CHAPTER 16

Assessment of Cardiac Geometry and Stroke Volumes by 4D Fetal Echocardiography

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Abstract: Accurate and reliable methods to assess fetal cardiac function would be useful in evaluating fetuses with cardiac disease (structural or otherwise). Traditionally, two-dimensional echocardiography has been used to estimate fetal ventricular volume, and assess cardiac function. However, the unique and complex geometry of the fetal ventricles makes analysis of cardiac function using this modality a challenge, and hence, the interest in using three- and four-dimensional ultrasound. Although theoretically appealing, three-dimensional echocardiography had to overcome several difficulties, including: gating, suboptimal image quality, and lack of real-time observation. Four-dimensional fetal echocardiography is a method to assess ventricular volume and cardiac function, and can overcome many of the pitfalls of conventional methods. Thus, this modality offers an important method for the assessment of fetal cardiac function.

Key Words: 4D Ultrasound, Fetal Echocardiography, Cardiac Volumes, Stroke Volume.

INTRODUCTION

The cardiovascular system is the first to functionally develop in the human embryo (Esh-Broder UOG 2004) [1]. Fetal disease (structural congenital heart disease, or systemic disorders affecting the heart) may change the anatomy and physiology of the cardiac chambers. Examples include hydrops, diabetic cardiomyopathy, intrauterine growth restriction, and twin to twin transfusion syndrome. Therefore, quantifying ventricular volume and calculating cardiac function parameters (e.g. stroke volume, cardiac output, ejection fraction, etc.) contributes to the evaluation of congenital heart disease (Figs. **1** and **2**).

Several methods to obtain ventricular volume measurements through four-dimensional (4D) echocardiography and STIC (Spatio-Temporal Image Correlation) are available. These methods allow calculation of stroke volume, cardiac output, and ejection fraction. We will review the methodology and normal values to assess cardiac function in this chapter. Additionally, we will briefly discuss the roles and limitations of two- and three-dimensional fetal echocardiography in estimating ventricular volumes.

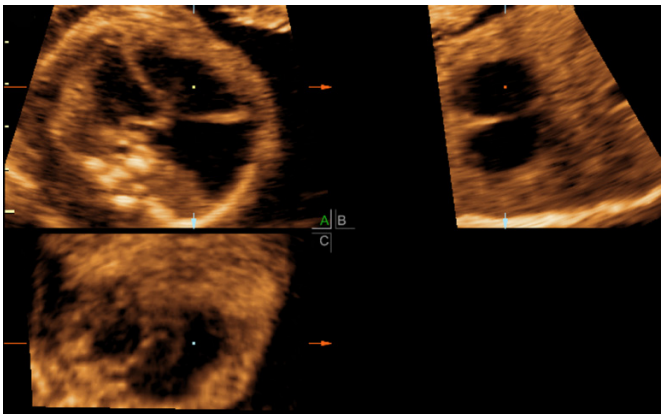

Figure 1: Multiplanar examination of a fetal heart showing the four-chamber view (A-plane) from a spatio-temporal image correlation (STIC) acquisition in a 24-week fetus at end-diastole. In this multiplanar reconstruction, the navigation point is placed in the right ventricle in the A-plane. The fetus has pulmonary atresia and a dysplastic tricuspid valve with severe regurgitation. The right atrium is severely enlarged, and the right ventricle is moderately/severely dilated.

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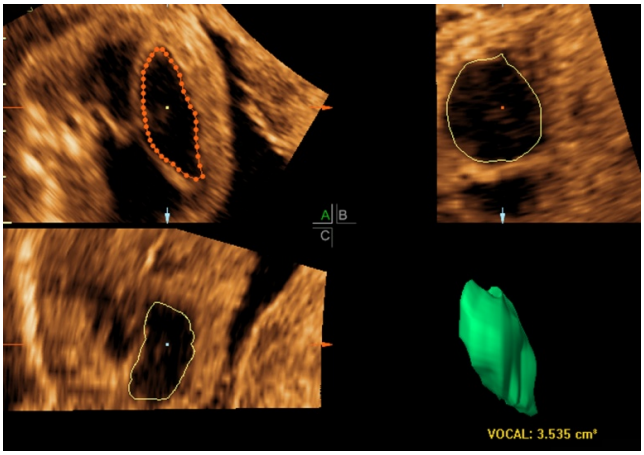

Figure 2: Same fetus as Figure 1. Quantification of fetal right ventricular volume during end-diastole, using the VOCAL™ (Virtual Organ Computer-aided AnaLysis) tool and manual trace. The right ventricular end-diastolic volume (3.5 mL) is above the 95th centile for gestational age.

FETAL VENTRICULAR VOLUME (TWO-DIMENSIONAL AND THREE-DIMENSIONAL ECHOCARDIOGRAPHY)

Traditionally, two-dimensional (2D) echocardiography has been used to estimate fetal ventricular volume; however, this technology has limitations. Simpson and Cook reported a prospective study of normal human fetuses (measurement of 32 different variables) to determine the repeatability of: 1) 2D ultrasound measurements in real-time B-mode; 2) M-mode; and 3) Doppler measurements (Simpson UOG 2002) [2]. The formula employed to estimate the left ventricular (LV) volume was the method of discs (Simpson's rule), which divides this ventricle into 20 equal thickness discs, where each disc is assumed to be circular. The technique involves tracing the LV endocardial border at end-diastole and at end-systole. However, this formula can not be applied to the right ventricle (RV) due to its complex geometric shape. For M-mode measurements, all volumetric data were computed using the Teichholz formula (Teichholz AJC 1976) [3]. Simpson and Cook found that the repeatability of most echocardiographic measurements was poor, and this applied particularly to ventricular volumes and volume flow estimations (Simpson UOG 2002) [2]. Using Simpson's rule, the coefficient of variation exceeded 10% in the estimation of both LV end-diastolic volume (13%) and stroke volume (14%). Moreover, inter-observer errors were consistently higher than intra-observer errors, suggesting that for sequential measurements, the same observer should conduct the assessment. Computing ventricular volumes using the Teichholz formula also did not appear to be very repeatable; the coefficient of variation was over 30% for both the estimation of LV end-diastolic volume (32%) and stroke volume (36%). Observer errors reported in estimation of LV end-diastolic volume, stroke volume, and ejection fraction were lower for 2D techniques than for equivalent measurements made by M-mode echocardiography.

The reasons for these limitations are several. First, Simpson's rule is restricted to monoplanes and assumes a prolate (elongated) ellipsoid shape of the LV, with a ratio of long axis to short axis of 2:1 (Bhat Circulation 2004) [4]. However, this assumption may not apply to the developing fetal heart. Second, when using Simpson's rule, it may be impossible to achieve two orthogonal planes of the ventricle, in the absence of reliable scanning plane landmarks (Esh-Broder UOG 2004) [1]. Third, quantitative echocardiographic methods perform poorly in distorted ventricles, in which standard geometric assumptions become tenuous (Wyatt AHJ 1980) [5]. Fourth, performing cross-sectional measurements of the ventricles assumes that the endocardium is smooth, which is not accurate (Sedmera Anat Rec 2000) [6]. Finally, in the rapidly contracting fetal heart, it may be difficult to identify the precise points of end-diastolic and end-systolic frames. For these reasons, it is generally accepted that 2D echocardiography has some limitations in assessing fetal cardiac function (Simpson UOG 2002) [2] (Simpson Prenat Diagn 2004) [7].

