Development of fetal growth charts in twins stratified by chorionicity and mode of conception: a retrospective cohort study in China

Yuan-Qing Xia[1,2], Shu-Ping Lyu[1], Jun Zhang[3], Yi-Ting Chen[2], Li Gao[1], An-Da Zhao[4], Yan-Lin Wang[1], Sheng-Hui Li[2]

[1]Department of Prenatal Diagnosis, International Peace Maternity & Child Health Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China;
[2]School of Public Health, Shanghai Jiao Tong University, Shanghai 200025, China;
[3]MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China;
[4]Department of Clinical Nutrition, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China.

Abstract

Background: Twin pregnancies continue to increase worldwide; however, the current clinical prenatal evaluation for the intrauterine growth of twins still relies on the growth standards of singletons. We attempted to establish a set of fetal biometric references for Chinese twin pregnancies, stratified by chorionicity and conception mode as spontaneously conceived monochorionic diamniotic (SC-MCDA), spontaneously conceived dichorionic diamniotic (SC-DCDA), and assisted reproductive technology dichorionic diamniotic (ART-DCDA) twins.
Methods: From 2016 to 2019, the ultrasonographic fetal biometric measurements were longitudinally collected in pregnant women, including fetal weight, biparietal diameter, head circumference, abdominal circumference, femur length, and humerus length. The linear mixed models were used to test the difference of growth patterns between groups, and the growth curve of each biometric parameter was modeled by a generalized additive model for location scale and shape.
Results: A total of 929 twin pregnant women and 2019 singleton pregnant women, met the inclusion criteria. Among twin pregnancies, 148 were SC-MCDA, 215 were SC-DCDA, and 566 were ART-DCDA twins. Overall, SC-DCDA twins grew faster than SC-MCDA twins, while slower than ART-DCDA twins (all $P < 0.05$), and all of the three groups showed significant differences comparing with singletons, especially during the third trimester. Hence, the customized fetal growth charts of each fetal biometric parameter were, respectively, constructed for SC-MCDA, SC-DCDA, and ART-DCDA twins.
Conclusions: The fetal biometric trajectories demonstrated characteristic patterns according to chorionicity and conception mode. To fill the gap, we modeled fetal biometric parameters for Chinese SC-MCDA, SC-DCDA, and ART-DCDA twin pregnancies, hoping to provide a reference for the further establishment of fetal growth reference values for Chinese twin fetuses.
Keywords: Twin pregnancy; Ultrasonography; Fetal growth; Chorionicity; Spontaneously conceived; Assisted reproductive technology

Introduction

In recent years, the rate of twin pregnancies has continued to rise due to the growing utilization of assisted reproductive technology (ART) and delayed childbirth.[1,2] Available data suggest that ART accounts for a third of twin pregnancies.[3,4] It has been well documented that fetal growth of twin fetuses is slower than that of singletons, usually starting from 28 to 32 weeks of gestation,[5-8] owing to the limited uterine space.[9]

Up to date the clinical examination for the intrauterine growth of twins still largely relies on the growth standards of singletons, and it has been an increasing need to develop a twin-specific biometry chart to monitor fetal growth trajectory for twin pregnancy.[10,11] In recent years, several ultrasonographic reference charts of twins have been established.[5-8,12-17] However, they were derived from small populations[5,12,16] or did not exclude high-risk pregnancies.[5,13,17] In addition, evidence suggested that compared with dichorionic diamniotic (DCDA) twins, monochorionic diamniotic (MCDA) twins showed a slower growth rate,[6,12,17] and ART may affect the perinatal outcome of twin pregnancies.[18-21] Therefore, both chorionicity and conception mode should be taken into consideration while developing fetal biometric reference for twins. A newly published study from Italy established the first longitudinal growth charts for fetal

Access this article online

Quick Response Code:

Website:
www.cmj.org

DOI:
10.1097/CM9.0000000000001616

ultrasound biometry customized for chorionicity. However, the data did not show a statistical difference in fetal growth over gestational age between DCDA and MCDA twins.[6] To date, no study explored the differences in fetal intrauterine growth between ART and spontaneously conceived (SC) twin pregnancies. To fill the knowledge gap, our study examined the growth difference of twins with varied chorionicity and conception mode, aiming to establish chorionicity- and conception mode-specific fetal biometric parameters reference.

Existing studies modeled the growth curve adopting linear mixed model,[5-7,13,14] multilevel linear models,[15,16] or hierarchical Bayesian models.[8] Compared to a linear model, the generalized additive model for location, scale, and shape (GAMLSS) extends to model all the fourth-order variations, including median, standard deviation, skewness, and kurtosis, demonstrating a strong strength in improving the accuracy of fitting smoothed percentile curves.[22] Since 2006, World Health Organization (WHO) performed GAMLSS to establish child growth standards.[23]

Fetal growth can differ by race or ethnicity.[24,25] In 2015, a Chinese study initially established a standard for twin fetal weight growth.[26] However, the study was based on birth weight data but not ultrasonographic biometric parameters, while the birth weight data can be biased by preterm delivery since preterm delivery is usually associated with pregnancy complications and fetal growth abnormalities. Therefore, the standard established in this study somewhat sacrificed the sensitivity to identify the early onset of growth restriction and cannot convey the longitudinal pattern of fetal growth from early pregnancy.[26]

The present study used the GAMLSS model-based longitudinal growth trajectories among Chinese pregnant women by using ultrasonographic biometry data. We aim to develop a fetal growth chart for Chinese twin pregnancies stratified by both chorionicity and mode of conception and compare it with singleton charts.

Methods

Ethical approval

The ethical application and consent procedure were approved by the Ethics Committee of Shanghai Jiao Tong University School of Medicine (No. SJUPN-201717, dated December 28, 2017).

Study population

Based on fetal ultrasonographic biometry electronic datasets, a retrospective longitudinal study was conducted among pregnant women from the Department of Prenatal Diagnosis, International Peace Maternity & Child Health Hospital, Shanghai, China. Those pregnant women who delivered twins, as well as singletons between January 2016 and December 2019, were included.

This study attempted to construct ultrasonic biometry standards to monitor fetal growth for twin pregnancy.

