CLINICAL SCIENCE

Longitudinal reference ranges for fetal ultrasound biometry in twin pregnancies

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OBJECTIVE: The purpose of this study was to establish longitudinal reference ranges for fetal ultrasound biometry measurements and growth parameters in twin pregnancies.

METHOD: A total of 200 uncomplicated twin pregnancies before 21 weeks of gestation were recruited for this prospective, longitudinal study. Women who abandoned follow-up, pregnancies with unknown outcomes or pregnancies with complications were excluded. Ultrasound scans were performed every three weeks, and biparietal and occipitofrontal diameters, head and abdominal circumferences, and femur diaphysis length measurements were obtained for each fetus at each visit. Estimated fetal weight, biparietal/occipitofrontal diameter, head circumference/abdominal circumference, and femur diaphysis length/abdominal circumference ratios were also calculated. Multilevel regression analysis was performed on normalized data.

RESULTS: A total of 807 ultrasound examinations were performed in 125 twin pregnancies between 14 and 38 weeks of gestation (6.5 ± 1.4 scans/pregnancy). Regression analysis demonstrated significant correlations for all variables with gestational age, namely log of the biparietal diameter ($r = 0.98$), log of the occipitofrontal diameter ($r = 0.98$), log of the head circumference ($r = 0.99$), log of the abdominal circumference ($r = 0.98$), square root of the femur length ($r = 0.99$), log of the estimated fetal weight ($r = 0.99$), biparietal/occipitofrontal ratio ($r = -0.11$), head/abdomen circumference ratio ($r = -0.56$), and log of the femur length/abdominal circumference ratio ($r = 0.61$). Values corresponding to the 10^{th}, 50^{th}, and 90^{th} percentiles for estimated fetal weight at 28, 32, and 36 weeks, respectively, were as follows: 937, 1,096, 1,284 g; 1,462, 1,720, 2,025 g; and 2,020, 2,399, 2,849 g.

CONCLUSION: In twin pregnancies, fetal ultrasound biometry measurements and growth parameters show a significant correlation with gestational age.

KEYWORDS: Twins; Pregnancy; Ultrasonography; Biometry; Reference Values.

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INTRODUCTION

The mean birth weight adjusted for gestational age in twin pregnancies is lower compared to singletons at the end of the second trimester (1). Nevertheless, neonatal mortality appears to be similar in both groups (2). Therefore, it is unclear whether smaller growth in twins should be interpreted as normal or pathological (3,4).

Fetal size and weight can be evaluated prenatally by ultrasound. However, most centers still use singleton reference charts in the assessment of twin pregnancies, which leads to frequent diagnosis of fetal growth restriction (5). Under these circumstances, subsequent management usually includes serial growth scans and fetal well-being tests. These measures will inevitably increase parental anxiety and financial costs. Moreover, false-positive results may eventually lead to mismanagement of the pregnancy and unnecessary iatrogenic deliveries.

The aim of this study was to establish longitudinal reference ranges for ultrasound fetal biometry and growth parameters in uncomplicated, twin pregnancies from our study population.

MATERIALS AND METHODS

This was a prospective study conducted at the Twin's Clinic, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, Department of Obstetrics and Gynecology, between May 2007 and June 2010. The study protocol was approved by the hospital's ethics committee (418/04).

No potential conflict of interest was reported.

Participants

Women at less than 21 weeks of gestation and with an uncomplicated, naturally conceived, diamniotic, twin pregnancy were included in the study. Those who abandoned follow-up, pregnancies with unknown outcomes or pregnancies that were complicated by oligohydramnios, increased umbilical artery pulsatility index (above the 95th percentile), pre-eclampsia, gestational diabetes, placenta previa, twin-to-twin transfusion syndrome, fetal structural malformation, chromosomal abnormalities, or fetal death were excluded.

Pregnancy outcome information was obtained from hospital notes and delivery records or by direct phone contact with the patients. Chorionicity was confirmed by histological examination of the placenta after delivery.

Gestational age was calculated from the first day of the last menstrual period (LMP) and confirmed either by an ultrasound crown-rump length measurement during the first trimester or by an estimate based on multiple ultrasound parameters (biparietal diameter [BPD], head circumference [HC], abdominal circumference [AC] and femur length [FL]) of the larger fetus during the second trimester. When the first day of the LMP was uncertain or unknown, or when there was a discrepancy between gestational age based on the LMP and ultrasound dates, gestational age was determined based on the earliest ultrasound findings.