It is generally agreed upon that three-dimensional (3D) volume quantitation is more accurate than 2D-derived methods, because it avoids geometric assumptions and magnification of small errors (inherent in the latter modality) (Bhat JUM 2004) [8]. We have shown that there are changes in cardiac geometry with advancing gestational age (Espinoza JUM 2007) [9]. Therefore, three-dimensional (3D) echocardiography has been utilized to evaluate cardiac

volume and function. However, some investigators have noted the assessment of ventricular volume and ejection fraction to be arduous, with processing of acquired data to be time-consuming (Esh-Broder UOG 2004) [1]. Levental *et al.* found that non-gated 3D volume acquisition of the fetal heart and the subsequent planar reformatting generated suboptimal image quality (Levental 1998 JUM) [10]. Moreover, the inability to observe the heart in real-time was a limitation. Other investigators have noted gating difficulties in using 3D echocardiography (Meyer-Wittkopf JUM 2001) [11]. Meyer-Wittkopf *et al.* found that both the quantitative 3D analysis and endocardial tracing were time-consuming and required considerable expertise (Meyer-Wittkopf JUM 2001) [11]. Therefore, the applicability and reproducibility of this technique might be limited in a clinical setting. Meyer-Wittkopf *et al.* also noted problems with the anatomic accuracy of tracing the endocardium, which can influence the accuracy of quantitative 3D echocardiography. Therefore, while 3D echocardiography can overcome some of the limitations of 2D echocardiography, there are still challenges in utilizing this modality to evaluate fetal ventricular volume and cardiac function.

Recently, a novel approach involving semi-automatic segmentation of fetal cardiac cavities to assess ventricular volume was presented (Tutschek and Sahn UOG 2008) [12]. Tutschek and Sahn retrospectively analyzed STIC cardiac volumes off-line using a commercially available segmentation algorithm designed for ovarian folliculometry (SonoAVC, or Sonography-based Automatic Volume Count). Individual "cavities" in a static volume were selected and assigned individual colors, and diameters and volumes were calculated. For two normal fetuses (21 and 29 weeks of gestation), end-diastolic (0.45-1.35 mL) and end-systolic (0.20-0.57 mL) ventricular volumes were determined, and ejection fraction was calculated (49% and 58%). Tutschek and Sahn proposed that their technique was an important step towards an automated fetal volume echocardiogram. However, the authors acknowledged that because this technique required manual editing (introducing operator-dependency), it was not yet suited for routine fetal echocardiography, and its quantitative aspects still needed to be validated (Tutschek and Sahn UOG 2008) [12].

FETAL VENTRICULAR VOLUME (FOUR-DIMENSIONAL ECHOCARDIOGRAPHY)

Four-dimensional (4D) fetal echocardiography minimizes the effect of several technical factors. When STIC datasets are acquired, examination times are dramatically reduced since acquisitions generally take no more than 12.5 seconds to complete (Uittenbogaard UOG 2008) [13]. Moreover, when computing ventricular volumes, no geometric assumptions are made and measurements are not angle-dependent. This is important, because RV geometry *in-utero* differs from that of the LV. The RV is tripartite, and has inflow, apical, and outflow portions. Because of its complex shape, calculating RV volume is difficult and must be measured using non-geometric techniques (Huhta 2009) [14]. Several investigators have evaluated the utility of fetal cardiac datasets obtained with 4D echocardiography in calculating cardiovascular parameters (Messing UOG 2007) [15] (Molina UOG 2008) [16] (Rizzo Prenat Diagn 2007) [17] (Uittenbogaard UOG 2009) [18].

Four-dimensional STIC is a novel approach to clinically assess the fetal heart (DeVore 2003 UOG) [19] (Gonçalves 2006 UOG) [20]. The technology uses a slow regular sweep of high line density 3D data, with the data realigned into its correct temporal domain in 3D space, to yield a 4D cine sequence (Bhat 2004 JUM) [8]. Therefore, it acquires a cardiac volume dataset and displays a cine loop of a complete single cardiac cycle in motion. The volume set can be manipulated both spatially and temporally. The principles used by the STIC algorithm to synchronize spatial and temporal information (3D images plus motion) in cardiac volume datasets are similar to the non-ECG motion Fourier analysis gating method proposed by Nelson *et al.* in 1996 (Nelson JUM 1996) [21]. STIC avoids the need for gated acquisition, making it ideal for fetal echocardiography. From the cine loop, a specific cardiac phase can be identified and analyzed by observing the opening and closing of the atrioventricular and semilunar valves. Because STIC captures end-diastolic and end-systolic time points in the cardiac cycle, it allows volume measurements at these specific points. From this, fetal cardiac parameters such as stroke volume and ejection fraction can be calculated.

In 2004, Bhat *et al.* investigated the ability of 4D STIC to produce quantitatively accurate dynamic fetal heart images using an *in vitro* pulsatile balloon model and apparatus (Bhat 2004 JUM) [8]. Volume determination was undertaken to correspond to systolic and end-diastolic volumes within the inner balloon, as well as stroke volume.

Volume data was analyzed by customized radial summation techniques using 4D data analysis software, and was compared with known volumes (2.5 to 10 mL) and masses. 4D STIC was found to be feasible, practical, and an acceptably accurate method for volume and mass estimations in the ranges comparable with the mid- and late-gestation fetal heart. Good correlation was found between observed and actual systolic volumes (R^2 = 0.92), diastolic volumes (R^2 = 0.90), and stroke volumes (R^2 = 0.92), and there was good overall correlation across all volumes (R^2 = 0.91). However, there was a wider range of percentage error in the lowest volumes tested (2.5 mL), attributed to difficulties in spatial resolution or from distortions within the model apparatus itself. 4D STIC was found to be particularly accurate for diastolic estimations, and at volumes of greater than 2.5 mL.

Using 4D fetal echocardiography and STIC, different methods have been used to obtain ventricular volume measurements (Messing UOG 2007) [15] (Molina UOG 2008) [16] (Rizzo Prenat Diagn 2007) [17] (Uittenbogaard UOG 2009) [18]. Messing et al. used inversion mode (Messing UOG 2007) [15], Rizzo et al. and Molina et al. used VOCAL™ (Virtual Organ Computer-aided AnaLysis) (Rizzo Prenat Diagn 2007) [17] (Molina UOG 2008) [16], and Uittenbogaard et al. used the 3D Slice method (Uittenbogaard UOG 2009) [18]. VOCAL™ is the most frequently used method to obtain volume measurements from 3D datasets. Inversion mode is a rendering algorithm which transforms echolucent structures into solid voxels, thus "inverting" their presentation. Thus, anechoic structures such as cardiac chambers, lumen of the great vessels, stomach, and bladder appear echogenic on the rendered image, while structures that are normally echogenic prior to gray-scale inversion (e.g. bones) become anechoic (Gonçalves UOG 2004) [22]. By adjusting the thresholding level within the inversion mode, this allows fine-tuning to eliminate speckle within the volume. Messing et al. reported nomograms for fetal ventricular volume by using inversion mode and STIC (Messing UOG 2007) [15]. Inversion mode was used because it allowed superior demonstration of fluid-filled fetal anatomical structures and much better segmentation based on thresholding within the region of interest. VOCAL™ was then used to perform rotational measurements of the volume. By combining the VOCAL™ volume with inversion mode thresholding, only the fluid-filled portion of the ventricle was ultimately measured, and a new intraventricular model created (Fig. **3**).

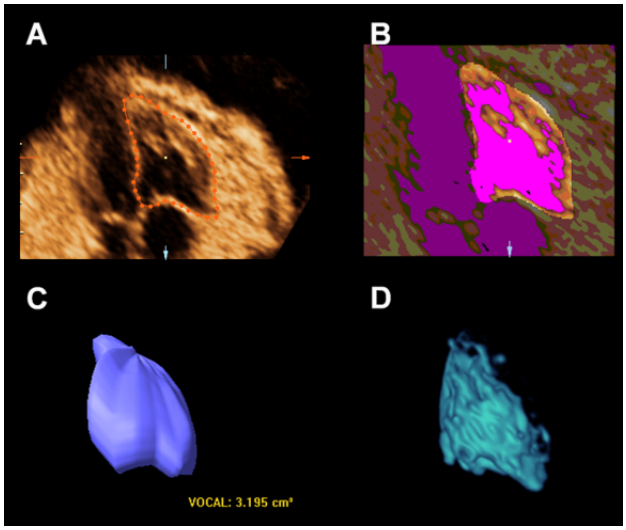

Figure 3: Post-processing quantification of right ventricular volume in end-diastole. (A) The VOCAL™ trace (drawn including the myocardium) in the A frame at the level of the four-chamber view; (B) the same frame with the inversion mode activated; (C) the three-dimensional model created by the VOCAL™ tool, which includes the entire traced volume; and (D) the final intraventricular volume model based only on the fluid-filled portion of the ventricle.