Only deliveries at or beyond 34 weeks of gestation, with at least two sets of measurements during the whole pregnancy, were considered to be qualified. Exclusion criteria were listed as follows: uncertain chorionicity; monoamnionicity; key information deficit, mainly gestational age or ultrasonic measurements being unavailable; spontaneous or iatrogenic reduction from a multifetal gestation; fetal death; fetal structural or chromosomal anomalies; occurrence of twin-to-twin transfusion syndrome or twin anemia-polycythemia sequence; selective fetal growth restriction or a birth weight below the 3rd percentile for the national birthweight charts[27]; pre-existing maternal disease such as hypertension, diabetes, or renal and autoimmune disorders; and the development of obstetric complications such as gestational hypertensive disease and diabetes. In addition, very few ($n = 23$) were MCDA twins conceived by ART and, therefore, they were not included in the final analyses. Thus, this study focused on SC-MCDA, SC-DCDA, and ART-DCDA. While for a singleton pregnancy, only delivery at or beyond 37 weeks of gestation were selected, and the inclusion/exclusion criteria were defined in accordance with those of twin pregnancy where appropriate.

Gestational age was calculated by the date of ovulation, the date of embryo transfer, last menstrual period, or the crown-rump length, as appropriate. The diagnosis of chorionicity was based on the number of gestational sacs at 7 to 8 weeks of gestation and "T sign," or "lambda sign" obtained by ultrasonography at the first trimester.

Maternal age at delivery was grouped into two categories: <35 vs. ≥35 years, and ≥35 years was defined as advanced maternal age. Maternal pre-pregnancy body mass index (BMI) was grouped into four categories: <18.5, 18.5 to 23.9, 24.0 to 27.9, and ≥28.0 kg/m^2.

Fetal ultrasonic measurements

Ultrasound examinations were performed every 3 to 4 weeks between 14 and 32 weeks, then every 2 weeks until delivery in singleton and DCDA twins; while for MCDA twins, every 2 weeks between 14 and 32 weeks and weekly beyond 32 weeks of gestation. At each visit, the fetal biparietal diameter (BPD), head circumference (HC), femur length (FL), humerus length (HL), anteroposterior trunk diameter (APTD), and transverse trunk diameter (TTD) for each fetus were measured and recorded. The BPD was measured from the outer to the inner edge of the fetal skull at the level of the thalami. The HC was measured as an ellipse around the perimeter of the fetal skull. The FL was measured from one end of the femoral diaphysis to the other, not including the distal femoral epiphysis. The HL was measured from one end of the humeral diaphysis to the other with the borders clearly visible. Both APTD and TTD extended from the outer aspects of the lateral abdominal wall, and TTD usually was perpendicular to APTD. Estimated fetal weight (EFW) was calculated based on BPD, APTD, TTD, and FL according to Hadlock formulas.[28,29] Abdominal circumference (AC) was calculated based on the mean arithmetic diameters of APTD and TTD.[30]

Statistical analysis

The statistical description was made by use of percentage for categorical variables, and mean and standard deviation, for continuous variables. The group difference was examined using the Chi-squared test, variance analysis, or Kruskal-Wallis rank test where appropriate, and the pairwise comparisons were further checked.

The linear mixed models were used to test the fetal growth difference by varied chorionicity and mode of conception. Both the overall longitudinal change including trajectory curve and growth velocity across the pregnancy and week-specific discrepancy were taken into the examination. For the purpose of this study, the difference was compared between any two groups. For those ultrasound measurements which showed statistically difference either between SC-MCDA and SC-DCDA twins or between SC-DCDA and ART-DCDA twins, week-specific comparisons were further conducted after adjusting for maternal age, height, weight, gravidity, and parity.[5,6] Moreover, all of the three groups were in comparison with uncomplicated singletons, which were delivered in the same hospital at the same time.

The GAMLSS was applied to fit the growth curve of each biometric parameter in relation to gestational age. The GAMLSS is highly flexible as it is capable of modeling not only mean (or location) but also other parameters (such as standard deviation, skewness, and kurtosis).[22,23] Box-Cox t distribution was used for modeling fetal biometric parameters as non-parametric cubic spline functions of gestational age. Model selection was based on generalized Akaike Information Criterion (GAIC), and the model with the smallest value of GAIC was selected. Worm plots were used for visual inspection of the fit of the smoothed curves. Centile curves for each biometric parameter were constructed according to placental chorionicity and mode of conception.

All analyses were performed with the Statistical Package for the Social Sciences (version 24; SPSS Inc., Chicago, IL, USA) and the GAMLSS and lme4 package for R statistical software (version 3.5.1; http://www.R-project.org). Significance was defined as a two-tail probability value of <0.05.

Results

A total of 4055 pregnancies, 2142 singleton and 1913 twin, were included in this study. The flowchart of twin participants enrolment was shown in Figure 1. The final sample consisted of 2019 singleton with a total of 9787
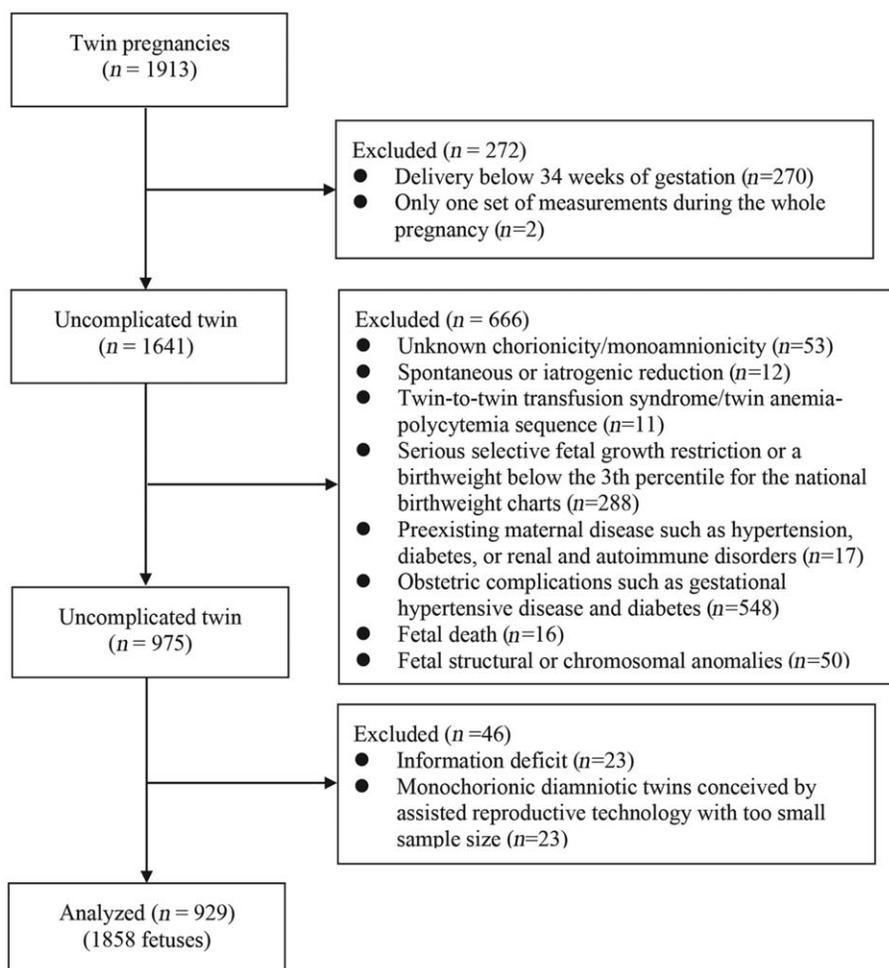
Figure 1: Flowchart of Chinese twin pregnancies selection.

