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Development of the Z-score for the measurement of myocardial thickness by two-dimensional echocardiography in normal fetuses

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Abstract

Background: In the current literature, there is a tendency to describe normal values of echocardiographic measurements by means of the Z-score. In fetal cardiology, these Z-score equations are still being established. Measurement of myocardial thickness is an important assessment, especially in fetuses of diabetic mothers, because of the risk of developing myocardial hypertrophy secondary to elevated maternal blood glucose levels.

Objective: To determine the percentiles and to develop the Z-score equations of right and left ventricular lateral walls and interventricular septum measurements using two-dimensional echocardiography in normal fetuses between 24 and 34 weeks of gestation.

Methods: This is a prospective cross-sectional study that was performed in single fetuses with normal heart from nondiabetic pregnant women. Measurements of the lateral walls of the right and left ventricles and the interventricular septum were made.

Results: Eight hundred and seventy three pregnant women were included. We determined the percentiles of the measurements for each gestational age. The Z-score equation was developed for each of the measurements: right ventricular lateral wall measurement [$RVLW = x-(-1 + 0.109 * GA)/0.4$], left ventricle lateral wall measurement [$LVLW = x-(-1.366 + 0.12 * GA)/0.43$], and interventricular septum, both at the four-chamber view [$IVS_{4ch} = (x-(-1.113 + 0.107 * GA)/0.4$] and at the left ventricular outflow tract plane [$IVS_{LVOT} = (x-(-0.581 + 0.084 * GA)/0.35$].

Conclusion: The present study allowed the demonstration of the percentiles and the Z-score equations for each of the measurements studied.

KEYWORDS

echocardiography, fetal echocardiography

1 | INTRODUCTION

Structural measurements of the fetal heart are important for monitoring cardiac development in relation to fetal growth. Some congenital heart defects may be suspected early when nonstandard measurements for a given gestational age of the fetus are detected.[1] The literature shows a tendency to describe reference curves of echocardiographic measurements by means of Z-scores. In fetal cardiology, these Z-score equations are still being established.[2–13]

Measurement of myocardial thickness is important in fetuses of diabetic pregnant women, since there is a risk for developing myocardial hypertrophy. Several studies suggest that myocardial hypertrophy due to diabetes mellitus (DM) is caused by elevated serum insulin levels, which consequent increase in protein and fat synthesis, causing greater proliferation and hypertrophy of cardiac myocytes.[14–16] Myocardial hypertrophy may cause changes in fetal cardiac function: impairment of diastolic function, with alteration in myocardial relaxation/compliance and, at a later stage, of systolic function.

Some reference curves of fetal myocardial thickness in the fetus have already been published[15–18]; however, to our knowledge, no Z-score equations related to right and left ventricular lateral walls and interventricular septum thickness have been described.

This study was aimed to determine the percentiles and to develop fetal Z-score equations for right and left ventricular lateral walls and for the interventricular septum both at four-chamber and outflow tract views.

2 | METHODS

This is a prospective cross-sectional study conducted in normal pregnant women. They were consecutively recruited from spontaneous demand in the city of Manaus, Amazonas, from January 2017 to August 2018. This study was approved by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP PhD Program).

Inclusion criteria were as follows: single pregnancy, live fetus; gestational age defined according to the last menstrual period date (LMD) and/or confirmed by ultrasound performed until the 13th week; gestational age between 24 and 33 weeks and 6 days of gestation; absence of fetal malformations on ultrasound examination; and exclusion of anatomical or functional cardiac alterations by fetal echocardiography. Exclusion criteria were as follows: fetuses with cardiac conduction disorders—arrhythmias; maternal conditions that prevented adequate fetal cardiac imaging such as the presence of abdominal scars and obesity (body mass index >30 kg/m^2); chronic maternal disease such as high blood pressure, diabetes mellitus, nephropathy, or collagen disease; smoking or illicit drugs consumption; fetal hydrops; intrauterine growth restriction; oligohydramnios; and/or polyhydramnios (amniotic fluid index—ILA below the 5th percentile or above the 95th percentile, respectively, according to Moore and Cayle).[19]

Ultrasound studies were performed using a Vivid 7 (General Electric) equipment with convex (3.5C) and sector (3S and 7S) transducers. All fetal cardiac examinations were performed by the first author of this study (LARA).

All pregnant women who were referred to the service and signed an informed consent to participate in the study underwent a complete Doppler echocardiogram, with anatomical and functional analysis. After confirmation of fetal heart normality, clinical and echocardiographic data according to the protocol of this study were collected.

