity 0, maternal weight 60 kg, maternal height 160 cm, paternal height 180 cm, male fetal sex) and tables (Supplemental Tables 5-9) were generated to allow centile comparison. To allow an easy calculation of the growth curve percentiles in twin pregnancies with different combination of covariates we have created an Excel based file (Additional file 1).

The growth curves of DC twin pregnancies appeared to differ significantly from those of singletons, with the reference percentiles of each biometric parameter showing lower values along the whole gestational interval considered. The differences with singleton growth charts were more evident with advancing gestation (Figure 1). When the Wald test was applied to evaluate week-specific differences in the biometric variables between singleton and DC twins, BPD measurements appeared different from 31 weeks (p=0.05), HC from 29 weeks (p=0.04), AC from 27 weeks (p=0.05) and FL from 34 weeks (p=0.03) of gestation.

Similarly the growth curves of MCDA twin pregnancies appeared to differ significantly from those of singletons, the reference percentiles of each biometric parameter showing lower values along the whole gestational interval considered, Again, the differences with singleton growth charts became more evident with advancing gestation and for some parameter such as AC appeared to increase progressively during the third trimester (Figure 2). Significant differences were evidenced for BDP from 30 weeks (p=0.03), HC from 28 weeks (p=0.05), AC from 26 weeks (p=0.04) and FL from 34 weeks (p=0.05) of gestation.

Comparing DC with MCDA pregnancies, the measurements of each biometric index appeared slightly smaller in the latter group, with differences being statistically significant only for AC after 33 weeks of gestation (p=0.03)

Comment

Principal findings

In a large population of uncomplicated dichorionic and monochorionic twin pregnancies we documented a different growth pattern in comparison with singleton fetuses, with a flattening of the biometric curve starting at 26-28 weeks of gestation for all biometric parameters. Differences with singleton charts were larger in monochorionic twins, progressively increasing during the third trimester for some parameters such as AC. Moreover, as previously shown in singletons [19, 20], a relationship between fetal biometric data and parental characteristic and fetal gender was documented in both dichorionic and monochorionic twins.

Clinical and research implications

The use of twin-specific customized growth charts for ultrasound biometry may allow a more accurate assessment of the intrauterine biometry of twins for clinical purposes. In particular this approach may help the provider in distinguishing cases of true fetal smallness among a subgroup of pregnancies whose intrauterine growth potential compared with singleton is per se reduced. On this basis a precise sonographic diagnosis of fetal growth restriction among twin gestations is considered as a cornerstone to optimize their clinical management and to reduce the risk of adverse outcomes. Surprisingly, in common practice the reference charts for the intrauterine growth of twins are very often those in use for the evaluation of singletons. A recent theory has recently suggested that constraints to maternal metabolism increase in pregnancy may limit fetal growth; this may further explain why the intrauterine growth rate in twins might be reduced in comparison with singletons [21]. On this basis the construction of specific twin size charts has been claimed by some as a more reliable tool to assess the intrauterine fetal growth in multiple gestation [5, 22-24]. In principle, adjusting for multiple pregnancy, thereby shifting the normal range of fetal growth downward, has the potential to mask truly growth restricted twins and increase perinatal morbidity from failure to recognize growth restriction. However, having selected as a reference standard a large group of uncomplicated twin gestations delivered close to term with a fetal birthweight of both twins above the 5th percentile of population standards, this should reduce if not abolish the risk of overlooking or masking a fetal growth restriction of one of both fetuses using these charts. This is simply because the biometric data used to produce these charts come from super healthy twin gestations; altough the fetal measurements may appear smaller than those of a singleton a good placental function is in fact required to a normal twin to fit in our curves. The choice to exclude those twin pregnancies whose birthweight of one of both fetuses was below the 5th percentile of population standards was made with the aim of maintaining a low threshold to define fetal smallness in twins. Using the 5th percentile at 36 weeks or beyond (rather than the 10th percentile as in singletons) as the lower limit to define smallness at birth should account for the reduced intrauterine size of normally growing twins compared to singletons.