ultrasound observations, and 929 mothers (148 SC-MCDA, 215 SC-DCDA, and 566 ART-DCDA), with a total of 12,837 ultrasound observations (3099 in 296 SC-MCDA, 2542 in 430 SC-DCDA, and 7196 in 1132 ART-DCDA fetuses). A gestational age interval between 14 and 37 weeks was covered.

Characteristics of the study group

Description and comparison of the maternal and neonatal characteristics between SC-MCDA, SC-DCDA, and ART-DCDA twins are displayed in Table 1. Women in advanced maternal age accounted for 23 (15.5%) among SC-MCDA twins, 42 (19.5%) in SC-DCDA twins, and 137 (24.2%) in ART-DCDA twins ($H = 24.314$, $P < 0.001$). Maternal pre-pregnancy BMI showed significant differences only between SC-MCDA and ART-DCDA twins ($H = 9.639$, $P = 0.008$). In addition, the proportion of nulliparous women in ART-DCDA twins were higher than those of SC-MCDA twins ($\chi^2 = 13.809$, $P = 0.001$). SC-MCDA twins had a shorter gestational age than SC-DCDA and ART-DCDA twins ($H = 34.483$, $P < 0.001$). Meanwhile,

SC-MCDA twins were significantly lighter in birth weight and shorter in birth length than SC-DCDA and ART-DCDA twins (2468.82 g vs. 2643.62 and 2637.71 g, $F = 36.753$, $P < 0.001$; 47.21 cm vs. 47.82 and 47.89 cm, $H = 18.523$, $P < 0.001$). The description of singleton and comparison with twins are displayed in Supplementary Table 1, http://links.lww.com/CM9/A644.

Modeling fetal growth reference

As shown in Supplementary Tables 2, http://links.lww.com/CM9/A644, 3, http://links.lww.com/CM9/A644, and 4, http://links.lww.com/CM9/A644, both the overall growth curve through 14 to 37 weeks of gestation and each fetal biometric measurement by gestational age were compared among the three groups. The statistical differences occurred in the fetal biometric measurement curves for EFW, AC, FL, and HL between SC-DCDA and SC-MCDA twins, and the size of each biometric measurement appeared to be smaller in SC-MCDA (EFW: $F = 11.224$, $P < 0.001$; AC: $F = 7.062$, $P = 0.008$; FL: $F = 7.360$,

Table 1: Description and comparison of maternal and neonatal characteristics between SC-MCDA, SC-DCDA, and ART-DCDA twins.

Items	SC-MCDA ($n = 148$)	SC-DCDA ($n = 215$)	ART-DCDA ($n = 566$)	Statistics	P value
Maternal characteristics					
Age				24.314	<0.001[*]
<35 years	125 (84.5)	173 (80.5)	429 (75.8)		
≥35 years	23 (15.5)	42 (19.5)	137 (24.2)		
Mean ± SD	30.03 ± 4.10	31.03 ± 3.84	32.20 ± 3.41		
Maternal height, cm	162.90 ± 4.86	162.30 ± 4.71	162.16 ± 8.43	0.586	0.557[*]
Pre-pregnancy weight, kg	55.10 ± 8.13	56.21 ± 8.72	56.92 ± 8.58	2.696	0.068[*]
Pre-pregnancy BMI				9.639	0.008[*]
<18.5 kg/m^2	25 (16.9)	33 (15.3)	56 (9.9)		
18.5–23.9 kg/m^2	111 (75.0)	152 (70.7)	409 (72.3)		
24.0–27.9 kg/m^2	10 (6.8)	23 (110.7)	86 (15.2)		
≥28.0 kg/m^2	2 (1.3)	7 (3.3)	15 (2.6)		
Mean ± SD	20.73 ± 2.71	21.35 ± 3.24	21.56 ± 3.00		
Gravidity				23.463	0.001[†]
1	70 (47.3)	103 (47.9)	355 (62.7)		
2	43 (29.0)	62 (28.9)	115 (20.3)		
3	25 (16.9)	28 (13.0)	55 (9.7)		
≥4	10 (6.8)	22 (10.2)	41 (7.3)		
Parity				13.809	0.001[†]
0	89 (60.1)	152 (70.7)	427 (75.4)		
≥1	59 (39.9)	63 (29.3)	139 (24.6)		
Delivery mode				5.671	0.059[†]
Natural birth	145 (98.0)	211 (98.1)	564 (99.6)		
Cesarean/forceps/assisted breech delivery	3 (2.0)	4 (1.9)	2 (0.4)		
Neonatal characteristics					
N	296	430	1132		
Gender				5.216	0.074[†]
Male	128 (43.2)	215 (50.0)	573 (50.6)		
Female	168 (56.8)	215 (50.0)	559 (49.4)		
Gestational age at delivery, weeks	36.20 ± 0.95	36.68 ± 0.91	36.64 ± 0.82	34.483	<0.001[*]
Birth weight, g	2468.82 ± 305.99	2643.62 ± 316.38	2637.71 ± 315.13	36.753	<0.001[‡]
Birth length, cm	47.21 ± 1.89	47.82 ± 1.85	47.89 ± 1.60	18.523	<0.001[*]

Data are presented as n (%) or mean ± SD. Bold fonts mean significance appeared. [*]Kruskal-Wallis rank test. [†]Chi-squared test. [‡]Variance analysis test. ART-DCDA: Assisted reproductive technology dichorionic diamniotic; BMI: Body mass index; SC-DCDA: Spontaneously conceived dichorionic diamniotic; SC-MCDA: Spontaneously conceived monochorionic diamniotic; SD: Standard deviation.

