

## Reference Curve of the Fetal Ventricular Septum Area by the STIC Method: Preliminary Study

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### Abstract

**Background:** Early detection of septal changes such as septal hypertrophy commonly present in fetuses of diabetic mothers would help reduce the high rates of infant mortality.

**Objective:** Determine reference ranges for the fetal ventricular septal area through three-dimensional ultrasound (US3D) using the STIC method (Spatio-Temporal Image Correlation).

**Methods:** We conducted a cross-sectional study with 69 pregnant women between the 18<sup>th</sup> and 33<sup>rd</sup> weeks of pregnancy. We used as a reference the four-chamber plane with the ROI (Region of Interest) positioned from the ventricles; the septum area were manually marked. To assess the correlation of the interventricular septum area with gestational age (GA), we constructed scatter plots and calculated Pearson's correlation coefficient ( $r$ ), and the adjustment was performed by the coefficient of determination ( $R^2$ ). We calculated averages, medians, standard deviations (sd), as well as maximum and minimum values. To calculate the intraobserver reproducibility, we used the intraclass correlation coefficient (ICC). The interventricular septum thickness was measured and it was correlated with gestational age and the septal area rendering in 52 patients using the ICC.

**Results:** The interventricular septum area was highly correlated with gestational age ( $r = 0.81$ ), and the average increased from 0.47 cm<sup>2</sup> in the 18<sup>th</sup> week to 2.42 cm<sup>2</sup> in the 33<sup>rd</sup> of gestation. The intraobserver reproducibility was excellent with ICC = 0.994. No significant correlation was observed between the interventricular septum measurement and the GA ( $R^2 = 0.200$ ), as well as there was no correlation with the septal area rendering with ICC = 0.150.

**Conclusion:** Reference intervals for the interventricular septum area between the 18<sup>th</sup> and the 33<sup>rd</sup> pregnancy week were determined to be highly reproducible. (Arq Bras Cardiol 2011;96(5):386-392)

**Keywords:** Pregnancy; fetal heart; ventricular septum; reference values; imaging, three-dimensional.

### Introduction

The progressive increase in oxygen demand due to the rapid growth of the embryo during the early stages of embryogenesis determines the early heart formation around the 22<sup>nd</sup> - 23<sup>th</sup> days of gestation<sup>1</sup>. Progressively, the primitive fetal heart undergoes a series of remodeling processes capable of modifying their anatomy to reach the functional form we know<sup>1</sup>.

When the heart has its four chambers (atria and ventricles), its ventricles present the divider interventricular septum (IV) formed from the merger of the medial walls: the muscular part of the IV septum. Within the ventricles, with their cavitation, the muscle bundles are formed and these originate the papillary muscles and the chordae tendineae, or heart strings<sup>1</sup>, with the function of valve competence, preventing

the prolapse of the cuspid valves during the cardiac systole and blood reflux<sup>2</sup>.

In 50% of the cases, congenital heart defects are considered major factors determining the high rate of early and late infant mortality<sup>3,4</sup>, highlighting the need for screening and providing early diagnosis of such heart diseases. Despite the wide application of conventional ultrasonography in screening for heart defects, antenatal detection still has some diagnosis gaps, which drives the development of new image acquisition techniques for a better cardiac screening during routine antenatal tests<sup>5-7</sup>.

In recent decades, with the development of three-dimensional ultrasound (US3D) and the Spatio-Temporal Image Correlation technology (STIC), a new form of prenatal cardiac examination has emerged. This technique allows obtaining cardiac volume and its storage for later reconstruction and analysis of anatomy, presenting the image in the multiplanar and surface mode (rendered), identifying the cardiac chambers, semilunar and atrioventricular valves, and the positioning of vessels and their correlations, and it is also possible to track the cardiac motion by using the cine loop technique<sup>7-9</sup>.

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Manuscript received July 29, 2010; revised manuscript received November 17, 2010, accepted on December 14, 2010.