Clinical data included name, age, race, educational level, weight, height, gestational history, gestational age, and indication for fetal echocardiography. Complete fetal echocardiography was performed, with sequential segmental analysis. Structural measurements of the study variables were recorded. The acquired images were stored for further detailed analysis if necessary.

The following fetal cardiac structures were measured: right ventricular lateral wall, left ventricular lateral wall, and interventricular septum thickness, both at the four-chamber view, in a horizontal position, and with exposure of the left ventricular outflow tract, in a superior and anterior region of the septum, at the level of the mitral valve papillary muscle or the tip of the mitral valve leaflets, at end diastole (Figure 1).

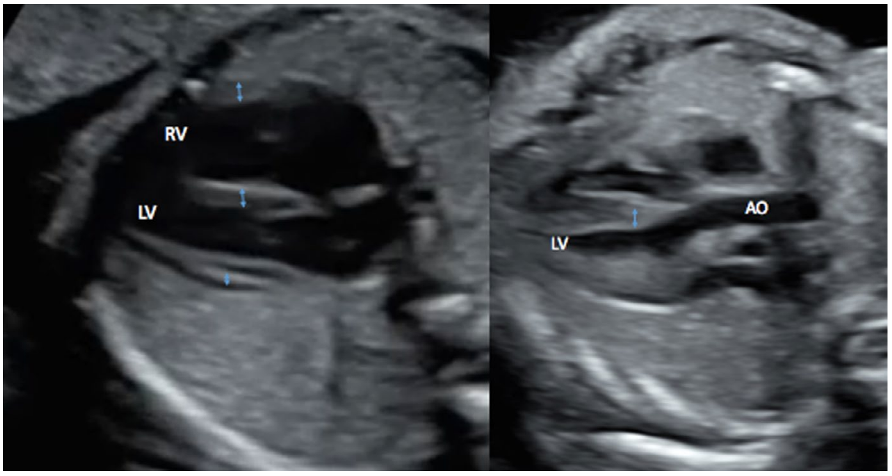

FIGURE 1 Axial section of the fetal chest showing the longitudinal plane of the heart. On the left, we observe a four-chamber plane with measurements of the right and left ventricular lateral walls and the middle portion of the interventricular septum inlet. On the right, measurement of the outflow tract portion of the interventricular septum. RV = right ventricle; LV = left ventricle; AO = aorta

2.1 | Statistical analysis

All data were collected and transferred to an Excel 2007 spreadsheet (Microsoft Corp.). Statistical analysis was performed using the MacBook STATA/IC 12.1 program (Apple Inc).

Initially, an exploratory analysis of echocardiographic data was performed using scatter plots and summary measurement tables. Due to the large number of data, this analysis was conducted to evaluate possible discrepancies in the database. Discrepant data found were reevaluated and corrected. Some echocardiographic variables showed missing measurements. As our sample presented a satisfactory number, we did not consider data imputation.

A descriptive analysis of the studied population was performed, reporting the absolute and relative numbers for the qualitative variables and the means with their variability (standard deviation and/or amplitude) in the quantitative variables. Using echocardiographic data, the 10th, 50th, and 90th percentiles were determined for each gestational age for whole weeks, as proposed by Altman and Chitty.[20] The 10th, 50th, and 90th percentile curves were constructed for each variable as a function of gestational age in weeks.

To assess normalization of the data, the Shapiro–Wilk test was performed and the histogram evaluation in all variables studied. Linear regression was used to evaluate the relationship between the measures studied and gestational age. The Z-score equations were generated respecting the coefficient of determination ($R^2 \neq 0$). If variables were independent of gestational age ($R^2 = 0$), the Z-score equations would be developed by the mean and standard deviation of the original measurements or the residuals of the normalized measurements. In the case of gestational age-dependent variables ($R^2 \neq 0$), the Z-score equations would be generated from the relationship between the original measurements or the normalized value residuals and the linear regression of the data obtained, divided by the respective standard deviation.

Thus, Z-score formulas were developed for measurements of the right ventricular lateral wall, left ventricular lateral wall, four-chamber interventricular septum, and left ventricular outflow tract.

3 | RESULTS

The recruitment profile performed in this study is recorded in Figure 2.

One thousand and seventy-four fetal echocardiograms were performed during the data collection period. Fifty-eight cases were excluded after the identification of some cardiac alteration in the fetus, and 203 pregnant women were also removed from the analysis because of their high BMI, among which, coincidentally, some were diabetic. A total of 873 pregnant women were included in the final analysis.