In this cross-sectional study of 100 normal fetuses (20.5-40 weeks of gestation), nomograms were created for right end diastolic volume (EDV), right end systolic volume (ESV), left EDV, left ESV, and total stroke volume vs. gestational age and estimated fetal weight. Table 1 shows various fetal cardiac parameters and their mean volumes (95% CI) (cm3) at midgestation, and at term.

Fetal cardiac parameters and their mean volumes (95% CI) (cm^3) at midgestation and term, as determined by 4D sonography (STIC combined with inversion mode).

Table 1

Fetal cardiac parameter	Mean volume (95% CI) (cm^3)	
	Midgestation	Term
LEDV	0.53 (0.39-0.66)	3.96(3.41-4.51)
LESV	0.17 (0.12-0.22)	1.56 (1.2-1.8)
REDV	0.68 (0.56-0.79)	5.44 (4.69-6.18)
RESV	0.26 (0.19-0.31)	2.29 (1.88-2.71)

LEDV, left end-diastolic volume; LESV, left end-systolic volume; REDV, right end-diastolic volume; RESV, right end-systolic volumeTable based upon the report from Messing et al. UOG 2007 [15]

The mean right EDV:left EDV ratio was 1.4 (95% CI: 1.3-1.5), and was relatively stable throughout pregnancy. The EDV and ESV of both ventricles were found to correlate strongly with gestational age and average estimated fetal weight; however, the correlation was slightly stronger with estimated fetal weight (Messing UOG 2007) [15]. Messing et al. also observed similarity between their data for near-term infants, and those derived from neonatal echocardiographic studies. Volume measurements were both reliable and reproducible (inter- and intraobserver variations <10% and <5%, respectively).

In 2008, Molina et al. established cross-sectional reference intervals (12-34 weeks of gestation) for fetal heart stroke volume and cardiac output in 140 normal singleton pregnancies (Molina UOG 2008) [16]. This was accomplished by initially measuring ventricular volumes (systole and diastole) using 4D STIC and the VOCAL™ technique (Fig. 4), and demonstrated its feasibility.

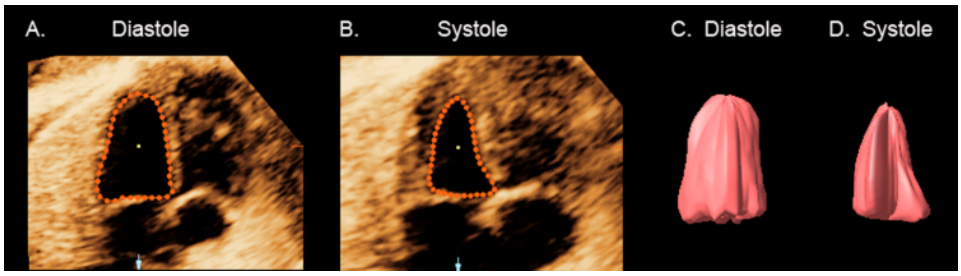

Figure 4: Three-dimensional ultrasound images of the left ventricle in diastole (A) and systole (B), and the cardiac volumes obtained from them using VOCAL™ (Virtual Organ Computer-aided AnaLysis) (C and D, respectively).

Factors that could affect the acquisition of 4D volumes included maternal obesity and breathing movements, fetal movements, and fetal position. Advantages of the VOCAL™ technique included: 1) when drawing the contours of the ventricle, the entire ventricle is visualized simultaneously in all planes; and 2) after the initial calculation of ventricular volume, it is possible to modify the contour in each plane. However, various limitations were noted when using the VOCAL™ technique: 1) its reproducibility in the first trimester was poor, due to small ventricular volume; 2) in the third trimester, factors obscured the accurate definition of the limits of the ventricles (fetal spine-up position, fetal breathing movements, shadows from ossified ribs); and 3) with advancing gestation, the anatomic accuracy of endocardial tracing became progressively worse because the myocardium and atrioventricular septa appear thicker, and have lower resolution in the orthogonal plane (vs. original data acquisition) (Molina UOG 2008) [16]. This finding may be related to spatial volume artifacts, slower frame rates, or erroneous rendering algorithms, as described by others (Meyer-Wittkopf JUM 2001) [11].

Uittenbogaard et al. performed a prospective, longitudinal study (12-30 weeks of gestation) using 4D STIC to provide reference values for left and right ventricular volumes, and cardiac function indices (stroke volume, ejection fraction, cardiac output) (Uittenbogaard UOG 2009) [18]. The relationships of ventricular volume and cardiac function indices with gestational age and estimated fetal weight were also determined. All volumetric data were obtained using the 3D Slice method (Fig. **5**), which is based on Simpson's rule. Multiple slices of the four-chamber view were manually traced, and the areas multiplied by the slice thickness and summed (Uittenbogaard UOG 2009) [18].

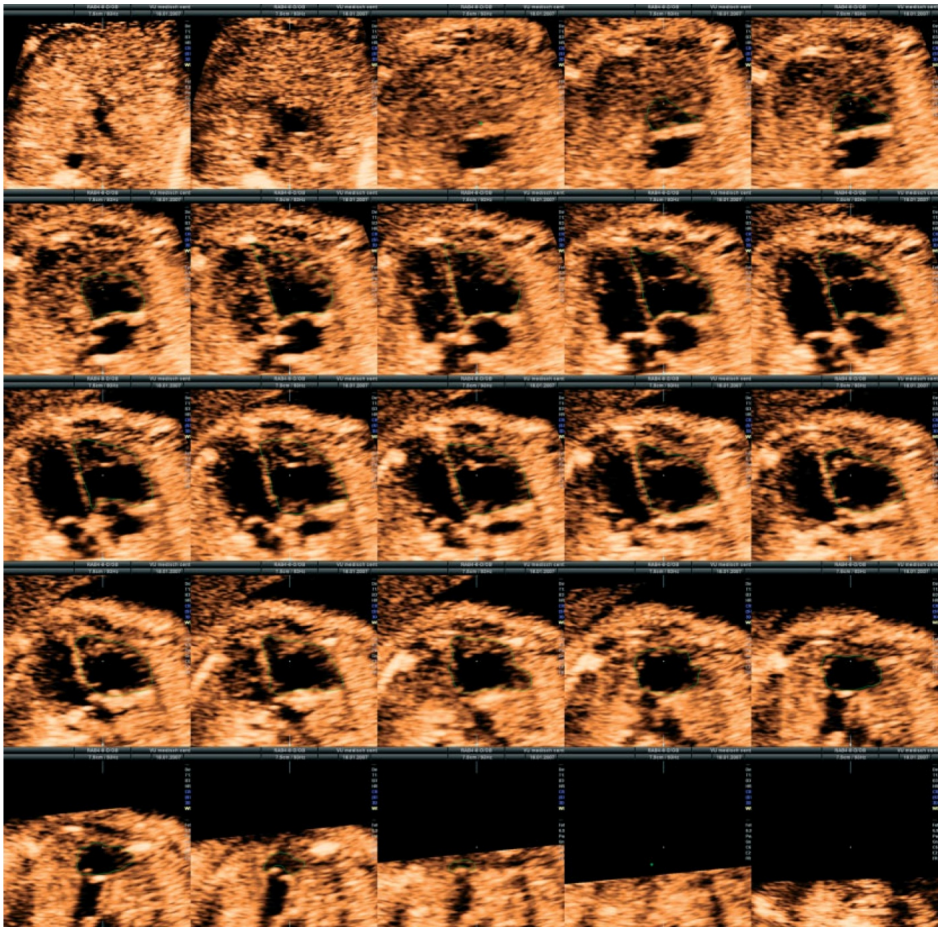

Figure 5: Compilation of images 1 mm apart obtained by multiplanar imaging of a spatiotemporal image correlation volume (3D Slice method) frozen in end-diastole. The outlines of the right ventricle have been traced as indicated by a thin green line. The figure demonstrates the complex geometric shape of the right ventricle. Reproduced with permission from Uittenbogaard *et al.* UOG 2009 [18]

From 63 fetuses, 202 STIC volumes were included in the analysis. Volumes were acquired longitudinally from 12 weeks of gestation onwards (with an interval of 3-4 weeks), as long as high-quality acquisition was possible. Volumes were acquired from 12-30 weeks of gestation only, because beyond 29 weeks of gestation, the failure rate of STIC acquisition increased remarkably. There were only three technically acceptable STIC volumes beyond 30 weeks of gestation. Tables **2** and **3** show the mean, 5th, and 95th centiles of left and right systolic and diastolic ventricular volumes in relation to gestational age. The RV/LV ratio remained constant at around 1.12, for both end-systole and end-diastole.