Despite the number of projects developed with the STIC technique, the literature does not provide its application in assessing the interventricular septum area in hearts of normal fetuses and/or patients with heart disease. This would be a way to directly analyze septal defects, which are the most common congenital heart defects, accounting for about 30% of heart diseases, especially membrane defects<sup>1</sup>, also analyzing indirectly other cardiac malformations (such as valvular stenoses with consequent ventricular hypertrophy, among others). Moreover, the valve area measurement will allow the early detection of septal hypertrophy in fetuses of diabetic pregnant women, by tracking fetuses with greater likelihood of developing macrosomia and cardiomyopathy<sup>10,11</sup>.

This study aims to determine reference values for the interventricular septum area of normal fetuses between the 18<sup>th</sup> and the 33<sup>rd</sup> pregnancy week through the US3D by using the method STIC.

## Methods

We conducted a cross-sectional study from December 2009 to May 2010 with 69 pregnant women between the 18<sup>th</sup> and the 33<sup>rd</sup> pregnancy week. This study was approved by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP) under number 0135/10, and patients who agreed to participate voluntarily signed a consent form.

All patients were selected in the low-risk prenatal sector of the Obstetrics Department of Unifesp, these being derived from the Brazilian Public Health System of the city and metropolitan area of São Paulo. Inclusion criteria were: singleton pregnancies with live embryo and gestational age determined by the last menstrual period (LMP) confirmed by ultrasound test performed until the 10<sup>th</sup> week, using the crown-rump length (CRL) as a parameter. The exclusion criteria were: oligohydramnios [amniotic fluid index (AFI) below the 5<sup>th</sup> percentile for gestational age, according to the table proposed by Moore and Cayle<sup>12</sup>]; fetus in the dorsal anterior position (between 11 and 1am); estimated fetal weight below and/or above two standard deviations from the average, according to the table proposed by Hadlock; pregnant women with chronic illnesses that could affect fetal growth and development, such as hypertension, diabetes mellitus, collagen diseases, among others; intense sound beam attenuation by conditions such as maternal obesity and abdominal scars; fetal malformations diagnosed at morphology ultrasound of 1<sup>st</sup> and 2<sup>nd</sup> quarters; smokers and/or users of illegal drugs. All pregnant women underwent fetal echocardiography between the 20<sup>th</sup> and 24<sup>th</sup> pregnancy week.

All examinations were performed in the Sector of Fetal Cardiology of the Department of Obstetrics of Unifesp, these being performed by a single examiner (LCR) with three years' experience in US3D in obstetrics. All examinations were performed on a device of the brand Voluson 730 Expert (General Electric Medical Systems, Kretztechnik, Zipf, Austria) equipped with a volumetric transducer (RAB4-8P). The offline analyses to measure the interventricular septum areas were performed by the same examiner using the software 4D View version 9.0 (GE, Medical Systems Kretztechnik, GmbH & Co OHG).

Cardiac volumes were collected in the four heart chambers section with the fetal back, if possible, at the 6 o'clock position, using an opening angle between 20° and 40° and an acquisition time between 10 and 15 seconds. After the three-dimensional scanning, the image was displayed on the device screen as three orthogonal planes: axial (A), sagittal (B) and coronal (C). Before starting the examinations, we used the standard proposed by Paladini<sup>13</sup> in order to guide the volumes obtained by the STIC, that is, for fetuses in the cephalic position, the left side of the heart coincides with the left side of the screen, while for pelvic fetuses, the left side of the heart coincides with the right side of the screen, where plane A should be rotated 180° around the y-axis.