Ultrasonography protocol

At the first evaluation, each twin was defined as ''1'' or ''2'' according to the relative position of its amniotic sac and the uterine internal cervical os. This definition was used throughout all subsequent examinations, which were performed every three weeks. Scans were carried out transabdominally with a 3.5-MHz curvilinear transducer and a Corevision SSA-350A (Toshiba, Japan), Envisor (Philips, Netherlands), or Voluson (General Electric, Austria) ultrasound machine.

At each visit, BPD, occipitofrontal diameter (OFD), HC, AC, and FL measurements were obtained for each fetus according to standard techniques (Figure 1) (6). Fetal weight was estimated according to the mathematical formula proposed by Hadlock et al. (7), and BPD/OFD, HC/AC, and FL/AC ratios were calculated for each fetus.

Statistical analysis

All data were prospectively recorded in a computer fetal database system and exported to a Microsoft Excel spreadsheet. Statistical analysis was performed with MLwiN version 2.19 (Centre for Multilevel Modelling, University of Bristol, United Kingdom).

The data were tested for normal distributions using the Kolmogorov-Smirnov test, and variables that were not normally distributed underwent transformation. Multilevel regression analysis was performed to examine the associations between each parameter and gestational age and to construct reference curves for the full gestation period. In the multilevel analysis, the first level was the variance between measurements obtained from the same fetus, the second was the variance between fetuses within the same pregnancy, and the third was the variance between different pregnancies. Values corresponding to the 5th, 10th, 50th, 90th, and 95th percentiles at each gestational week were determined for each fetal growth parameter.

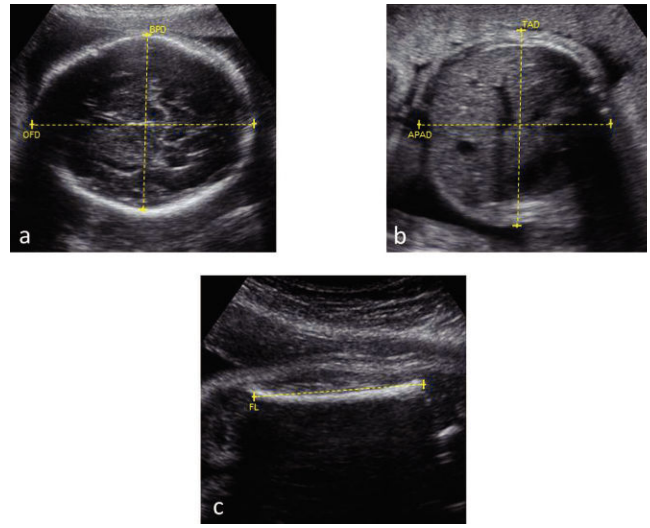

Figure 1 - Ultrasound images demonstrating the transverse section of the fetal head (A), abdomen (B) and femur length (C). Markers are placed depicting measurements of the biparietal (BPD), occipitofrontal (OFD), antero-posterior (APAD) and transverse abdominal (TAD) diameters and femur diaphysis length (FL).

RESULTS

Participants and pregnancy outcomes

A total of 200 women with twin pregnancies were recruited for the study, and 75 (37.5%) were excluded for the following reasons: three abandoned follow-up, seven had an unknown pregnancy outcome, and 65 developed clinical or obstetrical complications. The final study group included 125 women with normal pregnancy outcomes.

The mean gestational age at delivery was 35.5 $\pm$ 2.9 weeks, and the mean birth weight was 2,266 $\pm$ 546 g. Regarding chorionicity, 103 pregnancies (82.4%) were dichorionic, 16 (12.8%) were monochorionic diamniotic and chorionicity was not determined in six (4.8%) cases.

Ultrasound measurements

A total of 807 ultrasound examinations were performed between 14 and 38 weeks of gestation (6.5 $\pm$ 1.4 scans/pregnancy). Gestational age at the first scan was 17.9 $\pm$ 2.0 weeks, and the mean interval between ultrasound examinations was 3.0 $\pm$ 0.6 weeks. All examinations were performed by a group of eight experienced physicians, and measurements of both fetuses were successfully obtained in all examinations.

To normalize the distribution, the square roots of femur length measurements were calculated, and the biparietal and occipitofrontal diameters, head and abdominal circumferences, estimated fetal weight, and FL/AC ratio were log-transformed.

All of the ultrasound parameters showed significant correlations with gestational age. Table 1 presents polynomial regression equations for each parameter according to gestational age. Gestational age-specific reference values for the 5th, 10th, 50th, 90th, and 95th percentiles are presented in Tables 2-6.