Table 2: Mean, 5th, and 95th centiles of left systolic and diastolic ventricle volumes in relation to gestational age

	Left ventricle ESV (mL)			Left ventricle EDV (mL)		
Gestational Age (weeks)	**Mean**	**5th**	**95th**	**Mean**	**5th**	**95th**
12	0.03	0.02	0.04	0.04	0.03	0.06
13	0.04	0.03	0.05	0.07	0.05	0.09
14	0.06	0.04	0.08	0.10	0.07	0.13
15	0.08	0.04	0.13	0.15	0.09	0.20
16	0.12	0.05	0.18	0.21	0.12	0.30
17	0.16	0.07	0.26	0.30	0.17	0.43
18	0.22	0.09	0.35	0.41	0.23	0.59
19	0.29	0.12	0.45	0.55	0.31	0.78
20	0.37	0.16	0.58	0.72	0.42	1.02

Table 2: cont…

21	0.48	0.21	0.74	0.93	0.55	1.30
22	0.60	0.28	0.91	1.17	0.71	1.62
23	0.73	0.36	1.11	1.44	0.89	1.98
24	0.89	0.45	1.33	1.73	1.08	2.37
25	1.06	0.55	1.57	2.03	1.28	2.79
26	1.24	0.66	1.82	2.34	1.47	3.21
27	1.43	0.76	2.09	2.63	1.64	3.62
28	1.61	0.87	2.35	2.89	1.77	4.02
29	1.79	0.95	2.62	3.11	1.85	4.38
30	1.95	1.02	2.88	3.27	1.86	4.69

Data based on regression equations.

EDV, end-diastolic volume; ESV, end-systolic volume

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Table 3: Mean, 5th, and 95th centiles of right systolic and diastolic ventricle volumes in relation to gestational age.

Gestational Age (weeks)	Right ventricle ESV (mL)			Right ventricle EDV (mL)		
	Mean	**5th**	**95th**	**Mean**	**5th**	**95th**
12	0.03	0.02	0.03	0.05	0.03	0.06
13	0.04	0.03	0.05	0.07	0.04	0.10
14	0.06	0.04	0.08	0.11	0.06	0.15
15	0.09	0.05	0.13	0.16	0.09	0.23
16	0.13	0.07	0.18	0.23	0.13	0.34
17	0.17	0.09	0.26	0.33	0.18	0.47
18	0.20	0.12	0.35	0.45	0.26	0.64
19	0.32	0.17	0.47	0.61	0.36	0.86
20	0.41	0.22	0.60	0.80	0.49	1.11
21	0.53	0.29	0.76	1.03	0.64	1.41
22	0.66	0.37	0.95	1.29	0.83	1.75
23	0.81	0.47	1.16	1.59	1.04	2.13
24	0.98	0.57	1.38	1.90	1.26	2.54
25	1.16	0.68	1.63	2.22	1.48	2.97
26	1.34	0.80	1.88	2.54	1.69	3.39
27	1.52	0.90	2.14	2.84	1.87	3.80
28	1.69	0.99	2.39	3.09	2.00	4.18
29	1.85	1.06	2.63	3.29	2.08	4.51
30	1.98	1.10	2.85	3.43	2.07	4.78

Data based on regression equations. EDV, end-diastolic volume; ESV, end-systolic volume Slightly modified and reproduced with permission from Uittenbogaard *et al.* UOG 2009 [18]

Uittenbogaard *et al.* felt that the 3D Slice method was preferable and advantageous in obtaining volume measurements. From an *in-vitro* validation study using a balloon model (performed by the same group), the 3D Slice method was proven to be less time-consuming than the use of VOCAL™ or inversion mode. Moreover, manual adjustment of threshold settings when using inversion mode is operator dependent, and this is not the case in the 3D Slice method. Uittenbogaard *et al.* found significantly larger values for end-systolic and end-diastolic ventricular volume (compared to that of Messing *et al.*), and proposed that these differences could be explained by using inversion mode, since it is more dependent on B-mode gain, gray-scale curve, and dynamic range settings. (Uittenbogaard UOG 2009) [18].

Uittenbogaard *et al.* observed the following limitations in performing STIC acquisitions: 1) low image resolution at young gestational ages; 2) numerous acoustic shadows at advanced gestational ages; 3) high failure rate late in gestation; 4) abundant fetal movement; and 5) a persistent unfavorable fetal position (Uittenbogaard UOG 2009) [19].

An important concept when performing quantitative measurements is the potential for measurement error, which can have important consequences when applied clinically. This concept applies to measuring fetal ventricular volumes as well. *Repeatability* of measurements refers to the variation in repeat measurements made on the same subject under identical conditions, while *reproducibility* refers to the variation in measurements made on a subject under changing conditions (National Institute of Standards and Technology) [23]. A comprehensive understanding of fetal ventricular volume repeatability is limited, because previous work has not included assessments of both agreement and reliability, as recommended by Bartlett and Frost (Bartlett UOG 2008) [24]. Moreover, the reproducibility of fetal ventricular volume measurements (obtained by STIC and VOCAL™) is a subject that requires further investigation. Therefore, our group recently quantified both repeatability and reproducibility in the calculation of fetal ventricular volumes (systole and diastole) obtained using the STIC and VOCAL™ technique (Hamill JUM 2009) [25]. From the VOCAL™ application, the sub-feature "Contour Finder/Trace" was utilized (Figs. **6** and 7). This feature employs a sophisticated algorithm which helps to find the contour of the ventricle as the mouse is moved along the ventricular wall.

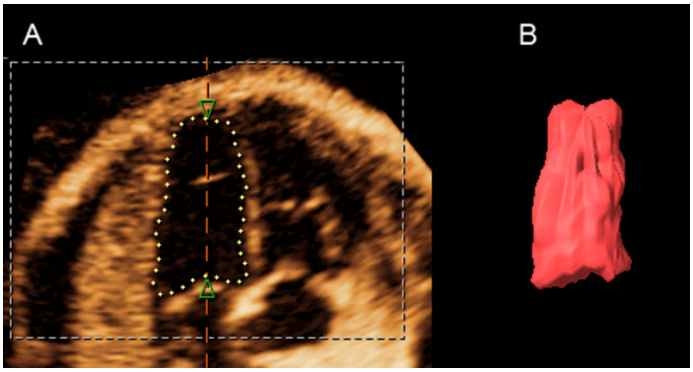

Figure 6: Quantification of left ventricular volume in end-diastole. (A) The VOCAL™ trace (using sub-feature "Contour Finder/Trace") in the A frame at the level of the four-chamber view; (B) the three-dimensional model created by the VOCAL™ tool, which includes the entire traced volume.