The plane of the four heart chambers (A) was selected as a reference, this being rotated around the z-axis so that the cardiac apex was laid off in 6 hours; then the RENDER key was turned off with the reference point of the box laid on the middle third of the interventricular septum. Three virtual planes for the evaluation of the interventricular septum were used. Two planes were determined by the green line position (active) of the rendering box parallel to the outer edge of the interventricular septum, with the midpoint maintained in its middle third. We used the option six of the ROI selection, delimiting the septal region; by convention, we chose to view it through the left ventricular side. The rendered image was magnified and then the MEASURE key was pushed and the TRACE AREA option was selected to manually mark off the septal area in cm<sup>2</sup>. The measurement of the septal area was performed during the initial phase of the ventricular diastole, when the septum was less contracted (Figure 1). The two-dimensional measure of the septal thickness was also obtained in four-chamber plane in mm (Figure 2).

The data were stored in an Excel spreadsheet (Microsoft, Redmond, WA, USA) and analyzed using the program Statistical Package for the Social Sciences (SPSS) for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). We calculated means, medians, standard deviations (SD), maximum and minimum values for each gestational interval evaluated, as well as the percentiles 5, 25, 50, 75 and 95. To assess the correlation between interventricular septum areas with gestational age (GA), scatter diagrams were determined by obtaining Pearson's correlation index (*r*), and the equation adjustment was given by the coefficient of determination (*R*<sup>2</sup>). For the construction of reference intervals for the interventricular septum areas as a function of gestational age, we followed the simple linear regression model<sup>14</sup> using the Altman's method<sup>15</sup> with a significance level of *p* < 0.05. To assess the extent of septal thickness as a function of gestational age, we also used linear regression. To assess the correlation between the two-dimensional thickness of the interventricular septum and its area, we used the intraclass correlation coefficient (ICC). To calculate the intraobserver reproducibility, the same examiner (LCR) took another measurement of the septal area of 40 fetuses, with these measures blinded from the results of the former. For that purpose, we also used the ICC and Bland-Altman's plot<sup>16</sup>. The Bland-Altman's plot outlines the average of the two measurements performed by the same examiner against the difference in their averages with 95% of confidence interval