The mean age of the pregnant women was 29.2 ± 6 years, and 19.7% were ≥35 years old. The mean body mass index was 26.9 kg/m^2. The most evident level of education was completed college and high school (44.8% and 39.4%, respectively). The mean gestational age at fetal echocardiography was 29 weeks. The number of cases by gestational age is provided in Figure 3. Approximately half of the mothers were brown (53.3%). Almost 85% of pregnant women were routinely screened, without risk factors for fetal cardiac disease, 10% for maternal risk and less than 1% for fetal or family risk. As for parity, 48.5% were nulliparous and 51.5% multiparous (Table 1).

The 10th, 50th, and 90th percentile curves of the right ventricular lateral wall, left ventricular wall, and interventricular septum measurements at the four-chamber view and at the left ventricular outflow tract are shown below (Figure 4).

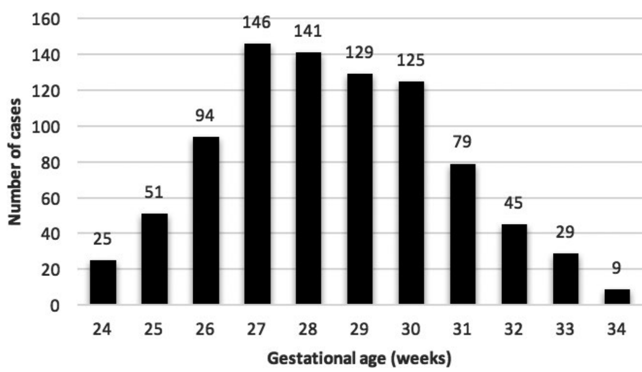

FIGURE 3 Histogram showing the number of cases by gestational age of the studied fetuses

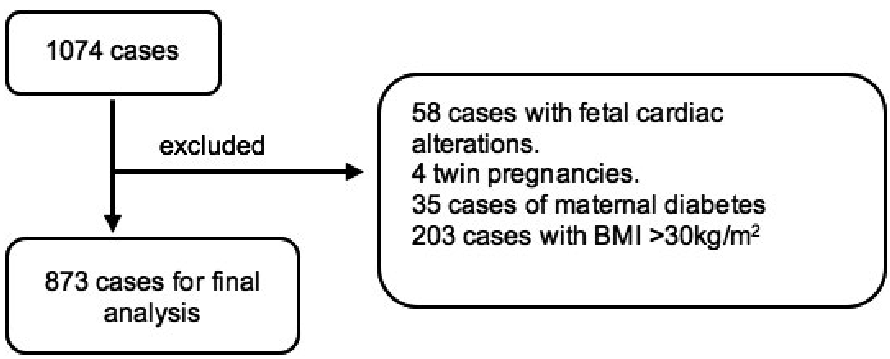

FIGURE 2 Flowchart of the study. BMI = body mass index

TABLE 1 Demographic data (n = 873)

Variables	
Maternal age in years, (mean, SD)	29.2 ± 6
Maternal age ≥ 35 years, n (%)	335 (19.7)
Fetal GA, in weeks (mean, amplitude.)	28.8 (24–34.8)
Body mass index, in kg/m^2 (mean, SD)	26.9 (2.9)
Race, n (%)	
Caucasian	384 (44)
Brown	464 (53.3)
Black	22 (2.5)
Level of instruction, n (%)	
Superior (complete)	389 (44.8)
Superior (incomplete)	121 (13.9)
Medium	342 (39.4)
Basic	16 (1.84)
Indication for fetal echocardiography, n (%)	
Routine screening	752 (86.4)
Maternal risk	92 (10.5)
Fetal risk	8 (0.9)
Familial risk	5 (0.57)
Gestational history (mean, amplitude)	
Gestation	1.8 (1–7)
Parity	0.6 (0–6)
Abortions	0.2 (0–3)

Abbreviations: GA = gestational age; n = absolute number; SD = standard deviation.

Development of equations for the calculation of the Z-score of measurements assessed in normal fetuses by two-dimensional echocardiography.

The basic Z-score equation for the development of the formulas of the studied variables was as follows:

$$Z(x) = \frac{(x) - E(x).}{SD\ (x).}$$

where (x) is the measure of the studied variable. E (x) is value generated through linear regression (considering that the variable depends on gestational age). SD (x) is the standard deviation.