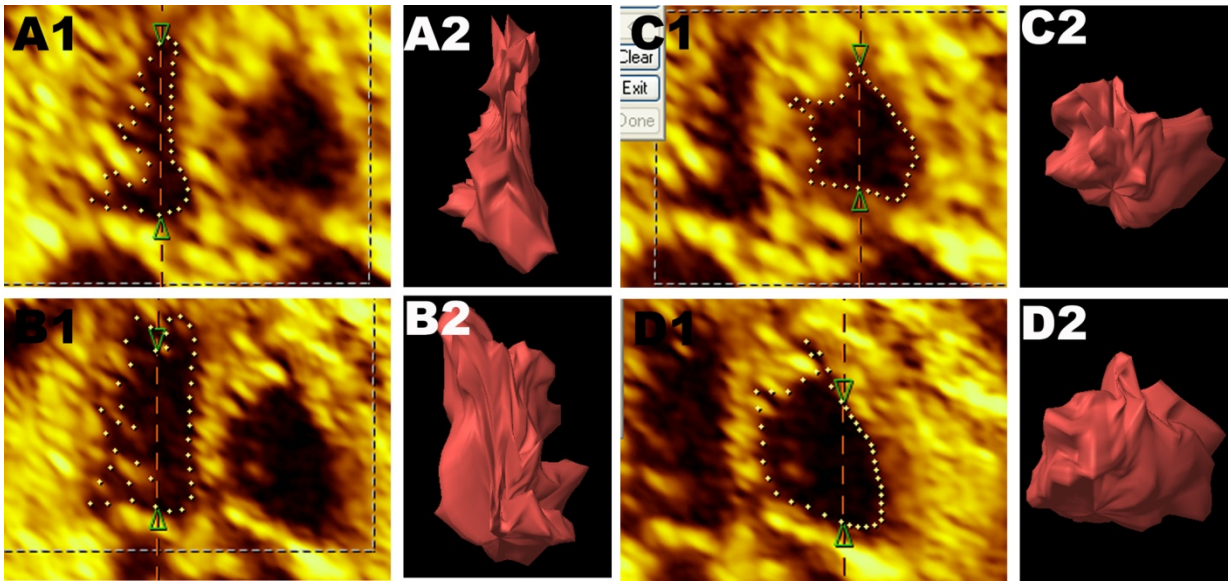

Figure 7: Selected VOCAL™ rotational steps utilizing "Contour Finder/Trace" for each ventricle in end systole and end diastole (A: Left ventricle in systole; B: Left ventricle in diastole; C: Right ventricle in systole; D: Right ventricle in diastole) at the level of the four chamber view (image 1: A, B, C, D) and the rendered image (image 2: A, B, C, D). Reproduced with permission from Hamill *et al.* JUM 2009 [25]

Twenty-five normal pregnancies were evaluated for the following: 1) to compare the coefficient of variation (CV) in the calculation of ventricular volumes, when the number of rotational steps is varied from 15° to 30°; 2) to compare the CV in volume calculations between 3 methods of quantifying ventricular volumes from STIC and VOCAL™ (manual trace, inversion mode, and "Contour Finder/Trace"); and 3) to determine *repeatability* (ventricular volumes

were measured twice by each of three observers). *Reproducibility* was assessed by obtaining two STIC datasets from each of 44 normal pregnancies; for each STIC dataset, 2 ventricular volume calculations were performed. Agreement and reliability were evaluated by computing CV and intraclass correlation (ICC), respectively, and the technique was considered *repeatable* if there was good agreement (CV < 10%), as well as good reliability (ICC > 0.90). The technique was considered *reproducible* if there was a negligible difference (<1%) in agreement between datasets, as well as good reliability (ICC > 0.90). Measurement error introduced into STIC acquisitions was examined by constructing a Bland-Altman plot.

The following results were observed: 1) ventricular volume calculations obtained with "Contour Finder/Trace" had better agreement (3.6%, 95% CI 3.0-4.2) than either inversion mode (6.0%, 95% CI 4.9-7.2; p < 0.001), or manual trace (10.5%, 95% CI 8.7-12.5; p < 0.001); 2) agreement in ventricular volume measurements using STIC and VOCAL™ ("Contour Finder/Trace") was better with 15º than 30º rotation (3.6%, 95% CI 3.0-4.2 vs. 7.1%, 95% CI 5.8-8.6; p < 0.001) (an effect likely due to the complicated geometry of the fetal ventricles) 3) ventricular volume measurements were *repeatable* with good agreement (CV < 10%) and excellent reliability (ICC > 0.95) for both intra-observer and inter-observer measurements (Table **4**); and 4) ventricular volume calculations were *reproducible* with a negligible difference in agreement (CV < 1%), and good reliability (ICC > 0.90) (Table **5**).

Table 4: Repeatability of ventricular volumes utilizing VOCAL™ with the sub-feature "Contour Finder/Trace"

	Observer 1 (n=25)	**Observer 2 (n=25)**	**Observer 3 (n=25)**
Agreement*	2.8% (2.3 – 3.3)	3.6% (3.0 – 4.3)	6.3% (5.2 – 7.6)
Intra-observer reliability#	0.998 (0.996– 0.998)	0.996 (0.994– 0.997)	0.990 (0.985– 0.993)
Inter-observer reliability#	0.96 (0.94 – 0.97)		

*Agreement expressed as mean percent coefficient of variation (95% CI) #Reliability expressed as intraclass correlation (95% CI) Reproduced with permission from Hamill *et al.* JUM 2009 [25]

Table 5: Reproducibility of ventricular volumes utilizing VOCAL™ with the sub-feature "Contour Finder/Trace."

	STIC Dataset A	**STIC Dataset B**
Agreement*	4.0% (3.5 – 4.7)	3.8% (3.2 – 4.6)¶
Intra-observer reliability#	0.998 (0.997– 0.998)	0.997 (0.996 – 0.998)
Inter-observer reliability#	0.94 (0.92 – 0.96)	

*Agreement expressed as mean percent coefficient of variation (95% CI) ¶Paired t-test not significant (t = 0.47; p = ns)#Reliability expressed as intraclass correlation (95% CI) Reproduced with permission from Hamill *et al.* JUM 2009 [25]

The Bland-Altman plot comparing the percent difference between volume calculations from each STIC acquisition indicated that minimal bias was introduced between acquisitions (<1%; mean percent difference -0.4%, 95% limits of agreement -5.4 – 5.9). Therefore, we found that fetal echocardiography using STIC and VOCAL™ allowed both *repeatable* and *reproducible* calculations of ventricular volumes, when using the sub-feature "Contour Finder/Trace" (Hamill JUM 2009) [25]. It is noteworthy that ventricular volume calculations obtained using VOCAL™ and manual trace achieved CVs greater than 10%, suggesting this may not be the most optimal method to quantify ventricular volumes.

Obtaining fetal ventricular volume measurements using 4D STIC technology, however, is not without limitations. STIC produces a computer-generated cine loop of a single cardiac cycle that is an assemblage of 20 to 30 real cardiac cycles. It is possible that some degree of smoothing, or averaging of the ventricular borders could occur, introducing a degree of error into the calculations performed. STIC volume datasets may also be limited by acoustic shadowing (signal loss in the sound path secondary to echogenic structures), dropout (signal loss in the sound path without intervening structures), motion artifact, fetal positioning, and respirations. Moreover, regardless of the method used in conjunction with STIC to determine ventricular volumes, (e.g. inversion mode, VOCAL™, etc), there is still a significant learning curve and time commitment required to orient, process, and analyze the data.

These factors have hindered the widespread adoption of multi-dimensional sonographic techniques. Nevertheless, we anticipate that this practice will change in the future as sonologists become more accustomed to these tools. Importantly, the use of 4D sonographic technology to obtain fetal ventricular volumes has advantages over 2D and 3D sonography, and it has been demonstrated that ventricular volumes obtained through STIC and VOCAL™

provide both repeatable and reproducible calculations. Therefore, the available literature supports the continued practice of this technique. Once ventricular volumes have been determined, cardiac function parameters (e.g. stroke volume, cardiac output, ejection fraction) can be subsequently calculated.