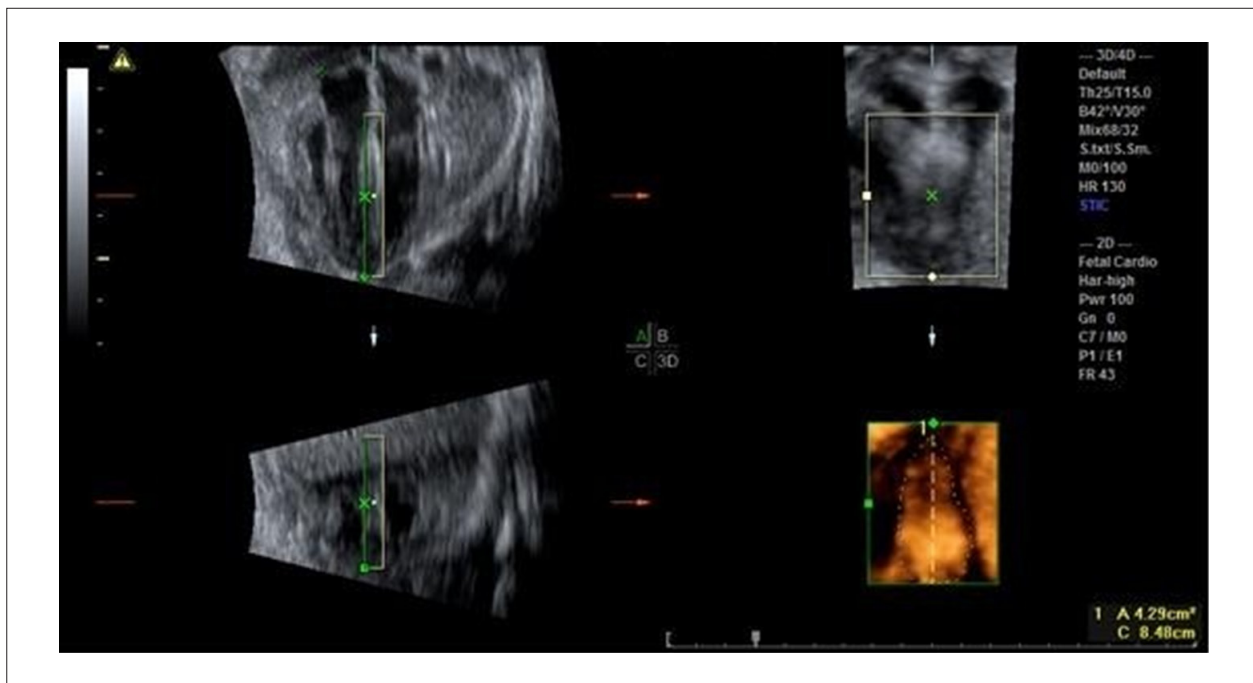


Figure 1 - Rendered image of the interventricular septum at the start of ventricular diastole, with the measurement of its area manually determined in cm<sup>2</sup>.

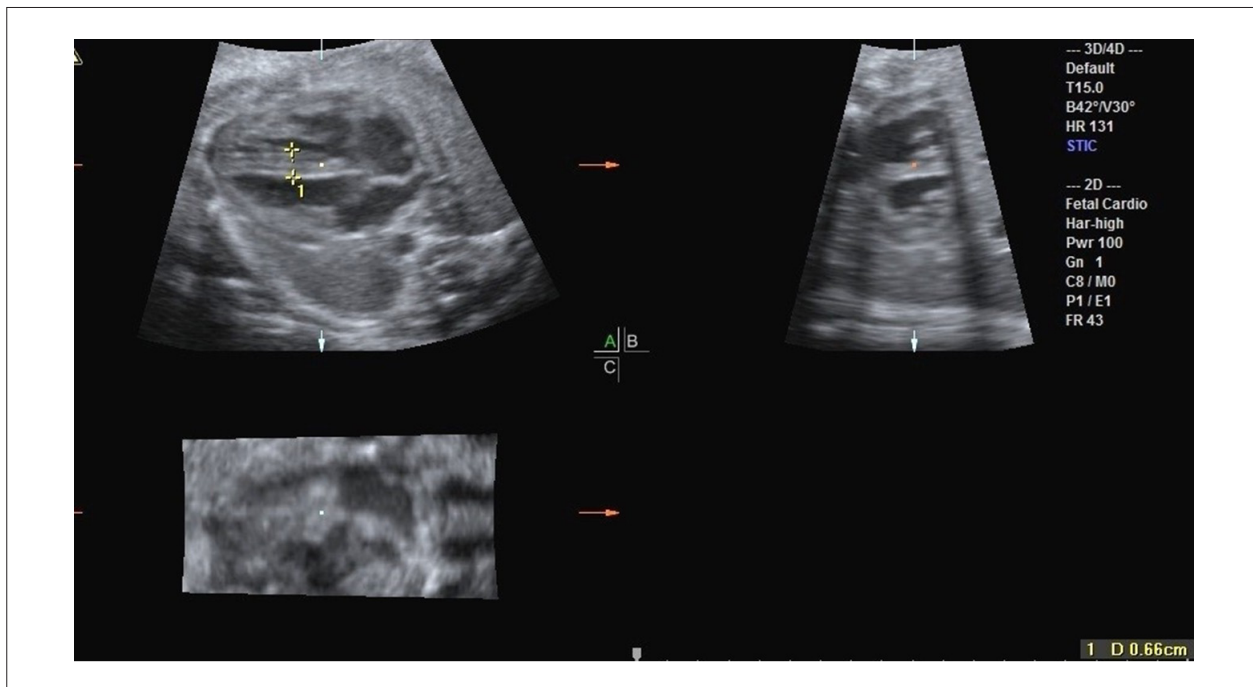


Figure 2 - Dimensional image of interventricular septum thickness in cm.

of 1.96 SD of the average. In all analyses, a significance level of 0.05 was used.