$P = 0.007$; HL: $F = 4.920$, $P = 0.027$). The growth velocity for EFW, BPD, HC, FL, and HL of SC-DCDA twins was also different from those of SC-MCDA twins (EFW: $F = 31.381$, $P < 0.001$; BPD: $F = 15.263$, $P < 0.001$; HC: $F = 23.712$, $P < 0.001$; FL: $F = 9.026$, $P = 0.003$; HL: $F = 14.595$, $P < 0.001$), but no statistical differences were found in AC. While evaluating week-specific differences in the biometric variables between SC-MCDA and SC-DCDA twins, EFW, FL, and HL growth slowed down in SC-MCDA twins compared with SC-DCDA during most of the gestational weeks ($P < 0.05$). In addition, the size of all the six fetal biometric measurements demonstrated smaller through 15, 21, and 25 weeks of gestation in SC-MCDA twins than that in SC-DCDA twins consistently ($P < 0.05$). When the comparison between SC-DCDA and ART-DCDA groups was examined, significant differences were observed in all six fetal biometric measurement curves and only in the growth velocity for HC (EFW: $F = 5.676$, $P = 0.017$; BPD: $F = 5.200$, $P = 0.023$; HC: curve: $F = 5.271$, $P = 0.022$, velocity: $F = 5.371$, $P = 0.021$; AC: $F = 4.524$, $P = 0.033$; FL: $F = 5.377$, $P = 0.020$; HL: $F = 3.168$, $P = 0.075$). Similarly, week-specific differences of all six fetal biometric measurements were significant at a few weeks of gestation (all $P < 0.050$). Further focusing on SC-MCDA and ART-DCDA twins, it was found that there were significant differences in all six fetal biometric measurement curves and the growth velocity of EFW, BPD, HC, FL, and HL. Meanwhile, week-specific differences of all the six fetal biometric measurements were statistically significant during most gestational weeks.

All of the SC-MCDA, SC-DCDA, and ART-DCDA twins were different from singletons, especially during the third trimester [Supplementary Tables 5–7, http://links.lww.com/CM9/A644]. Compared with singletons, the size of all six fetal biometric measurement appeared to be smaller in SC-MCDA twins, the size of EFW, AC, FL, and HL appeared smaller in SC-DCDA twins, and the size of EFW, BPD, HC, AC, and HL appeared smaller in ART-DCDA twins.

Figures 2–4 show the fitted growth curves of fetal biometric parameters, including EFW, BPD, HC, AC, FL, and HL for SC-MCDA, SC-DCDA, and ART-DCDA twins, respectively. The smoothed percentiles, such as the 5th, 10th, 50th, 90th, and 95th percentiles of fetal biometric parameters by gestational age are illustrated in Supplementary Table 8, http://links.lww.com/CM9/A644.

Discussion

Main findings

Based on a large data set of ultrasonographic biometric measurements, we developed fetal ultrasound biometric parameters, including EFW, BPD, HC, AC, FL, and HL, among Chinese uncomplicated twin pregnancies. The major strength of this study lies in taking both chorionicity and mode of conception into account, and the findings showed that SC-DCDA twins had a faster growth rate than SC-MCDA twins but slower than ART-DCDA twins. Comparing with singletons, all three twin charts show a slower growth pattern; however, the contrasts were mainly observed during the third trimester. The data enriched our understanding of the twin-specific fetal growth trajectories, and the parameters established in this study have the potential to promote a more accurate assessment of intrauterine growth trajectories for Chinese twins.