At the same time it should limit the risk of overlooking fetal growth restriction of twins and considering as biologically normal for two fetuses what is a pathologically reduced growth pattern [25].

We are aware that monochorionic and dichorionic pregnancies have different rates of IUGR and also that the threshold of physiological intertwin discordance of biometric data is varies according to the chorionicity On this point we feel that the use of growth reference charts which are customized for chorionicity may help the clinician also in the interpretation of the intertwin discordance as it should more accurately reflect the specific intrauterine growth pattern of dichorionic and monochorionic twin gestations. In other words using a reference charts which have been specifically designed for dichorionic and monochorionic twin pregnancies the clinician will be able to assess more accurately the degree of intertwin discordance and to determine, in a clinical context, if this difference should be considered physiological or pathological. We decided to include the measurements obtained from both twins at each visit rather than select the measurement of the largest twin. We are aware of the potential risk of downgrading the reference interval for fetal growth, and that this may eventually decrease the sensitivity in detecting antenatally pathological fetal smallness. However, the strict inclusion criteria of our study population should reduce the risk of overlooking fetal growth restriction.

Previous Studies

Some older studies have shown smaller values for all biometric parameters obtained sonographically in twin gestations compared to singletons. Ong et al. [6] assessed 884 twins between 1986 and 1999, and used a single random measurement of the dataset to construct the intrauterine nomograms. In their series the AC values of twins were smaller in comparison with singleton gestations only after 32 weeks of gestation, whereas BPD appeared reduced along the whole pregnancy. However, in the aforementioned study the fetal growth charts were not adjusted for the chorionicity, as the differentiation between dichorionic and monochorionic placentae has become accurate only more recently.

In 2012 Liao et al. [4] assessed a smaller group of 125 uncomplicated diamniotic twin gestations in a longitudinal prospective study, without differentiating for chorionicity. Using a multilevel regression approach they constructed specific charts for all biometric parameters, whose values appeared smaller compared with those obtained in singleton after 28 weeks.

Stirrup et al. [8], using a large database of twin pregnancies, retrospectively built reference charts for all fetal biometric parameters from 14 weeks to term adjusting for chorionicity. Similarly to our findings, they found that ultrasound measurements of fetal growth showed a significant reduction in twin pregnancies, particularly in the third trimester, compared with singletons. Also in their cohort, this reduction was more marked in MCDA gestations. However, they also included complicated twin gestations such as those with twin to twin transfusion or fetal growth restriction, which contributed to the construction of the reference growth charts. This should be acknowledged as a methodological limitation in building the twin specific nomograms for the intrauterine fetal growth.

Recently ultrasound based estimated fetal weight reference charts have been retrospectively built in 642 uncomplicated dichiorionic and monochorionic twin pregnancies [7]. In this study the reference centiles of fetal weight were significantly lower among monochorionic compared to dichiorionic twins along the whole pregnancy. Furthermore, similarly to previous studies, a significant flattening of the intrauterine fetal weight curve in twins compared to singleton in the third trimester was reported, starting earlier in the monochorionic than in the dichiorionic group (28 vs 32 weeks). Finally, a recent study from the National Institute of Child Health and Human Development has shown that compared with singleton fetuses, dichorionic twin fetuses have a progressively asymmetrical slower growth, beginning around 32 weeks of gestation [3].

In this study, as previously proposed by others [26, 27], we opted to customize all the fetal biometric parameters obtained at ultrasound and not only the estimated fetal weight. We believe indeed that this is a more appropriate approach when developing fetal growth charts as some parameters may vary according to the ethnicity or the constitutional characteristics of the parents, these differences not being specifically reflected by the changes in estimated fetal weight [7]. Some of our findings related to the association between fetal biometric parameters and parental characteristics are not easy to interpret: HC has many more significant associations than the BPD. This is not biologically plausible, and might easily be explained by the fact that the variance in BPD measurements is smaller than for HC and thus may not have a sufficient power to reveal associations of HC. Moreover, the fact that maternal weight does not seem to affect significantly the fetal growth charts of twins, as opposed to singletons [19, 20], may be explained by the fact that the mean maternal weight in twins is larger than in singletons.