## Results

We evaluated 69 pregnant women carrying normal fetuses between the 18<sup>th</sup> and the 33<sup>rd</sup> completed pregnancy week,

and all met the inclusion criteria and were allocated in the final statistical analysis. Average maternal age was 29.8 years with sd  $\pm$  5.1, with an average of 1.4 pregnancies (SD  $\pm$  0.5) and average rate of 0.3 births (SD  $\pm$  0.5).

The average area of the septal area was  $0.47 \pm 0.10$  cm<sup>2</sup> (ranging from 0.36 to 0.59 cm<sup>2</sup>) in the 18<sup>th</sup> week to  $2.42 \pm$

1.13 cm<sup>2</sup> (ranging from 1.26 to 3.92 cm<sup>2</sup>) in the 33<sup>rd</sup> pregnancy week. Table 1 shows the means, medians, standard deviations and minimum and maximum values of the interventricular septum area, while Table 2 shows the percentiles 5, 25, 50, 75 and 95 in each gestational interval evaluated.

The septal area showed a strong correlation with the gestational age ( $r = 0.81$ ). We performed polynomial regression models. The best adjustment with the exponential equation was: interventricular septum area =  $\exp(0.124 \times \text{GA} + 0.043)$ , with  $R^2 = 0.65$  (Figure 3).

In 52 patients, the septal thickness measurement was taken in the two-dimensional mode, but it did not present a significant correlation with gestational age ( $R^2 = 0.20$ ), showing an almost linear pattern between the 18<sup>th</sup> and the 33<sup>rd</sup> complete pregnancy week (Figure 4). Furthermore, there was no statistically significant correlation between the measure of septal thickness and area in the gestational interval studied with  $\text{ICC} = 0.150$  [95% CI -0.480; 0.512], with  $p < 0.005$ .

There was an excellent intraobserver reproducibility for the measure of the interventricular septum area, with  $\text{ICC} = 0.994$  (95% CI 0.988 to 0.997). The Bland-Altman's plot demonstrated this good reproducibility with the mean difference between the measurements of  $0.01 \text{ cm}^2$  (SD  $\pm 0.06 \text{ cm}^2$  and 95% CI  $\pm 0.12 \text{ cm}^2$ ) (Figure 5).

## Discussion

The STIC technology has been available on some US3D devices in mid-2010. STIC consists in the acquisition of the fetal heart volume with its vascular connections, allowing its evaluation in the multiplanar or rendered mode in the form of a cineloop sequence of a complete cardiac cycle. For the acquisition of the volume, we must select a region of interest (ROI), which determines the height and width of the volume, covering the entire heart with its vascular connections. Whenever possible, the acquisition should be performed with the fetus in the dorsal position (6 hours) and with no maternal breathing movements. The acquisition angle, which determines the depth of the volume varies with gestational age, and angles between 20° and 25° are sufficient in second-quarter fetuses. The acquisition time is determined by the operator, ranging from 7.5 to 15 seconds, and corresponds to the speed at which the transducer scans the ROI. The grayscale volume can be associated to the color Doppler mode, and post-processing such as the inversion mode and B-flow imaging<sup>8,17</sup>. The advantage of the STIC over dimensional echocardiography is that this method is less operator-dependent, allowing the identification of abnormalities in the outflow tracts of the fetal heart by examiners with little to intermediate experience<sup>18</sup>.

**Table 1 - Descriptive analysis of fetal interventricular septal area through the rendering technique in each gestational interval evaluated**

Gestational interval (weeks)	Average (cm <sup>2</sup> )	Median (cm <sup>2</sup> )	Standard deviation (cm <sup>2</sup> )	Minimum (cm <sup>2</sup> )	Maximum (cm <sup>2</sup> )
18 to 19+6 days	0.47	0.47	0.10	0.36	0.59
20 to 21+6 days	0.65	0.54	0.48	0.26	2.22
22 to 23+6 days	0.76	0.70	0.24	0.33	1.19
24 to 25+6 days	0.90	0.97	0.25	0.52	1.13
26 to 27+6 days	1.12	1.06	0.18	0.99	1.37
28 to 29+6 days	2.09	2.17	0.24	1.75	2.28
30 to 31+6 days	1.91	1.96	0.44	1.25	2.68
32 to 33+6 days	2.42	2.26	1.13	1.26	3.92