Interpretation

In our study, it was demonstrated that the fetal biometric measurements including EFW, AC, FL, and HL of SC-DCDA twins were statistically different from those of SC-
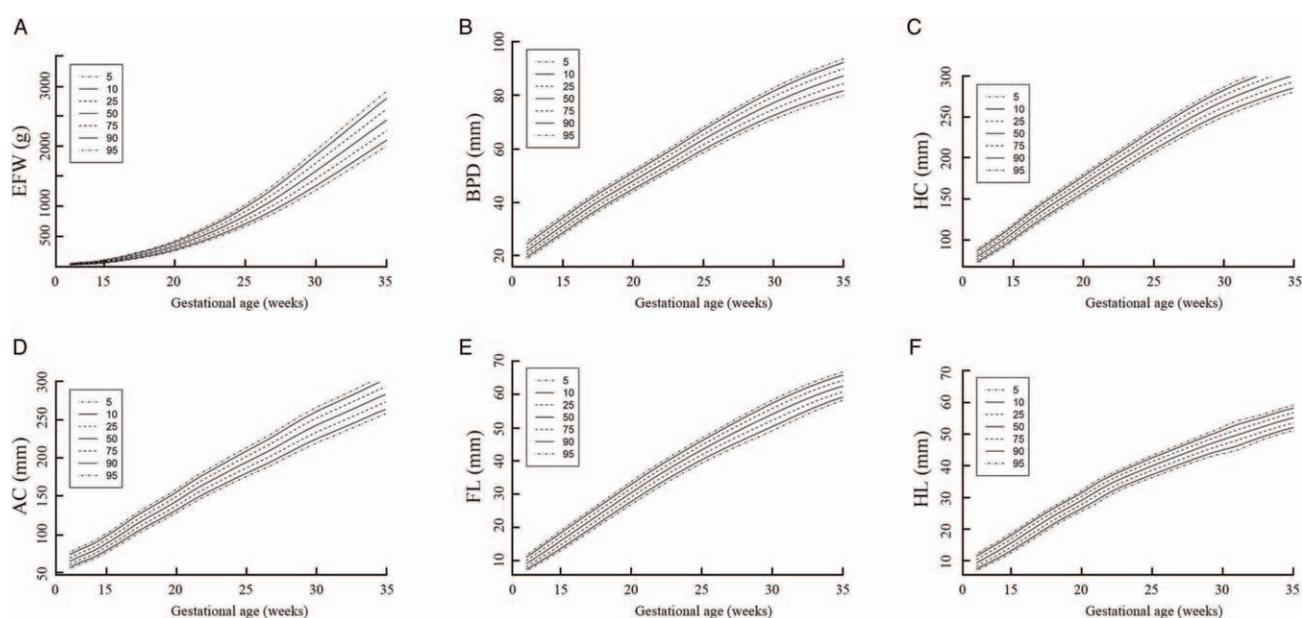
Figure 2: Estimated percentiles for (A) EFW, (B) BPD, (C) HC, (D) AC, (E) FL, and (F) HL in SC-MCDA twins. AC: Abdominal circumference; BPD: Biparietal diameter; EFW: Estimated fetal weight; FL: Femur length; HC: Head circumference; HL: Humerus length; SC-MCDA: Spontaneously conceived monochorionic diamniotic.
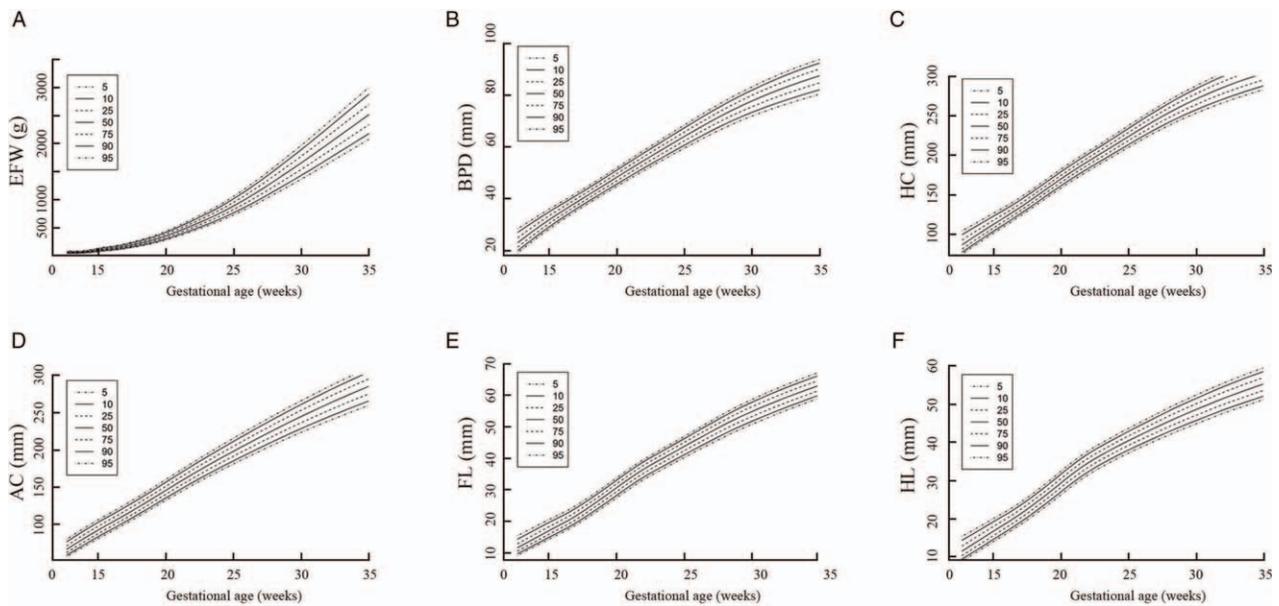
Figure 3: Estimated percentiles for (A) EFW, (B) BPD, (C) HC, (D) AC, (E) FL, and (F) HL in SC-DCDA twins. AC: Abdominal circumference; BPD: Biparietal diameter; EFW: Estimated fetal weight; FL: Femur length; HC: Head circumference; HL: Humerus length; SC-DCDA: Spontaneously conceived dichorionic diamniotic.
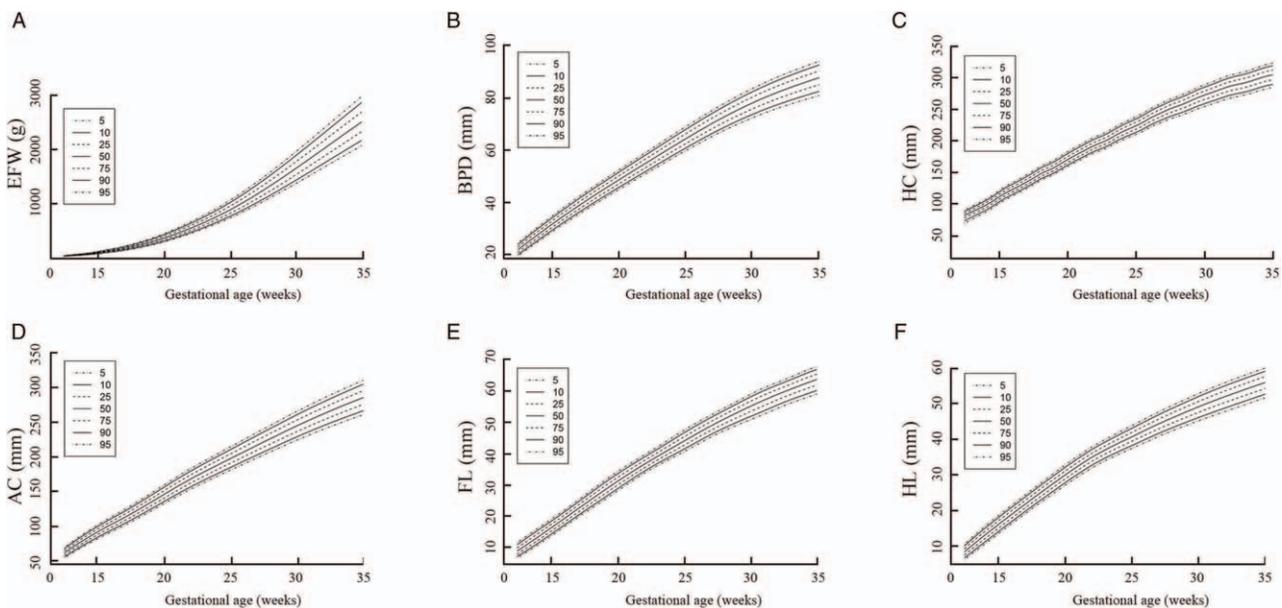
Figure 4: Estimated percentiles for (A) EFW, (B) BPD, (C) HC, (D) AC, (E) FL, and (F) HL in ART-DCDA twins. AC: Abdominal circumference; ART-DCDA: Assisted reproductive technology dichorionic diamniotic; BPD: Biparietal diameter; EFW: Estimated fetal weight; FL: Femur length; HC: Head circumference; HL: Humerus length.