The studied variables presented normal distribution, being unnecessary to perform any transformation function in the elaboration of the Z-score equation, thus leaving the formula with a better applicability. We also observed that the cardiac measurements performed were dependent on the gestational age of the fetus, thus with a nonzero coefficient of determination (R^2 ranging from .27 to .37; Table 2).

As the variables studied were dependent on gestational age ($R_2 \neq 0$), the Z-score equations were generated from the relationship between the original measurements and the linear regression of the data obtained, divided by the respective standard deviation (Table 3).

4 | DISCUSSION

In the present study, the curves for the 10th, 50th, and 90th percentiles were determined for four myocardial measurements: right ventricular lateral wall, left ventricular lateral wall, four-chamber interventricular septum, and left ventricular outflow tract interventricular septum. In addition, equations were developed for the Z-score calculation for all these variables.

With the determination of these percentiles, during the gestational period assessed (between 24 weeks and 33 weeks and 6 days), it was confirmed that these measures changed during pregnancy. This modification is a subtle increase, probably due to the architectural development of fetal myocardial structures.[21] In special situations, such as in diabetic pregnancies, an exaggerated myocardial thickening secondary to maternal inadequate glycemic control may be observed.[14,15]

In two published studies, no statistically significant variation in the measurements of the interventricular septum of the fetus with advancing gestational age was shown.[16,17] However, these studies had a small sample size and the evaluated periods of gestational age were also restricted (28–37 weeks[16] and 32–35 weeks of gestation,[17] respectively). Another study, published in 1992, performed a longitudinal evaluation in fetuses 18 weeks to term, showed a growing curve according to gestational age, being the first developed reference curve and the most used up to now.[18]

Fetal myocardial reference curves are important to assist in early detection and follow-up of myocardial hypertrophy.[16–18] The development of a Z-score equation for fetal myocardial measurements is the main original contribution of our study. The adequate sample size allowed creation of a less complex equation, and as a consequence its applicability to routine daily clinical practice in fetal cardiology is predictable.

A value considered normal for the Z-score would be between + 2 and −2. For example, in a hypothetical 29-week fetus with an IVS measuring 3.5 mm, the Z-score would be + 3.3, according to the equation developed for this structure (Table 3). This value would indicate interventricular septum hypertrophy, with implications to follow-up, prenatal management, and birth planning.

Some limitations can be pointed out in this study. First, no echocardiograms were routinely performed in the neonatal period to confirm fetal examination findings. The gestational age range of this sample was limited to the period between 24 and 33 weeks and 6 days of gestation, in order to avoid possible inadequate images for reference curves. Another potential limitation of the study is that the curves demonstrate the most common values to be found and thus considered normal for the populational sample studied; thus, values found outside this curve should not be considered in isolation, being necessary a sequential evaluation of these measurements to ascertain the diagnosis of myocardial hypertrophy in a particular fetus.

In conclusion, the 10th, 50th, and 90th percentile curves and Z-score equations were developed for thickness of fetal right and left ventricular walls and interventricular septum, increasing reliability in