The clinical usefulness of customization has been the object of debate in the last years [10, 28, 29]. However a number of publications have shown that in singleton pregnancies the use of customized growth charts is more accurate in identifying the true small fetuses whose risk of perinatal complications is actually increased. [10, 11]. Also in multiple pregnancies, the use of customized birth weight charts for twins rather than those for singletons seems more accurate in predicting adverse fetal and neonatal outcomes [5, 24]. Following the publication of the large prospective INTERGROWTH-21st study [30], which failed to demonstrate a significant impact of the ethnicity on the variability of fetal biometric data, the use of normative universal growth charts has been claimed as more appropriate. However the concept of an optimal fetal growth pattern that should ideally be followed by each fetus has been challenged from a theoretical point of view [31]. Moreover, recent evidence from singleton pregnancies suggests that the INTERGROWTH-21st standards may be less effective than population [32] or customized [9] charts in indentifying those small fetuses at risk of perinatal mortality or morbidity. Similar evidence is currently not available for twin pregnancies and also the current study does not allow to conclude that customized biometric models, in comparison to population-derived reference ranges, perform better in terms of their ability to identify individual fetuses at risk of adverse perinatal outcomes. The clinical usefulness of these models should be evaluated in a clinical trial and only if they are shown to be superior should they be considered for use in a clinical context. Altough such a validation can be carried also in retrospect, only a prospective study would be able to provide a convincing demonstration that the use of customized charts produces a measurable benefit in terms of reduction of perinatal morbidity or mortality compared to the use of the standard curves, as recently shown for singletons [9].

Strenghts and limitations

The main strength of our study is that complicated twin pregnancies were excluded in order to construct unbiased reference charts. In particular as the objective of this study was to build the normal intrauterine biometric charts of healthy uncomplicated dichorionic and monochorionic twin gestations, we decided a priori to exclude from the retrospective data collection the twins whose birthweight was below the 5th centile for population standards, and those who were delivered before 36 weeks. This may affect the construction of the reference interval and determine a selection bias between monochorionic and dichorionic pairs. However the rationale for this choice was to avoid the data contamination with measurements obtained from complicated twin pregnancies.

Thanks to a multilevel regression model that takes into account fixed and random effects, we adjusted our curves for chorionicity, parental variables and fetal gender. In particular, a main difference from previous studies is that these two latter factors, both constitutional variables of both parents and fetal gender, were considered in the model and were shown to have a significant impact on the different fetal biometric data, as previously documented in singleton [20]. The decision to include also paternal variables seems biologically plausible due to the presumably relevant contribution of the father to the fetal growth potential; however in this study the genetic paternity was based upon maternal report and remained unproven. Differently from our previous study on singleton gestations [19], no association was found between the twins biometry and the parental ethnicity, although the lack of significance may depend on the low number of non-European women enrolled in the current study.

The participation to this multicentric trial was arbitrarily restricted to units with long standing experience in obstetric ultrasound whose operators are certified by the Italian Society of Ultrasound in Obstetrics and Gynecology, and this is certainly a further strength point of this study. Finally this is to date the study with the largest number of biometric data longitudinally collected in twins used to construct the nomograms of intrauterine fetal growth.