**Table 2 - Descriptive analysis of the percentiles (5, 25, 50, 75 and 95) of the fetal interventricular septum area through the rendering technique in each gestational interval evaluated**

Gestational interval (weeks)	N = 69	Percentil 5	Percentil 25	Percentil 50	Percentil 75	Percentil 95
18 to 19+6 days	4	0.37	0.41	0.47	0.52	0.58
20 to 21+6 days	19	0.28	0.36	0.54	0.67	0.67
22 to 23+6 days	20	0.53	0.59	0.70	1.01	1.14
24 to 25+6 days	6	0.56	0.74	0.97	1.09	1.13
26 to 27+6 days	4	0.99	1.00	1.06	1.18	1.33
28 to 29+6 days	4	1.80	2.01	2.17	2.24	2.27
30 to 31+6 days	8	1.32	1.68	1.96	2.09	2.48
32 to 33+6 days	4	1.37	1.79	2.26	2.89	3.71

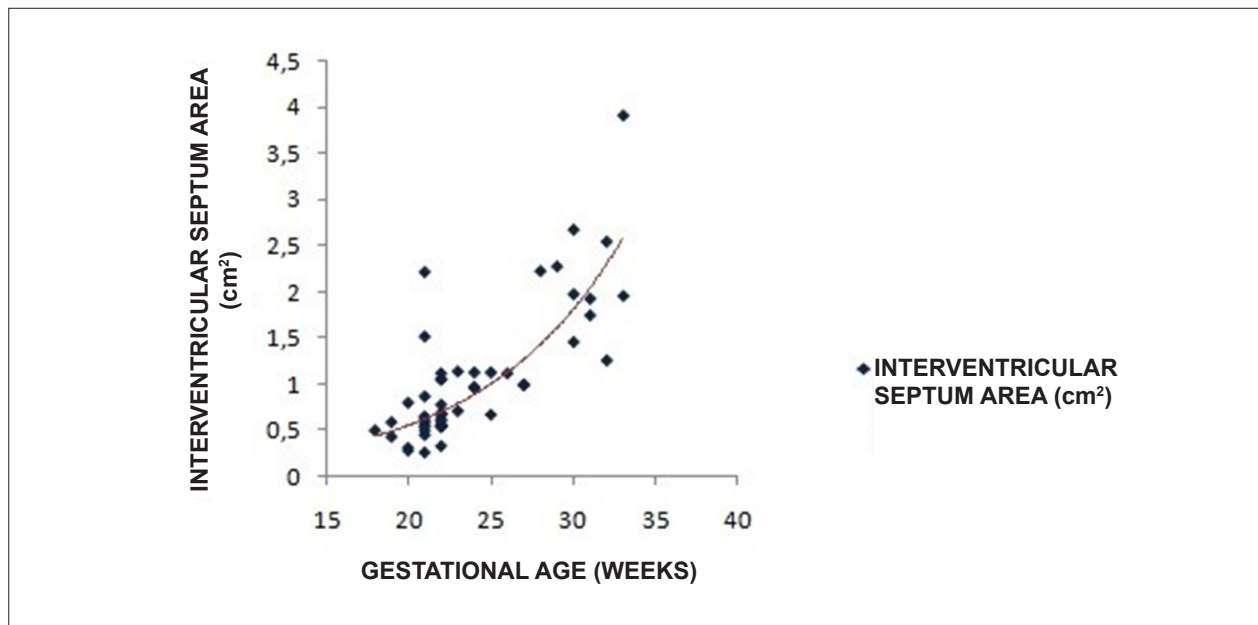


Figure 3 - Scatter diagram of the interventricular septum area in terms of gestational age through the rendering technique.