DISCUSSION

In this study, longitudinal reference ranges for traditional ultrasound fetal growth parameters in uncomplicated, twin pregnancies were established from our study population.

Table 1 - Polynomial regression formulas for ultrasound fetal biometry parameters according to gestational age in 125 uncomplicated, twin pregnancies.

Parameter	Equation	SD	r
log BPD	$0.3910099 + 0.1048263 \times GA - 0.0024624 \times GA^2 + 0.0000206 \times GA^3$	0.0220379	0.98
log OFD	$0.5214858 + 0.1011816 \times GA - 0.0022833 \times GA^2 + 0.000018 \times GA^3$	0.0219996	0.98
log HC	$0.95837 + 0.1028437 \times GA - 0.002362 \times GA^2 + 0.0000193 \times GA^3$	0.0194823	0.99
log AC	$0.7591763 + 0.1168791 \times GA - 0.0028582 \times GA^2 + 0.000026 \times GA^3$	0.0269806	0.98
$FL^{0.5}$	$-4.4415145 + 0.8054619 \times GA - 0.0184606 \times GA^2 + 0.0001606 \times GA^3$	0.1906277	0.99
log EFW	$0.234723 + 0.1450527 \times GA - 0.0016023 \times GA^2$	0.0550537	0.99
BPD/OFD	$79.0423431 - 0.0569206 \times GA$	3.7258641	-0.11
HC/AC	$1.2913035 - 0.0068408 \times GA$	0.0621849	-0.56
log FL/AC	$0.5179342 + 0.085255 \times GA - 0.0028784 \times GA^2 + 0.0000318 \times GA^3$	0.0290451	0.61

AC: abdominal circumference; BPD: biparietal diameter; EFW: estimated fetal weight; FL: femur length; HC: head circumference; OFD: occipitofrontal diameter; SD: standard deviation.
$p<0.001$ for all equations.

Although twin ultrasound reference ranges have been published based on large sets of cross-sectional data (8), studies based on longitudinal data are more appropriate to evaluate fetal growth (9-11). From a strictly statistical perspective, ranges derived from cross-sectional data should be considered to indicate size curves, which are suitable for single observations, rather than growth curves.

In a longitudinal study, serial measurements are obtained from the same fetus at different gestational ages. However, previous longitudinal studies have applied simple polynomial regression analysis. On the other hand, we based our statistical analysis on multilevel modeling (12), which takes into account variance in measurements obtained from the same fetus at different occasions, variance related to fetuses within the same pregnancy, and variance related to different pregnancies.

Moreover, due to sample size limitations, the results presented in some previous studies are limited to mean values (10,11) or clinically inadequate percentiles (13). The present study defines values for different percentiles throughout the second and third trimesters of pregnancy, which will be useful in the clinical setting. In most clinical situations, values at the 5^{th} - 10^{th} and 90^{th} - 95^{th} percentiles are used to define the limits of normality.

The present data were collected prospectively and evaluated by only a small number of experienced physicians. These study aspects guaranteed strict adherence to a single, standardized fetal biometry technique and are

Table 2 - Longitudinal reference ranges for biparietal diameter based on 807 ultrasound examinations performed on 250 fetuses from 125 uncomplicated, twin pregnancies.

Gestational age (weeks)	Biparietal diameter (mm)				
	P^5	P^{10}	P^{50}	P^{90}	P^{95}
14	24.5	25.1	27.1	29.2	29.9
15	27.4	28.0	30.1	32.4	33.1
16	30.3	31.0	33.3	35.8	36.5
17	33.4	34.0	36.5	39.2	40.0
18	36.4	37.2	39.8	42.7	43.5
19	39.6	40.3	43.2	46.2	47.1
20	42.7	43.5	46.5	49.7	50.6
21	45.8	46.7	49.8	53.1	54.1
22	48.9	49.8	53.1	56.6	57.6
23	51.9	52.8	56.2	59.9	61.0
24	54.8	55.8	59.3	63.1	64.2
25	57.6	58.6	62.3	66.3	67.4
26	60.3	61.3	65.2	69.2	70.5
27	62.8	63.9	67.9	72.1	73.4
28	65.2	66.3	70.4	74.8	76.1
29	67.4	68.6	72.9	77.4	78.7
30	69.5	70.7	75.1	79.8	81.2
31	71.4	72.7	77.3	82.1	83.5
32	73.2	74.5	79.2	84.3	85.8
33	74.9	76.2	81.1	86.3	87.8
34	76.4	77.8	82.8	88.2	89.8
35	77.8	79.2	84.5	90.1	91.7
36	79.1	80.6	86.0	91.8	93.5
37	80.3	81.9	87.5	93.5	95.3
38	81.5	83.1	88.9	95.2	97.0

P: percentile.