FETAL STROKE VOLUME AND CARDIAC OUTPUT (USING TWO-DIMENSIONAL AND DOPPLER ECHOCARDIOGRAPHY)

Cardiac output (CO) is the volume of blood that is pumped over a unit of time. The formula for calculating cardiac output is: *stroke volume (SV) x heart rate*. Traditionally, 2D and Doppler echocardiography have been used to calculate fetal SV and CO (Mielke Circulation 2001) [26]. SV is calculated by multiplying the time velocity integral (TVI) of the Doppler tracing, with the flow cross-sectional area. TVI is the area measured under the Doppler velocity envelope for one heartbeat. The cross-sectional area is calculated from $\pi d^2/4$, where d is the valve diameter. Therefore, the formulas for calculating SV and CO are:

$$SV = TVI \times \pi d^2/4$$

$$CO = SV \times \text{fetal heart rate}$$

Animal experimentation has demonstrated that Doppler echocardiography can be used to accurately quantify volumetric flow through the aortic and pulmonary valves (Stewart JACC 1985) [27]. However, it is important to consider methodological problems with this technique. Rizzo *et al.* assessed the agreement of fetal SV (measured with 2D and Doppler sonography) with 4D STIC sonography (Rizzo Prenat Diagn 2007) [17]. Both techniques were found to measure SV reliably and with good reproducibility. However, Rizzo *et al.* noted that the performance of 2D Doppler echocardiography was operator-dependent, and required various views of the fetal heart in order to measure Doppler velocity waveforms of the outflow tracts and valve diameter. Based on the formulas described above, errors in SV (and therefore, CO) may arise from inaccuracies in vessel diameter and Doppler recordings (Mielke Circulation 2001) [26]. Moreover, errors in measuring the TVI and valve area (even if small) will greatly influence volume flow measurements, particularly because the area of the valve is related to the square of the radius, thus accentuating any errors (Eik-Nes UMB 1984) [28]. Even by including just one vessel wall thickness (measuring vessel diameter from outer surface to inner surface), this will overestimate the diameter and consequently, the volume of blood flow. Indeed, vessel areas represent the most important source of error in flow calculations, and particularly in vessels with a small diameter, the error will be substantial (Mielke Circulation 2001) [26]. Simpson and Cook determined the repeatability of Doppler echocardiographic measurements in the human fetus (Simpson UOG 2002) [2]. They found that intra- and inter-observer errors were high for Doppler variables, such as vessel dimension, SV, and CO. For aortic Doppler measurements, the CV was greater than 10% for both the calculated SV (16%), and CO (16%). Performing Doppler echocardiography is also time-consuming, and requires both a favorable position of the fetus and an experienced sonologist. Therefore, these factors have limited its application in clinical practice, and measures of absolute volume flow using this modality have largely fallen out of favor.

FETAL STROKE VOLUME, CARDIAC OUTPUT, EJECTION FRACTION (FOUR-DIMENSIONAL ECHOCARDIOGRAPHY)

In adults and children, cardiac function is commonly expressed as SV and ejection fraction (EF). Both indices can be calculated from end-systolic and end-diastolic ventricular volumes. SV is the volume of blood pumped from one ventricle of the heart with each contraction, and SV applies equally to both ventricles. Its value is obtained by subtracting end-systolic volume (ESV) from end-diastolic volume (EDV) (or preload) for a given ventricle. Hence, the formula for calculating SV is:

$$SV = EDV - ESV$$

SV depends on various factors, such as heart size, contractility, duration of the contraction, preload, and afterload. The most commonly used index of LV function is the EF. This is the amount of blood pumped per contraction (SV), compared to the maximum left ventricular volume prior to contraction (EDV), and as the term states, it is calculated as a *fraction*. Even in healthy hearts, some blood always remains in the ventricles after each contraction. Therefore,

EF is the percentage of the blood within the ventricles that is ejected during systole, and is a measure of the effectiveness of the heart as a pump. EF is often used as a clinical index to evaluate the inotropic status of the heart. The formula for calculating EF is:

$$EF = SV \times 100\% / EDV$$

In the fetus, both ventricles pump blood to the systemic arterial circulation. Since pulmonary vascular resistance is higher in the fetus (vs. postnatally), most of the RV output enters the systemic arterial circulation via the ductus arteriosus. Because the pulmonary and systemic circulations are separate in fetal life, each ventricle has a SV which is determined by the preload, contractility, and afterload of that chamber. However, because of the large volume and pressure work required from the RV, it is a large contributor to the work output of the fetal heart (Huhta 2009) [14]. Indeed, it has been shown that the RV volume that is ejected is greater than that of the LV by echocardiography measurements throughout gestation (Chaoui Geburtshilfe Frauenheilkd 1995) [29]. Chaoui *et al.* found that the SV and CO of the RV had a higher ratio (1.3:1) than that of the LV, thus expressing RV dominance in the fetus (Chaoui Geburtshilfe Frauenheilkd 1995) [29]. This distribution of CO is maintained, in spite of significant changes in the pulmonary vascular resistance and flow between 20-37 weeks of gestation (Rasanen Circulation 1996) [30]. Further evidence that the RV performs more work than the LV is shown by data in fetal lambs, that measured the coronary blood flow of both ventricles (Thornburg AJP 1999) [31]. The RV coronary flow was found to be consistently one-third greater than that of the LV.

Prior studies (not utilizing 4D echocardiography) that evaluated fetal SV, EF, or CO either: 1) could not overcome the limitations of differentiating ventricular volume from the myocardium (due to the non-uniform shape of the endocardium); or 2) relied on sophisticated and time-consuming mathematical calculations (Meyer-Wittkopf JUM 2001 [11] Schmidt AJC 1995 [32] Mielke Circulation 2001) [26] that could not be applied in routine clinical settings.

After first establishing ventricle volumetry (using 4D STIC and inversion mode) in fetuses ranging from 20.5-40 gestational weeks, EF and SV were also calculated (Messing UOG 2007) [15]. Right, left, and total SV were found to correlate strongly with estimated fetal weight and gestational age: 1) total SV and EFW, $r^2 = 0.809$; and 2) total SV and gestational age, $r^2 = 0.766$. Left EF ranged from 42.5 to 86% in these fetuses. However, EF was found to have no correlation with estimated fetal weight or gestational age, but instead, remained fairly stable throughout gestation (Messing UOG 2007) [15].

Rizzo *et al.* assessed the agreement of fetal SV measured with 2D and Doppler sonography vs. 4D ultrasound with STIC (Rizzo Prenat Diagn 2007) [17]. Stroke volumes were cross-sectionally measured in a population of normal and growth-restricted fetuses (n=56) in the second half of pregnancy. The VOCAL™ technique was used to evaluate end-systolic and end-diastolic volumes of each ventricle, and the contour was traced manually (Figs. **8** and **9**).

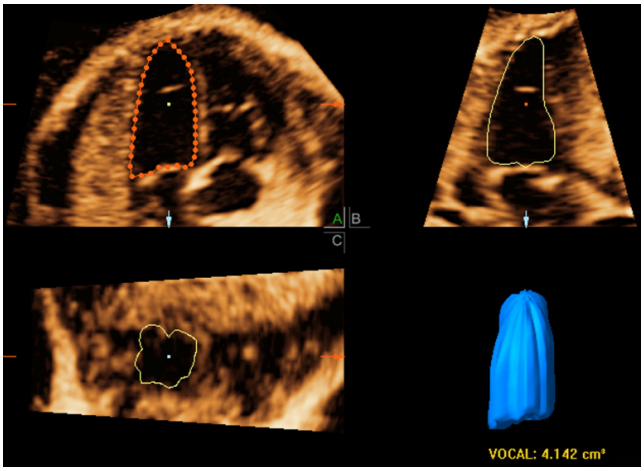

Figure 8: Example of quantification of the fetal left ventricular volume during end-diastole, using the VOCAL™ (Virtual Organ Computer-aided AnaLysis) tool and manual trace.

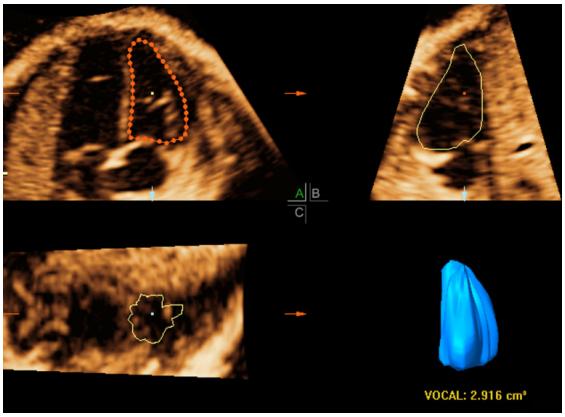

Figure 9: Example of quantification of the fetal right ventricular volume during end-diastole, using the VOCAL™ (Virtual Organ Computer-aided AnaLysis) tool and manual trace.