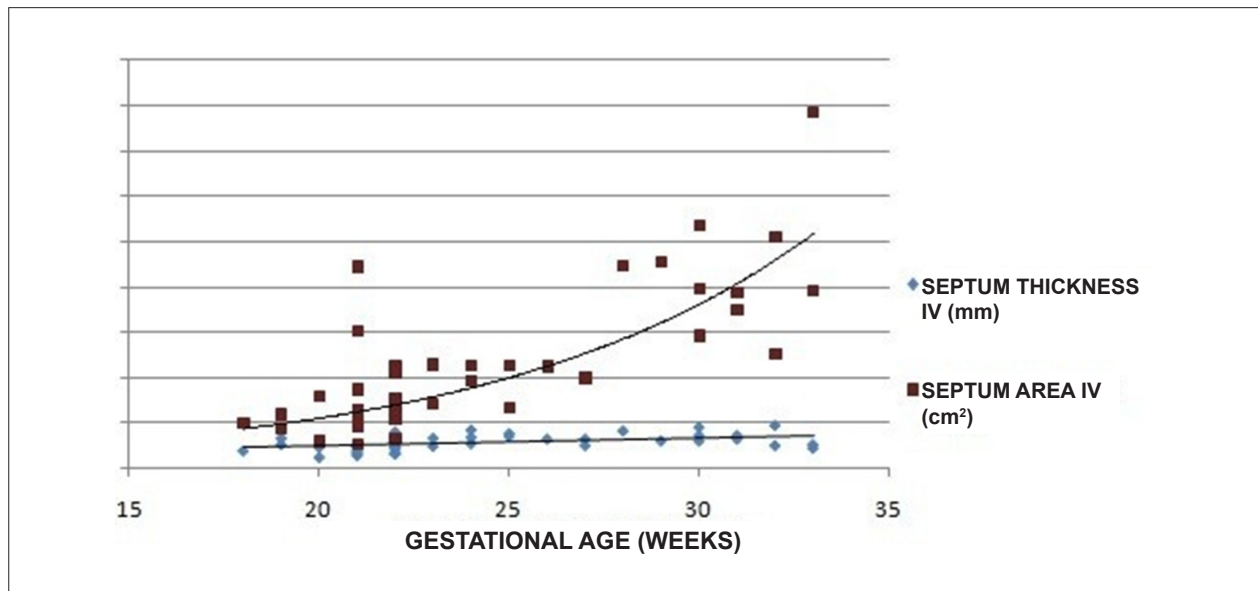


Figure 4 - Scatter diagram of the interventricular septum thickness in the two-dimensional mode as a function of the septal area by the rendering mode.

Regarding the rendering of cardiac structures, Yagel et al<sup>19</sup> evaluated 136 pregnant women, of which 35 carried fetuses with cardiac anomalies, using the rendering method get planes for the evaluation of interatrial and interventricular septum, atrioventricular annulus and alignment of large vessels. In 13 fetuses with cardiac anomalies, the interventricular septum plane improved assessment of the septal defect, and in 4 of them, the atrial septum plane contributed to the evaluation of the foramen ovale. In five cases, the atrioventricular annulus plane immediately distal to the semilunar valves (coronal atrioventricular) improved the evaluation of the alignment of the large vessels in relation to the atrioventricular annulus, and

in three of them, the evaluation of the semilunar valves, with or without malalignment of large vessels. Paladini et al<sup>20</sup> have recently evaluated a series of 30 cases of partial atrioventricular septal defects using in fetuses using echocardiography, and 14 of these cases was associated with STIC. In all of the cases, the echocardiographic markers for this anomaly were: ostium primum atrial septal defect and loss of normal appearance of the atrioventricular valves.