Among the main weaknesses of this study it is the retrospective design which prevented us from validating our growth curves in the clinical practice and assessing if this tool allows a more accurate assessment of intrauterine twins biometry, thus reducing the risk of adverse perinatal outcome. However the design of the study which by definition has retrospectively selected the largest group of normal uneventful twin gestation to construct the reference charts did not allow to test the clinical usefulness of these customized curves in the management of twin gestations. Moreover the rather homogenous racial mix, with approximately 90% of women of European origin probably led to an underestimation of the effect of parental racial origin on twin growth, which was not statistically significant for any biometric parameter. Some population studies on the customization of twin birth weight charts have actually proven an effect of maternal ethnicity on birthweight [23]. The unavailability of the father or the lack of certainty on paternal data is an additional limitation of our model which supports customization of fetal biometry in accordance to the characteristics of both parents. We customized our growth curves according to the maternal weight prepregnancy weight at the time of the first ultrasound scan. Altough the pre-pregnancy weight is a more reliable index of maternal characteristics independently from the effect of the pregnancy, its exact value may be uncertain or unknown to the woman; therefore we pragmatically decided to use the weight which was actually measured by the midiwife and reported in the antenatal notes. Furthermore, availability of the full set of study variables was a criterion of inclusion, and all participating centers shared only datasets containing this information: we were therefore unable to analyze details on the exclusions and the number of each type of exclusion in order to assess the representativity of the population.

The two fetuses within the twin pair were in fact treated as two independent fetuses and provided two distinct set of measurement for each visit. However as previously suggested by others [33] the measurements within a twin pair are not completely independent from each other and they are correlated to each other. This is biologically consistent with the fact that we may sonographically diagnose chorionicity but not zygosity and this latter factor may significantly affect the interdependency of the biometric data within a dichorionic twin pair. On this base, the use of a regression mixed model which accounts for the correlation of twin measurements has been suggested by some when assessing fetal biometry and growth of dichorionic twin gestation. This has been done also in our study as specified in the methods section although we acknowledge that this method, despite being widely used, cannot completely adjust for those biological and environmental factors which determine the interdependency of the biometric data within a twin pair.

We decided to include the dataset obtained from both twins at each visit rather than select the measurement of the best twin. We are aware of the potential risk of downgrading the reference interval for fetal growth and that this may eventually decrease our sensitivity in detecting antenatally a pathological fetal smallness. However having built our curves with the biometric data of superhealthy uncomplicated dichorionic and monochorionic twin gestations should keep high enough our reference interval reducing the risk of overlooking fetal growth restriction [25]. Moreover, a recent analysis suggests that increasing intertwin birthweight discordance is not associated with long-term neuropscychological disadvantages. However it carries an increased risk of neonatal complications and infant mortality which might be, at least in part, iatrogenic [34]. To this effect, our standards may help to better identify those discordant twins who may benefit from increased intervention better than currently used standards.

A major issue remains whether fetal growth in twins should be measured against a singleton reference: given the higher morbidity associated with twins, correcting for the presence of twins might not be appropriate. However, there is evidence that optimal birthweights are different in twins and in singletons [5, 23, 24], and this justifies adopting specific size and growth references for twins. A prospective validation study is needed to prove that our curves are superior to singleton or non-customized twin curves in clinical practice.

Conclusion

In conclusion, this large retrospective study has confirmed that the intrauterine growth of uncomplicated twin pregnancies is reduced in comparison with singletons starting from 26-28 weeks. This reduction is more evident in monochorionic twins. The growth pattern of the fetal biometric parameters is significantly influenced by parental variables and fetal gender. The reference ranges for gestation constructed in this study may provide an useful tool for a more accurate assessment of fetal growth in twin pregnancies.

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Table 1. Characteristics of the twin pregnancies according to chorionicity. Data are expressed as mean±SD or No (%)

	Dichorionic twin N=1289	Monochorionic diamniotic twin N=492	P value
Mother			
maternal age (years)	34.23±5.48	32.63±5.25	0.679
nulliparous	963 (74.68%)	313 (63.16%)	0.001
height (cm)	165.53±6.15	165.01±6.01	0.236
weight (kg)	62.72±10.73	61.07±10.99	0.258
Ethnic gruop			
European	1192 (92.40%)	442 (89.7%)	
East Asian	9 (0.70%)	23 (4.8%)	
Central African	41 (3.20%)	9 (1.8)	
North African	47 (3.7%)	18 (3.7)	0.222
Conception by in vitro fertilization (IVF)	422 (32.37%)	54 (10.98%)	0.0001
Father			
height	177.48±8.28	177.08±6.70	0.355
Ethnic group			
European	1204 (93.4%)	443 (90.2)	
East asia	8 (0.6%)	24 (4.8%)	
Central African	27 (2.1%)	6 (1.1)	
North African	50 (3.9%)	19 (3.9)	0.16
Fetus/newborn			
gestational age at delivery (weeks)	37.36±0.710	36.7±0.65	0.001
Birthweight(g)	2648.37±308.34	2516.40±328.41	0.001
sex			
male	637 (49.4%)	227 (46.2)	
female	652 (50.6)	265 (53.8%)	0.216