MCDA twins. To date, a few numbers of studies compared the intrauterine growth pattern of DCDA twins to that of MCDA twins,[6-8,12-14,17] and the results were generally consistent and similar to the findings of our study. A study in Brazil had a consistent result with ours in that there were statistically differences in EFW, BPD, AC, and FL between monochorionic and dichorionic twin pregnancies.[12] A multicentric study from Italy also reported that the measurements of EFW, BPD, HC, AC, and FL appeared smaller in the MCDA group in comparison with DCDA twins; however, the difference did not show statistical significance.[6] When further exploring the week-specific

difference for fetal biometric measurements between DCDA and MCDA twins, a study conducted in the US found that DCDA twins had higher sonographic EFW at almost all gestational ages until 34th gestational weeks,[14] and the data from Italy similarly found that the differences for AC were statistically significant after 33 weeks of gestation.[6] The evidence from the two studies provided support for our findings.

Fetal growth velocity has been identified as an important indicator to assess fetal growth and development.[31] Recently, a national study in the US examined the growth

velocity of six parameters including EFW, BPD, HC, AC, HL, and FL among singletons and compared the overall differences of these parameters between different racial/ethnic-specific curves.[32] To our knowledge, no study yet paid attention to the fetal growth velocity among twins. ART has become more common, and an understanding with regard to its perinatal outcome is becoming essential.[18-21] Several studies have, more often than not, focused on the possible impact of ART on birth weight among twin pregnancies, failing to cover intrauterine fetal growth.[18-21] As an initial study, we, for the first time, observed the gestational week-specific fetal size and growth velocity among ART-DCDA twins, revealing ART-DCDA twins had the highest growth rate, followed by SC-DCDA, and then SC-MCDA.

Clinical implications

The accuracy of intrauterine growth assessment for twins depends on the establishment of twin-specific growth charts, and the longitudinal ultrasonographic standards have benefits in recognizing growth pattern variations at different gestational ages.[33] The international society of obstetrics and gynecology ultrasound emphasized the clinical significance of development and use of twin-specific growth charts while assessing fetal growth of twins since 2016.[34] In medical practice, it has been well-known that growth restriction in twins is prevalent due to the slower growth rate in the third trimester.[5-8] In our study, all the median (50th percentile) biometric parameters of SC-MCDA, SC-DCDA, and ART-DCDA twins were lower at each gestational week compared with fetal biometry reference of Chinese singleton.[10,35] Therefore, over-diagnosis of restricted intrauterine growth should be a common concern while adopting the diagnostic criteria based upon the standard of singletons. For a long time, although there are the continuous efforts to establish a fetal growth curve for twins, the issue has not been resolved. The existing data have inherent limitations. For example, some of the data came from birth weight and cannot be applied to the growth and development assessment during pregnancy,[36-39] while others recruited subjects by small sample size[5,12,16] or without exclusion of high-risk pregnancy.[5,13,17] Our study overcame the limitations and largely promised our growth reference chart customized for chorionicity and mode of conception a more reliable tool to distinguish cases with fetal growth restriction in twins.[40]

We are aware that reducing the fetal growth reference value may sacrifice the sensitivity to identify pathological fetal growth restriction. Our subject enrolment strategy adopted strict inclusion criteria to ensure a qualified sensitivity in the early screening of fetal growth restriction. Notably, our sampled population included pregnancies via ART. Since twin pregnancies via ART account for an increasing proportion of twins, our growth charts customized for conception mode would be more generalizable to the current population of twins in China. The results of this study illustrated an asymmetric pattern of growth velocity between DCDA and MCDA twins, which has been confirmed by previous studies.[6,12,17] In addition, it has been suggested that chorionicity has a significant independent effect on birth weight,[41] and the

threshold of physiological intertwin size discordance of fetal biometry also varies by chorionicity.[42] Thus, developing growth reference charts customized for chorionicity is necessary.

In this study, six fetal biometric measurements obtained from ultrasound were opted to customize the growth reference, but not only the EFW. A previous study found that some biometric measurements may vary according to parental ethnicity or different constitutional characteristics. Not all the differences can be specifically explained by the changes in EFW.[6] As proposed by previous studies,[43,44] we developed all fetal biometric parameter growth charts rather than only EFW in most studies.[8,13,14] The full-spectrum parameters could enrich our knowledge on fetal changes in the uterus, which should be clinically significant to promote a more comprehensive evaluation of fetal intrauterine growth.

Our study has several strengths. First, only healthy uncomplicated twin pregnancies were included. Second, our reference charts were based on longitudinal data and adequate sample size, which made it available to develop growth centiles to identify patterns and differences in fetal growth at different gestational ages.[15] Third, taking both chorionicity and mode of conception into account made our growth reference chart a more reliable and sensitive tool in identifying growth restriction in twins. Fourth, the GAMLSS model has unique advantages in fitting the changing curve over time, which has been applied by WHO to establish child growth standards.[23] Moreover, making a comparison with data from singleton provided evidence to confirm that twin has intrinsic growth pattern, emphasizing the importance of this study.