The SV from both ventricles was calculated by taking the difference between the end-diastolic and end-systolic volumes. Doppler velocity waveforms were recorded from the outflow tracts, and the aortic and pulmonary valve diameters were measured. Intraclass correlation was used to evaluate the agreement between left and right SV obtained by the two techniques, and proportionate Bland-Altman plots were constructed. The amount of time necessary to obtain SV using both techniques was also analyzed.

For the left ventricle SV (measured by 4D STIC), intraobserver and interobserver correlation coefficients were 0.98 (95% CI 0.92-0.99) and 0.95 (95% CI 0.88-0.97), respectively. For the right ventricle SV (measured by 4D STIC), intraobserver and interobserver correlation coefficients were 0.97 (95% CI 0.91-0.98) and 0.93 (95% CI 0.87-0.95), respectively. There was good agreement found between SV measured either by 2D Doppler or 4D STIC. The intraclass correlation coefficient between 2D Doppler and 4D STIC measurements were 0.977 (95% CI 0.963-0.986) and 0.980 (95% CI 0.968-0.988), for the LV and RV, respectively. The proportionate limits of agreement between the two methods were 18.7 to 23.9% and -20.9 to 21.7%, for the LV and RV, respectively. Importantly, the average time necessary to acquire a 4D STIC volume and analyze both left and right SV was 3.1 ± 0.84 minutes, which was significantly lower than the time necessary to obtain the same measurements using 2D Doppler (7.9 ± 2.3 minutes; $p<0.0001$).

Rizzo *et al.* noted several advantages in using 4D STIC (vs. 2D Doppler): 1) SV can be measured by only obtaining the four-chamber view, and then automatically acquiring a cardiac volume dataset; 2) STIC avoids the "operator dependency" of the measurements necessary when using 2D Doppler, which also requires various cardiac views in order to measure valve diameter and Doppler velocity waveforms; and 3) it dramatically reduces the time necessary to measure SV. Therefore, 4D STIC was a simple and rapid technique to estimate fetal SV, and is likely to become the method of choice (Rizzo Prenat Diagn 2007) [17].

Molina *et al.* reported cross-sectional reference intervals (12-34 weeks of gestation) for fetal SV and CO in 140 normal singleton pregnancies (Molina UOG 2008) [16]. This was accomplished by initially measuring ventricular volumes (systole and diastole) using 4D STIC and the VOCAL™ technique (30º rotation), and demonstrated its feasibility. In 50 cases, SV were measured by the same sonologist twice, and intraobserver agreement of measurements was calculated. Mean left and right SV and CO increased exponentially with gestation (Table **6**).

Table 6: Mean (5^{th}, 95^{th} centiles) of left and right stroke volume and left and right cardiac output with gestation.

Gestational age(weeks)	Left stroke volume (mL)	Right stroke volume (mL)	Left cardiac output (mL/min)	Right cardiac output (mL/min)
12	0.02 (0.01, 0.03)	0.01 (0.01, 0.02)	2.39 (1.45, 3.92)	1.80 (1.01, 3.21)
13	0.02 (0.02, 0.04)	0.02 (0.01, 0.03)	3.80 (2.31, 6.25)	3.15 (1.78, 5.58)
14	0.04 (0.02, 0.06)	0.03 (0.02, 0.06)	5.86 (3.57, 9.64)	5.24 (2.98, 9.22)
15	0.06 (0.04, 0.09)	0.05 (0.03, 0.09)	8.78 (5.34, 14.43)	8.33 (4.76, 14.56)

Table 6: cont…

16	0.08 (0.05, 0.14)	0.08 (0.05, 0.14)	12.75 (7.76, 20.97)	12.68 (7.30, 22.02)
17	0.12 (0.07, 0.20)	0.12 (0.07, 0.21)	18.02 (10.96, 29.62)	18.54 (10.75, 31.98)
18	0.17 (0.10, 0.27)	0.18 (0.10, 0.30)	24.78 (15.08, 40.73)	26.10 (15.23, 44.72)
19	0.22 (0.14, 0.37)	0.24 (0.14, 0.40)	33.22 (20.21, 54.60)	35.48 (20.84, 60.41)
20	0.30 (0.18, 0.48)	0.32 (0.19, 0.54)	43.45 (26.44, 71.42)	46.72 (27.63, 79.02)
21	0.38 (0.23, 0.62)	0.41 (0.24, 0.69)	55.54 (33.79, 91.28)	59.75 (35.57, 100.38)
22	0.48 (0.29, 0.78)	0.51 (0.31, 0.87)	69.44 (42.25, 114.13)	74.42 (44.59, 124.19)
23	0.59 (0.36, 0.97)	0.63 (0.37, 1.06)	85.04 (51.74, 139.77)	90.52 (54.60, 150.06)
24	0.72 (0.44, 1.17)	0.76 (0.45, 1.27)	102.12 (62.13, 167.85)	107.83 (65.48, 177.59)
25	0.85 (0.52, 1.39)	0.89 (0.53, 1.50)	120.41 (73.26, 197.91)	126.16 (77.12, 206.40)
26	0.99 (0.61, 1.62)	1.03 (0.61, 1.74)	139.58 (84.92, 229.41)	145.38 (89.45, 236.27)
27	1.14 (0.69, 1.86)	1.18 (0.70, 1.99)	159.24 (96.89, 261.73)	165.46 (102.49, 267.13)
28	1.28 (0.79, 2.10)	1.34 (0.79, 2.25)	179.04(108.93,294.26)	186.51 (116.29, 299.13)
29	1.43 (0.88, 2.34)	1.50 (0.89, 2.53)	198.61(120.84,326.43)	208.82 (131.07, 332.69)
30	1.58 (0.96, 2.58)	1.69 (1.00, 2.84)	217.65(132.42,357.72)	232.86 (147.13, 368.55)
31	1.71 (1.05, 2.80)	1.88 (1.12, 3.17)	235.91(143.53,387.73)	259.37 (164.97, 407.79)
32	1.84 (1.13, 3.01)	2.11 (1.25, 3.55)	253.21(154.06,416.18)	289.36 (185.27, 451.93)
33	1.97 (1.20, 3.21)	2.37 (1.41, 3.98)	269.48(163.96,442.91)	324.25 (208.99, 503.08)
34	2.08 (1.27, 3.40)	2.67 (1.59, 4.50)	284.70(173.22,467.93)	365.98 (237.45, 564.09)

Reproduced with permission from Molina *et al.* UOG 2008 [16]

Moreover, the ratio of right to left SV increased significantly with gestation, from about 0.97 (12 weeks) to 1.13 (34 weeks). In the Bland-Altman plot, the mean percentage difference and 95% limits of intraobserver agreement for left SV and right SV were -2.1% (-18.4, 14.2) and -0.8% (-16.4, 18.0), respectively.

It is noteworthy that right and left CO values from this study are smaller than those in prior reports, which estimated CO from either 2D sonographic assessment of ventricular dimensions or from cross-sectional area measurements and Doppler velocity waveforms of the outflow tracts (Table **7**).

Table 7: Fetal cardiac output in previous studies using two-dimensional ultrasound in comparison with the results of the present study.

		Left cardiac output (mL/min)			**Right cardiac output (mL/min)**		
Reference	**Method of measurement**	**16 weeks**	**24 weeks**	**34 weeks**	**16 weeks**	**24 weeks**	**34 weeks**
Mielke and Benda [26]	Vessel area and TVI	20	125	380	30	175	575
Allan *et al.* [33]	Vessel area and TVI	33	127	409	44	171	547
De Smedt *et al.* [34]	AVV area and TVI	50	150	550	75	175	625
Kenny *et al.* [35]	Vessel area and TVI	83	167	400	116	226	518
Veille *et al.* [36]	M-mode echocardiography	48	142	362	55	166	433
Schmidt *et al.* [32]	Method of discs	23	102	367	34	133	423
Molina *et al.* [16]	4D ultrasound and VOCAL	13	102	285	13	108	366

4D, four-dimensional; AVV, atrioventricular valve; TVI, time velocity integral; VOCAL, Virtual Organ Computer-aided AnaLysis Slightly modified and reproduced with permission from Molina *et al*. UOG 2008 [16]

Molina *et al.* suggested that the results of the Doppler studies may be inaccurate, because fetal myocardium is greatly limited in its ability to contract when compared to the postnatal period; therefore, the Frank-Starling law (on which the Doppler technique relies) applies in a different manner (Molina UOG 2008) [16].