Table 3 - Longitudinal reference ranges for head circumference based on 807 ultrasound examinations performed on 250 fetuses from 125 uncomplicated, twin pregnancies.

Gestational age (weeks)	Head circumference (mm)				
	P^5	P^{10}	P^{50}	P^{90}	P^{95}
14	88.9	90.7	97.3	104.4	106.5
15	99.2	101.2	108.3	116.0	118.2
16	109.9	112.0	119.7	128.0	130.4
17	121.0	123.2	131.5	140.3	142.9
18	132.3	134.7	143.5	152.9	155.6
19	143.8	146.3	155.6	165.5	168.5
20	155.3	158.0	167.8	178.3	181.3
21	166.8	169.7	180.0	190.9	194.1
22	178.3	181.2	192.0	203.4	206.7
23	189.5	192.5	203.8	215.6	219.1
24	200.4	203.6	215.2	227.6	231.2
25	211.0	214.3	226.3	239.1	242.8
26	221.1	224.6	237.0	250.2	254.1
27	230.8	234.4	247.2	260.8	264.8
28	240.0	243.7	257.0	271.0	275.1
29	248.7	252.5	266.1	280.5	284.8
30	256.8	260.7	274.8	289.6	294.0
31	264.4	268.4	282.9	298.2	302.6
32	271.5	275.6	290.5	306.2	310.8
33	278.0	282.2	297.6	313.8	318.5
34	284.0	288.4	304.2	320.9	325.8
35	289.6	294.1	310.4	327.6	332.7
36	294.8	299.4	316.3	334.1	339.3
37	299.7	304.4	321.8	340.2	345.6
38	304.2	309.2	327.1	346.2	351.8

P: percentile.

Table 4 - Longitudinal reference ranges for abdominal circumference based on 807 ultrasound examinations performed on 250 fetuses from 125 uncomplicated, twin pregnancies.

Gestational age (weeks)	Abdominal circumference (mm)				
	P^{5}	P^{10}	P^{50}	P^{90}	P^{95}
14	72.0	73.8	80.7	88.1	90.4
15	81.0	83.0	90.6	98.8	101.3
16	90.4	92.6	100.9	110.0	112.7
17	100.1	102.6	111.6	121.5	124.5
18	110.2	112.8	122.6	133.3	136.5
19	120.4	123.2	133.8	145.3	148.7
20	130.7	133.7	145.0	157.3	161.0
21	141.0	144.2	156.3	169.4	173.3
22	151.2	154.7	167.5	181.4	185.5
23	161.4	165.0	178.5	193.2	197.6
24	171.3	175.2	189.4	204.8	209.4
25	181.0	185.1	200.0	216.1	220.9
26	190.5	194.7	210.3	227.2	232.2
27	199.7	204.0	220.3	237.9	243.1
28	208.5	213.1	230.0	248.3	253.7
29	217.0	221.8	239.4	258.3	264.0
30	225.3	230.2	248.4	268.1	273.9
31	233.2	238.3	257.2	277.6	283.6
32	240.9	246.2	265.8	286.8	293.1
33	248.5	253.9	274.1	296.0	302.5
34	255.9	261.5	282.4	305.0	311.7
35	263.2	269.0	290.7	314.0	321.0
36	270.5	276.6	299.0	323.2	330.4
37	278.0	284.3	307.5	332.6	340.1
38	285.7	292.1	316.2	342.3	350.1

P: percentile.

Table 5 - Longitudinal reference ranges for femur diaphysis length based on 807 ultrasound examinations performed on 250 fetuses from 125 uncomplicated, twin pregnancies.

Gestational age (weeks)	Femur length (mm)				
	P^{5}	P^{10}	P^{50}	P^{90}	P^{95}
14	11.0	11.5	13.4	15.4	16.0
15	13.6	14.2	16.2	18.4	19.1
16	16.4	17.0	19.2	21.5	22.2
17	19.2	19.8	22.1	24.6	25.3
18	22.0	22.7	25.1	27.7	28.4
19	24.8	25.5	28.1	30.8	31.5
20	27.6	28.4	31.0	33.8	34.6
21	30.4	31.1	33.9	36.7	37.5
22	33.1	33.8	36.6	39.6	40.4
23	35.6	36.4	39.3	42.4	43.2
24	38.1	39.0	41.9	45.0	45.9
25	40.6	41.4	44.4	47.6	48.5
26	42.9	43.7	46.8	50.1	51.0
27	45.0	45.9	49.1	52.4	53.4
28	47.1	48.0	51.3	54.7	55.7
29	49.1	50.1	53.4	56.9	57.9
30	51.0	52.0	55.4	59.0	60.0
31	52.8	53.8	57.3	61.0	62.0
32	54.6	55.6	59.2	62.9	64.0
33	56.2	57.2	60.9	64.8	65.9
34	57.8	58.9	62.7	66.6	67.7
35	59.3	60.4	64.3	68.4	69.6
36	60.8	62.0	66.0	70.1	71.3
37	62.3	63.5	67.6	71.9	73.1
38	63.8	65.0	69.2	73.7	74.9