Table 2. Mixed regression models for biparietal diameter (BPD) in dichorionic and monochorionic diamniotic twins.

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-158.23	0.76	209.25	0.0001
log gestational age	68.57	0.19	366.23	0.0001
mother weight	0.02	0.01	2.99	0.003
sex (female)	-0.71	0.13	5.39	0.0001
Monochorionic diamniotic				
Intercept	-157.64	0.73	217.42	0.0001
log gestational age	68.68	0.22	313.98	0.0001
sex (female)	-0.78	0.20	3.94	0.0001

Table 3. Mixed regression models for head circumference (HC) in dichorionic and monochorionic diamniotic twins.

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-591.74	7.72	673.18	0.0001
log gestational age	244.33	0.48	510.55	0.0001
mother weight	0.07	0.03	2.80	0.005
mother height	0.08	0.04	2.00	0.04
father height	0.07	0.03	2.44	0.015
sex (female)	-2.69	0.34	7.86	0.001
Monochorionic diamniotic				
Intercept	-622.31	12.41	50.42	0.0001
log gestational age	245.53	0.62	403.76	0.0001
mother height	0.14	0.07	2.16	0.032
father height	0.17	0.06	2.84	0.005
sex (female)	-3.72	0.76	4.93	0.0001

Table 4. Mixed regression models for abdominal circumference (AC) in dichorionic and monochorionic diamniotic twins.

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-646.97	8.51	76.06	0.0001
log gestational age	257.15	0.62	413.39	0.0001
mother weight	0.06	0.03	2.99	0.05
mother height	0.16	0.05	1.91	0.0029
sex (female)	-1.68	0.43	3.88	0.0001
Monochorionic diamniotic				
Intercept	-643.36	12.85	50.07	0.0001
log gestational age	255.82	0.79	324.81	0.0001
mother height	0.17	0.08	2.20	0.029
sex (female)	-2.10	0.94	2.22	0.027

Tab 5 Mixed regression models for femur length (FL) in dichorionic and monochorionic diamniotic twins.

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-163,20	2,12	76,99	0,0001
log gestational age	60,17	0,13	469,35	0,0001
mother height	0,05	0,01	4,34	0,0001
father height	0,04	0,01	4,51	0,0001
Monochorionic diamniotic				
Intercept	-157,43	2,64	-59,585	0,0001
log gestational age	60,37	0,17	365,67	0,0001
father height	0,05	0,01	3,214	0,001

LEGENDS

Figure 1: Estimated 5th, 50th and 95th percentiles for BPD (a), HC (b), AC (c) and FL (d) in DC twins (red lines) as obtained from linear mixed models. Data are compared with corresponding reference percentiles in singleton pregnancies (black lines). In both groups values were customized for the same paternal and obstetrical covariates and for fetal sex.

Figure 2: Estimated 5th, 50th and 95th percentiles for BPD (a), HC (b), AC (c) and FL (d) MCDA twins (blue lines) as obtained from linear mixed models. Data are compared with corresponding reference percentiles for in singleton pregnancies (black lines). In both groups values were customized for the same paternal and obstetrical covariates and for fetal sex.

Figure 3: Estimated 5th, 50th and 95th percentiles for estimated fetal weight in DC twins (**panel a** red lines) and MCDA twins (**panel b** blue lines). Data are compared with corresponding reference percentiles for in singleton pregnancies (black lines). In both groups values were customized for the same paternal and obstetrical covariates and for fetal sex.