However, several weaknesses also warrant acknowledgment. First, this study was a single-center design, there might be a selection bias. However, International Peace Maternity & Child Health Hospital is the top quality maternal and child health hospital in Shanghai, which attracts pregnant women all around Shanghai, China. The hospital has opened a twin pregnancy clinic since 2015, which is one of the three specialist consultation settings for twin pregnancy in Shanghai. Therefore, the twin pregnant women recruited through 4 years should have certain representativeness in the Shanghai area. Second, very few ($n = 23$) were MCDA twins conceived by ART, therefore, they were not included in this study. Finally, although we adopted strict inclusion criteria to acquire a qualified target population, another high-risk individual with unclear adverse perinatal outcomes was possible.

In conclusions, we established a set of fetal growth charts stratified by chorionicity and mode of conception for Chinese twins, which fills the gap and the clinical significance lies in providing a useful tool for a more accurate and comprehensive assessment of fetal growth in twins. This study provides new evidence that SC-MCDA twins grow slower than SC-DCDA twins, and the growth velocities of EFW, BPD, HC, FL, and HL were statistically different between them. Meanwhile, our results show that conception mode could affect the growth pattern of twins.

Conflicts of interest

None.

References

1. Smits J, Monden C. Twinning across the developing world. PLoS One 2011;6:e25239. doi: 10.1371/journal.pone.0025239.
2. Ananth CV, Chauhan SP. Epidemiology of twinning in developed countries. Semin Perinatol 2012;36:156–161. doi: 10.1053/j.semperi.2012.02.001.
3. Calhaz-Jorge C, de Geyter C, Kupka MS, de Mouzon J, Erb K, Mocanu E, et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. Hum Reprod 2016;31:1638–1652. doi: 10.1093/humrep/dew151.
4. Gill P, Van Hook MJ. Twin Births. Treasure Island, FL: StatPearls Publishing LLC; 2019.
5. Grantz KL, Grewal J, Albert PS, Wapner R, D'Alton ME, Sciscione A, et al. Dichorionic twin trajectories: the NICHD fetal growth studies. Am J Obstet Gynecol 2016;215:221.e1–221.e16. doi: 10.1016/j.ajog.2016.04.044.
6. Ghi T, Prefumo F, Fichera A, Lanna M, Periti E, Persico N, et al. Development of customized fetal growth charts in twins. Am J Obstet Gynecol 2017;216:514.e1–514.e17. doi: 10.1016/j.ajog.2016.12.176.
7. Hiersch L, Okby R, Freeman H, Rosen H, Nevo O, Barrett J, et al. Differences in fetal growth patterns between twins and singletons. J Matern Fetal Neonatal Med 2019;33:2546–2555. doi: 10.1080/14767058.2018.1555705.
8. Sekiguchi M, Mikami M, Nakagawa C, Ozaki M, Tanigaki S, Kobayashi T, et al. An ultrasonographic estimated fetal weight reference for Japanese twin pregnancies. J Med Ultrason (2001) 2019;46:209–215. doi: 10.1007/s10396-018-0921-y.
9. Hayes EJ. Review of "multiple pregnancy: epidemiology, gestation & perinatal outcome. Second edition". J Exp Clin Assist Reprod 2005;2:1–3. doi: 10.1186/1743-1050-2-12.
10. Zhang Y, Meng H, Jiang Y, Xu Z, Ouyang Y, Li S, et al. Chinese fetal biometry: reference equations and comparison with charts from other populations. J Matern Fetal Neonatal Med 2019;32:1507–1515. doi: 10.1080/14767058.2017.1410787.
11. Leung TN, Pang MW, Daljit SS, Leung TY, Poon CF, Wong SM, et al. Fetal biometry in ethnic Chinese: biparietal diameter, head circumference, abdominal circumference and femur length. Ultrasound Obstet Gynecol 2008;31:321–327. doi: 10.1002/uog.5192.
12. Araujo Junior E, Ruano R, Javadian P, Martins WP, Elito J Jr, Pires CR, et al. Reference charts for fetal biometric parameters in twin pregnancies according to chorionicity. Prenat Diagn 2014;34:382–388. doi: 10.1002/pd.4318.
13. Shivkumar S, Himes KP, Hutcheon JA, Platt RW. An ultrasound-based fetal weight reference for twins. Am J Obstet Gynecol 2015;213:224.e1–224.e9. doi: 10.1016/j.ajog.2015.04.015.
14. Gabbay-Benziv R, Crimmins S, Contag SA. Reference values for sonographically estimated fetal weight in twin gestations stratified by chorionicity: a single center study. J Ultrasound Med 2017;36:793–798. doi: 10.7863/ultra.16.02049.
15. Torres X, Bennasar M, Eixarch E, Rueda C, Gonce A, Munoz M, et al. Gender-specific antenatal growth reference charts in mono-
chorionic twins. Fetal Diagn Ther 2018;44:202–209. doi: 10.1159/000484555.
16. Liao AW, Brizot Mde L, Kang HJ, Assuncao RA, Zugaib M. Longitudinal reference ranges for fetal ultrasound biometry in twin pregnancies. Clinics (Sao Paulo, Brazil) 2012;67:451–455. doi: 10.6061/clinics/2012(05)08.
17. Stirrup OT, Khalil A, D'Antonio F, Thilaganathan B. Fetal growth reference ranges in twin pregnancy: analysis of the Southwest Thames Obstetric Research Collaborative (STORK) multiple pregnancy cohort. Ultrasound Obstet Gynecol 2015;45:301–307. doi: 10.1002/uog.14640.
18. Shebl O, Ebner T, Sir A, Sommergruber M, Tews G. The role of mode of conception in the outcome of twin pregnancies. Minerva Ginecol 2009;61:141–152.
19. Vasario E, Borgarello V, Bossotti C, Libanori E, Biolcati M, Arduino S, et al. IVF twins have similar obstetric and neonatal outcome as spontaneously conceived twins: a prospective follow-up study. Reprod Biomed Online 2010;21:422–428. doi: 10.1016/j.rbmo.2010.04.007.
20. Yang X, Li Y, Li C, Zhang W. Current overview of pregnancy complications and live-birth outcome of assisted reproductive technology in mainland China. Fertil Steril 2014;101:385–391. doi: 10.1016/j.fertnstert.2013.10.017.
21. Qin JB, Wang H, Sheng X, Xie Q, Gao S. Assisted reproductive technology and risk of adverse obstetric outcomes in dichorionic twin pregnancies: a systematic review and meta-analysis. Fertil Steril 2016;105:1180–1192. doi: 10.1016/j.fertnstert.2015.12.131.
22. Stasinopoulos DM, Rigby RA. Generalized additive models for location scale and shape (GAMLSS) in R. J Stat Softw 2007;23:1–43. doi: 10.18637/jss.v023.i07.
23. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76–85. doi: 10.1111/j.1651-2227.2006.tb02378.x.
24. Madan A, Holland S, Humbert JE, Benitz WE. Racial differences in birth weight of term infants in a northern California population. J Perinatol 2002;22:230–235. doi: 10.1038/sj.jp.7210703.
25. Buck Louis GM, Grewal J, Albert PS, Sciscione A, Wing DA, Grobman WA, et al. Racial/ethnic standards for fetal growth: the NICHD fetal growth studies. Am J Obstet Gynecol 2015;213:449.e1–449.e41. doi: 10.1016/j.ajog.2015.08.032.
26. Zhang J, Mikolajczyk R, Lei X, Sun L, Yu H, Cheng W. An adjustable fetal weight standard for twins: a statistical modeling study. BMC Med 2015;13:159. doi: 10.1186/s12916-015-0401-9.
27. Zhu L, Zhang R, Zhang S, Shi W, Yan W, Wang X, et al. Chinese neonatal birth weight curve for different gestational age (in Chinese). Chin J Pediatr 2015;53:97–103. doi: 10.3760/cma.j.issn.0578-1310.2015.02.007.
28. Shinozuka N, Okai T, Kohzuma S, Mukubo M, Shih CT, Maeda T, et al. Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes. Am J Obstet Gynecol 1987;157:1140–1145. doi: 10.1016/s0002-9378(87)80278-8.
29. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements - a prospective study. Am J Obstet Gynecol 1985;151:333–337. doi: 10.1016/0002-9378(85)90298-4.
30. Tamura RK, Sabbagha RE, Pan WH, Vaisrub N. Ultrasonic fetal abdominal circumference: comparison of direct versus calculated measurement. Obstet Gynecol 1986;67:833–835. doi: 10.1097/00006250-198606000-00017.
31. Deter RL, Lee W, Yeo L, Erez O, Ramamurthy U, Naik M, et al. Individualized growth assessment: conceptual framework and practical implementation for the evaluation of fetal growth and neonatal growth outcome. Am J Obstet Gynecol 2018;218:S656–S678. doi: 10.1016/j.ajog.2017.12.210.
32. Grantz KL, Kim S, Grobman WA, Newman R, Owen J, Skupski D, et al. Fetal growth velocity: the NICHD fetal growth studies. Am J Obstet Gynecol 2018;219:285.e1–285.e36. doi: 10.1016/j.ajog.2018.05.016.
33. Johnsen SL, Rasmussen S, Wilsgaard T, Sollien R, Kiserud T. Longitudinal reference ranges for estimated fetal weight. Acta Obstet Gynecol Scand 2006;85:286–297. doi: 10.1080/00016340600569133.
34. Khalil A, Rodgers M, Baschat A, Bhide A, Gratacos E, Hecher K, et al. ISUOG practice guidelines: role of ultrasound in twin pregnancy.