Measuring the interventricular septum area is of particular importance in fetuses at risk for hypertrophic cardiomyopathy, such as the children of diabetic mothers, where there is significant thickening of the interventricular septum, causing



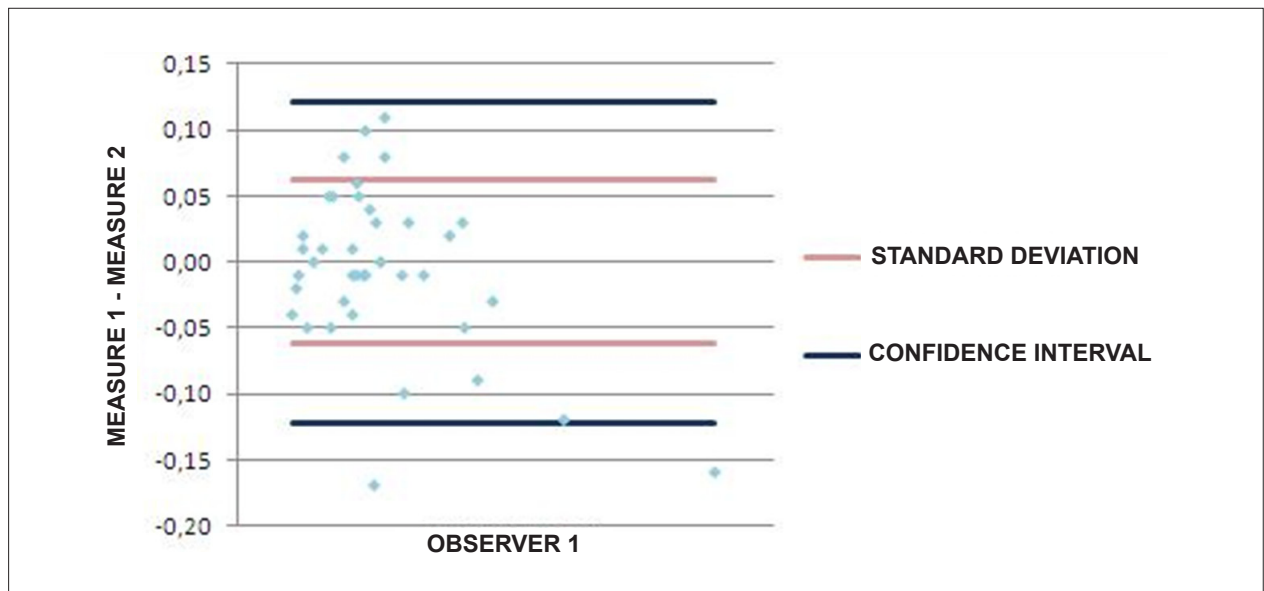


Figure 5 - Difference of the interventricular septum area measures performed by one examiner (measure 1 and measure 2) plotted against the difference of their averages.

obstructions in the left ventricular outflow tract<sup>21</sup>. The severity of symptoms of respiratory failure and congestive heart failure correlate with the severity of hypertrophic cardiomyopathy<sup>22,23</sup>. The measurement of the interventricular septum thickness has been traditionally performed by fetal echocardiography using the M mode<sup>10,11,24</sup>, however, this measure has inaccuracies, since the interventricular septum is not a uniform structure.

In this study, we used the STIC technique to measure the interventricular septum area, a methodology so far unprecedented in the literature. The septum area was strongly correlated with gestational age, and intraobserver reproducibility was confirmed. The septal thickness measure revealed a weak correlation with gestational age, showing a virtually linear pattern of the measure between the 18<sup>th</sup> and 33<sup>th</sup> pregnancy week without showing a statistically significant correlation with the area obtained by the STIC. This result makes the septal area a potentially more reliable ultrasound parameter to measure the interventricular septum thickness in the diagnosis of septal hypertrophy.

In summary, this study determined reference values for the fetal ventricular septal area, which can be applied to fetuses at risk for septal hypertrophy, such as the children of diabetic mothers, promoting early detection of morphological and functional changes in fetal hearts.

#### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

#### Sources of Funding

This study was funded by CAPES.

#### Study Association

This article is part of the thesis of doctoral submitted by Liliam Cristine Rolo, from Universidade Federal de São Paulo.

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