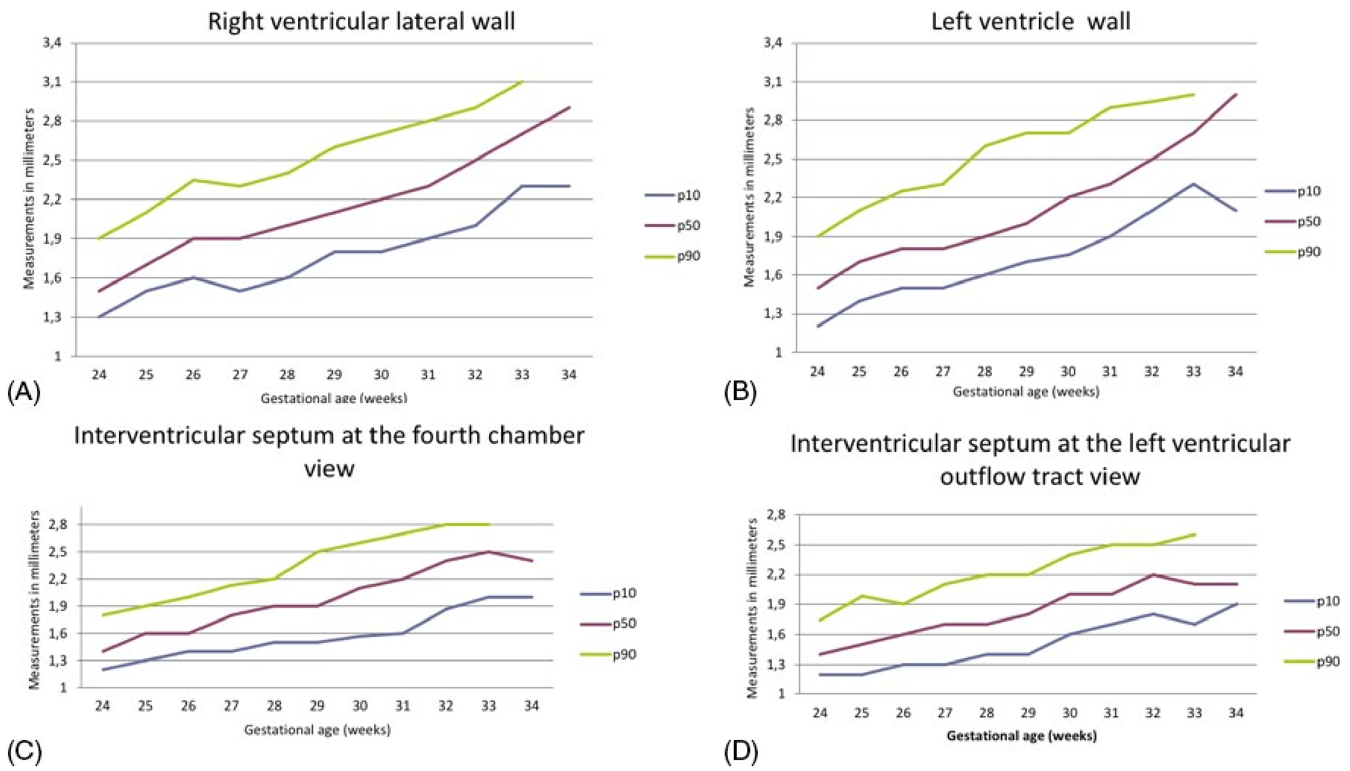

FIGURE 4 10Th, 50Th, and 90Th percentile curves for measurements in millimeters (y-axis) of the right ventricular lateral wall (A), left ventricle (B), and the interventricular septum at the fourth chamber view (C) and at the left ventricular outflow tract (D) view in normal fetuses between 24 and 33 wk and 6 d (x-axis)

TABLE 2 Linear regression parameters between the cardiac measurements studied and the gestational age of the fetus

Measures	ß	Intercept	*P*	R^2
RVLW	0.109	−1.001	<.001	.35
LVLW	0.120	−1.366	<.001	.37
IVS_4C	0.107	−1.113	<.001	.34
IVS_LVOT	0.084	−0.581	.002	.27

Abbreviations: 4C = four-chamber view; IVS = interventricular septum; LVLW = left ventricular lateral wall; LVOT = left ventricular outflow tract; R^2 = coefficient of determination; RVLW = right ventricular lateral wall; ß = multiplier.

TABLE 3 The Z-score equations for each cardiac measurement studied

Measures	n	Z-score equations
RVLW	873	(x−[−1.001 + 0.109.GA])/0.4
LVLW	873	(x−[−1.366 + 0.120.GA])/0.43
IVS_4C	873	(x−[−1.113 + 0.107.GA])/0.4
IVS_VSVE	873	(x−[−0.581 + 0.084.GA])/0.35

Note: (x) is the measure of the variable studied.

Abbreviations: 4C = four-chamber view; IVS = interventricular septum; LVLW = left ventricular lateral wall; LVOT = left ventricular outflow tract; RVLW = right ventricular lateral wall.

the echocardiographic diagnosis and follow-up of fetal myocardial hypertrophy and borderline cases. This is especially important when the possibility of fetal cardiac dysfunction is among the possible outcomes, such as fetal cardiac hypertrophy in maternal diabetes, intrauterine growth restriction, fetal anemia, agenesis of ductus venosus, and many others.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Allan L, Hornberger L, Sharland G. *Textbook of fetal cardiology*. New York, NY: Greenwich Medical Media; 2000:3–13.
2. Krishnan A, Pike JI, McCarter R, et al. Predictive models for normal fetal cardiac structures. *J Am Soc Echocardiogr*. 2016;29(12):1197–1206. https://doi.org/10.1016/j.echo.2016.08.019
3. Schneider CSC, McCrindle BW, Carvalho JS, Hornberger LK, McCArthy KP, Daubeney PEF. Development of Z-scores for fetal cardiac dimensions from echocardiography. *Ultrasound Obstet Gynecol*. 2005;26:599–605.
4. Pasquini L, Mellander M, Seale A, et al. Z - scores of the fetal aortic isthmus and duct: na aid to assessing arch hypoplasia. *Ultrasound Obstet Gynecol*. 2007;29(6):628–633.