Ultrasound Obstet Gynecol 2016;47:247–263. doi: 10.1002/uog.15821.

35. Xu Y, Lek N, Cheung YB, Biswas A, Su LL, Kwek KY, *et al*. Unconditional and conditional standards for fetal abdominal circumference and estimated fetal weight in an ethnic Chinese population: a birth cohort study. BMC Pregnancy Childbirth 2015;15:141. doi: 10.1186/s12884-015-0569-1.

36. Ananth CV, Vintzileos AM, Shen-Schwarz S, Smulian JC, Lai Y-L. Standards of birth weight in twin gestations stratified by placental chorionicity. Obstet Gynecol 1998;91:917–924. doi: 10.1016/s0029-7844(98)00052-0.

37. Dai L, Deng C, Li Y, Yi L, Li X, Mu Y, *et al*. Population-based birth weight reference percentiles for Chinese twins. Ann Med 2017;49:470–478. doi: 10.1080/07853890.2017.1294258.

38. Premkumar P, Antonisamy B, Mathews J, Benjamin S, Regi A, Jose R, *et al*. Birth weight centiles by gestational age for twins born in south India. BMC Pregnancy Childbirth 2016;16:64. doi: 10.1186/s12884-016-0850-y.

39. Zhang B, Cao Z, Zhang Y, Yao C, Xiong C, Zhang Y, *et al*. Birthweight percentiles for twin birth neonates by gestational age in China. Sci Rep 2016;6:31290. doi: 10.1038/srep31290.

40. Odibo AO, Cahill AG, Goetzinger KR, Harper LM, Tuuli MG, Macones GA. Customized growth charts for twin gestations to optimize identification of small-for-gestational age fetuses at risk of intrauterine fetal death. Ultrasound Obstet Gynecol 2013;41:637–642. doi: 10.1002/uog.12404.

41. Papageorghiou AT, Bakoulas V, Sebire NJ, Nicolaides KH. Intrauterine growth in multiple pregnancies in relation to fetal number, chorionicity and gestational age. Ultrasound Obstet Gynecol 2008;32:890–893. doi: 10.1002/uog.6140.

42. Stirrup OT, Khalil A, D'Antonio F, Thilaganathan B. Patterns of second- and third-trimester growth and discordance in twin pregnancy: analysis of the Southwest Thames Obstetric Research Collaborative (STORK) Multiple Pregnancy Cohort. Fetal Diagn Ther 2017;41:100–107. doi: 10.1159/000447489.

43. Pang MW, Leung TN, Sahota DS, Lau TK, Chang AM. Customizing fetal biometric charts. Ultrasound Obstet Gynecol 2003;22:271–276. doi: 10.1002/uog.196.

44. Johnsen SL, Wilsgaard T, Rasmussen S, Sollien R, Kiserud T. Longitudinal reference charts for growth of the fetal head, abdomen and femur. Eur J Obstet Gynecol Reprod Biol 2006;127:172–185. doi: 10.1016/j.ejogrb.2005.10.004.