P: percentile.

reflected in the success rate of obtaining adequate ultrasound images from both fetuses in all scans.

Accurate gestational age determination is crucial to correctly evaluate and classify fetal growth. In this regard, some previous studies did not report how gestational age was determined (10,11,14), and others were based solely on clinical parameters such as LMP and/or neonatal clinical examination (9). In the present study, gestational age was confirmed by ultrasound, and antenatal follow-up of all participants was conducted at our Twin's clinic according to previously established protocols. Only data from uncomplicated pregnancies were included in the statistical analysis.

Chorionicity was not a selection criterion for the present study. In fact, in a monochorionic pregnancy, a single placenta is shared by both fetuses, and fetal growth in such pregnancies may be slightly different from that of dichorionic twins. However, only approximately 12% of our cases were monochorionic twins.

Twin pregnancies resulting from assisted reproduction techniques are predominant in several clinical settings. However, due to the social and economic characteristics of our population, the reference ranges presented here are applicable to naturally conceived twins only.

A large dataset from the United States has shown that the mean birth weight in twin pregnancies is lower than that of singletons. Deviation begins at approximately 28 weeks and increases progressively throughout the pregnancy. For instance, at 38 weeks of gestation, the 50^{th} percentile for twins is equivalent to a singleton's 10^{th} percentile (1). Despite lower mean birth weights, perinatal outcomes in twin pregnancies are comparable to those in pregnancies with

Table 6 - Longitudinal reference ranges for estimated fetal weight based on 807 ultrasound examinations performed on 250 fetuses from 125 uncomplicated, twin pregnancies.

Gestational age (weeks)	Estimated fetal weight (g)				
	P^{5}	P^{10}	P^{50}	P^{90}	P^{95}
14	72	76	89	106	111
15	91	95	112	133	139
16	113	119	140	165	173
17	140	147	173	203	213
18	173	181	212	249	261
19	211	220	258	303	317
20	255	267	313	366	383
21	307	321	375	439	459
22	366	382	447	523	547
23	433	452	529	618	646
24	508	531	621	726	758
25	593	619	724	846	884
26	685	716	837	979	1023
27	787	822	962	1125	1176
28	896	937	1096	1284	1342
29	1012	1059	1241	1454	1521
30	1135	1188	1394	1635	1711
31	1264	1323	1554	1826	1912
32	1396	1462	1720	2025	2121
33	1530	1603	1890	2229	2336
34	1664	1745	2062	2437	2555
35	1796	1884	2232	2645	2775
36	1924	2020	2399	2849	2992
37	2045	2149	2559	3048	3203
38	2157	2269	2710	3237	3405

P: percentile.

singletons born at the same gestational age (2). Furthermore, some studies have shown a survival advantage despite lower birth weights in multiple pregnancies (15,16).

In multiple pregnancies, the uterine fundal height measurement does not allow an adequate evaluation of growth for each fetus. Therefore, fetal growth assessment is essentially based on serial ultrasound scans. However, most centers still use singleton reference ranges in the assessment of twins' fetal growth. This will inevitably result in frequent misdiagnosis of fetal growth restriction (5) and consequent misclassification of risk. Moreover, under these circumstances, repeat follow-up scans to monitor fetal growth and well-being surveillance are usually scheduled and may potentially lead to parental anxiety and increased risk of mismanagement due to additional false-positive results.

It is therefore plausible that the smaller fetal size characteristic of twin pregnancies constitutes a physiological phenomena; the use of appropriate reference charts for normal fetal growth in twin pregnancies should be advocated to help reduce false-positive diagnoses and unnecessary interventions.

AUTHOR CONTRIBUTIONS

Liao AW was responsible for the data collection, statistical analysis and the draft of manuscript. Brizot ML was responsible for the data collection and the draft of manuscript. Kang HJ and Assunção RA were responsible for the data collection. Zugaib M was responsible for the manuscript revision and discussion

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