5. Vigneswaran TV, Akolekar R, Syngelaki A, et al. Reference ranges for the size of the fetal cardiac outflow tracts from 13 to 36 weeks gestation: a single-center study of over 7000 cases. *Circ Cardiovasc Imaging*. 2018;11(7):e007575.
6. Gabbay-Benziv R, Turan OM, Harman C, Turan S. Nomograms for fetal cardiac ventricular width and right-to-left ventricular ratio. *J Ultrasound Med*. 2015;34(11):2049–2055.
7. Lee W, Riggs T, Amula V, et al. Fetal echocardiography: z-score reference ranges for a large patient population. *Ultrasound Obstet Gynecol*. 2010;35(1):28–34.
8. McElhinney DB, Marshall AC, Wilkins-Haug LE, et al. Predictors of technical success and postnatal biventricular outcome after in utero aortic valvuloplasty for aortic stenosis with evolving hypoplastic left heart syndrome. *Circulation*. 2009;120(15):1482–1490.
9. Comas M, Crispi F, Gomez O, Puerto B, Figueras F, Gratacos E. Gestational age- and estimated fetal weight-adjusted reference ranges for myocardial tissue Doppler indices at 24–41 weeks' gestation. *Ultrasound Obstet Gynecol*. 2011;37(1):57–64.
10. Gabbay-Benziv R, Turan OM, Harman C, et al. Nomograms for fetal cardiac ventricular width and right-to-left ventricular ratio. *J Ultrasound Med*. 2015;34(11):2049–2055
11. Rocha LA, Rolo LC, Nardozza LMM, Tonni G, Araujo JE. Z-Score reference ranges for fetal heart functional measurements in a large Brazilian pregnant women sample. *Pediatr Cardiol*. 2019;40(3):554–562. https://doi.org/10.1007/s00246-018-2026-1
12. Gagnon C, Bigras JL, Fouron JC, Dallaire F. Reference values and Z scores for pulsed-wave Doppler and M-mode measurements in fetal echocardiography. *J Am Soc Echocardiogr*. 2016;29(5):448–460.e9. https://doi.org/10.1016/j.echo.2016.01.002
13. Mao YK, Zhao BW, Zhou L, et al. Z-score reference ranges for pulsed-wave Doppler indices of the cardiac outflow tracts in normal fetuses. *Int J Cardiovasc Imaging*. 2019;35(5):811–825.
14. Hagemann LL, Zielinsky P. Prenatal study of hypertrophic cardiomyopathy and its association with insulin levels in fetuses of diabetic mothers. *Arq Bras Cardiol*. 1996;66:193–198.
15. Buchanan T, Kitzmiller J. Metabolic interactions of diabetes and pregnancy. *Annu Rev Med*. 1994;45:245–260.
16. Veille JC, Hanson R, Sivakoff M, et al. Fetal cardiac size in normal, intrauterine growth retarded, and diabetic pregnancies. *Am J Perinato*. 1993;10:275–279. Kafle P, Ansari MA, Khanal U. Fetal Cardiac Interventricular Septal Thickness at 28–37 weeks of gestation in Nepalese Population. NJR.2012;2(2):36–42.
17. Patchakapat L, Uerpairojkit B, Wacharaprechanont T, et al. Interventricular septal thickness of Thai Fetuses: at 32 to 35 weeks` gestation. *J. Med. Assoc Thai*. 2006;89(6):748–754.
18. Tan J, Silverman NH, Hoffman JIE, et al. Cardiac dimensions determined by cross-sectional echocardiography in the normal human fetus from 18 weeks to term. *Am. J Cardiol*. 1992;70(18):1459–1467.
19. Moore TR, Cayle JE. The amniotic fluid index in normal human pregnancy. *Am J Obstet Gynecol*. 1990;162:1168–1173.
20. Altman DG, Chitty LS. Charts of fetal size: 1. Methodology. *Br J Obstet Gynecol*. 1994;101:29–34.
21. Rudolph AM. *Congenital diseases of the heart: clinical physiological considerations*, 3rd edn. Hoboken, NJ: Wiley-Blackwell; 2009.

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