Molina *et al.* also point out that the findings of fetal CO are not comparable with postnatal Doppler study results because: 1) myocardial contractility is better and ventricular compliance greater in the neonatal period than in fetal life (Anderson 1990) [37] (Teitel 2003) [38]; and 2) in the first hours after birth (following closure of the ductus arteriosus and the foramen ovale), there is a decrease in CO. Indeed, Winberg *et al.* measured CO in normal newborns using Doppler sonography, and demonstrated a decrease from about 240 mL/min/kg (in the first 2 hours), to 190 mL/min/kg (at 24 hours) (Winberg ADC 1989) [39].

Uittenbogaard *et al.* performed a prospective, longitudinal study (12-30 weeks of gestation) using 4D STIC to provide reference values for left and right ventricular volumes and cardiac function indices (SV, CO, EF) (Uittenbogaard UOG 2009) [18]. Additionally, the relationships of cardiac function indices with gestational age and estimated fetal weight were determined. All volumetric data were obtained using the 3D Slice method (Fig. **5**), which is based on Simpson's rule. Table **8** shows the mean, 5th, and 95th centiles of left and right ventricular SV in relation to gestational age. The mean right to left SV ratio showed right dominance, remaining fairly constant (around 1.2) throughout gestation. Bland-Altman analysis showed a coefficient of variation for measured SV of 13.7% (intraobserver agreement).

Table 8 Mean, 5th, and 95th centiles of left and right ventricle stroke volume, in relation to gestational age

Gestational Age (weeks)	Left ventricle SV (mL)			Right ventricle SV (mL)		
	Mean	5th	95th	Mean	5th	95th
12	0.02	0.00	0.03	0.02	0.00	0.04
13	0.03	0.00	0.05	0.03	0.00	0.06
14	0.04	0.01	0.07	0.04	0.00	0.08
15	0.06	0.01	0.11	0.07	0.01	0.12
16	0.09	0.02	0.15	0.10	0.02	0.18
17	0.13	0.04	0.22	0.14	0.04	0.24
18	0.18	0.06	0.29	0.20	0.08	0.33
19	0.24	0.10	0.39	0.28	0.12	0.44
20	0.33	0.15	0.51	0.37	0.18	0.56
21	0.42	0.21	0.64	0.48	0.25	0.71
22	0.54	0.28	0.80	0.61	0.34	0.88
23	0.66	0.36	0.97	0.75	0.44	1.06
24	0.80	0.45	1.15	0.90	0.54	1.26
25	0.94	0.53	1.34	1.04	0.63	1.45
26	1.07	0.61	1.54	1.18	0.71	1.64
27	1.19	0.67	1.72	1.30	0.77	1.82
28	1.30	0.71	1.88	1.39	0.80	1.97
29	1.37	0.71	2.03	1.44	0.79	2.09
30	1.41	0.68	2.14	1.46	0.74	2.18

Data based on regression equations. SV, stroke volume. Slightly modified and reproduced with permission from Uittenbogaard *et al.* UOG 2009 [18]

Table **9** compares the results of combined SV determined from this study to that of prior studies, which used either 2D sonography and Doppler or 4D sonographic techniques, demonstrating varying results (Allan BHJ 1987) [33] (Kenny Circulation 1986) [35] (Rasanen Circulation 1996) [30] (Mielke Circulation 2001) [26] (Messing UOG 2007) [15] (Molina UOG 2008) [16] ([a]Uittenbogaard UOG 2009) [18]. Regardless of the method used, all studies using 4D echocardiography reported relatively small SV values, as compared with the larger values found in studies that used 2D sonography and Doppler (Table **9**).

Table 9 Combined left and right stroke volumes in previous studies using both two-dimensional and four-dimensional ultrasound imaging in comparison with the results of the present study

Reference	Method of measurement	20 weeks	24 weeks	30 weeks
Allan *et al.* (1987) [33]	Vessel area and TVI	1.16	2.13	4.49
Kenny *et al.* (1986) [35]	Vessel area and TVI	1.93	2.74	4.63
Rasanen *et al.* (1996) [30]	Vessel area and TVI	1.18	2.89	6.28
Mielke and Benda (2001) [26]	Vessel area ansd TVI	0.85*	2.06*	4.56*
Messing *et al.* (2007) [15]	STIC and inversion mode	0.41	1.26	2.95
Molina *et al.* (2008) [16]	STIC and VOCAL	0.55	1.27	2.69
Uittenbogaard *et al.* (2009) 18]	STIC and 3D Slice Method	0.72	1.72	2.63

*Obtained from figures. 3D, three dimensional; STIC, spatiotemporal image correlation; TVI, time velocity integral; VOCAL, Virtual Organ Computer-aided AnaLysis. [a]Slightly modified and reproduced with permission from Uittenbogaa

Uittenbogaard *et al.* suggested that this could be due to: 1) inaccuracies in Doppler calculations based on prenatal and postnatal differences in myocardial contractility (Molina UOG 2008 [16]; and 2) errors in measurement of the vessel cross-sectional area in Doppler studies, where the lumen is assumed to be round but instead, could be slightly oval. Therefore, Uittenbogaard *et al.* has proposed that the Doppler studies may have been erroneous, and led to overestimation of fetal SV (and subsequently CO) (Uittenbogaard UOG 2009) [18]. Since 4D echocardiographic estimations avoid geometric assumptions and are less susceptible to measurement errors, these results may provide a more accurate reflection of fetal cardiac volumes and function.

Table **10** shows the mean, 5th, and 95th centiles of left and right CO in relation to gestational age. To calculate left and right CO, SVs were multiplied by the fetal heart rate, as recorded within each STIC volume. The mean left CO increased from 2.40 (95% CI, 0.63-4.18) mL/min at 12 weeks, to 197.74 (95% CI, 98.40-297.08) mL/min at 30 weeks. The mean right CO increased from 2.60 (95% CI, 0.00-5.22) mL/min at 12 weeks to 204.81 (95% CI, 105.01-304.61) mL/min at 30 weeks.

Table 10: Mean, 5th, and 95th centiles of left and right cardiac output, in relation to gestational age.

Gestational Age (weeks)	Left cardiac output (mL/min)			Right cardiac output (mL/min)		
	Mean	5th	95th	Mean	5th	95th
12	2.40	0.63	4.18	2.60	0.00	5.22
13	3.81	0.78	6.83	4.17	0.17	8.16
14	5.88	1.10	10.65	6.51	0.67	12.34
15	8.84	1.84	15.85	9.88	1.73	18.04
16	12.98	3.25	22.71	14.61	3.67	25.56
17	18.58	5.64	31.52	21.03	6.82	35.24
18	25.93	9.28	42.58	29.46	11.51	47.41
19	35.29	14.45	56.13	40.18	18.01	62.34
20	46.82	21.29	72.34	53.32	26.47	80.18
21	60.58	29.88	91.28	68.89	36.87	100.90
22	76.42	40.06	112.79	86.62	48.97	124.27
23	94.01	51.49	136.53	106.02	62.26	149.78
24	112.77	63.60	161.94	126.31	75.96	176.65
25	131.90	75.59	188.20	146.47	89.07	203.87
26	150.43	86.50	214.36	165.32	100.38	230.25
27	167.28	95.24	239.33	181.62	108.68	254.56
28	181.40	100.74	262.05	194.22	112.80	275.64
29	191.80	102.05	281.55	202.16	111.78	292.53
30	197.74	98.40	297.08	204.81	105.01	304.61

Data based on regression equations. CO, cardiac output.

Slightly modified and reproduced with permission from Uittenbogaard *et al.* UOG 2009 [18]

Mean left and right ventricular EF remained constant with advancing gestational age. The mean left ventricle EF was 0.45 (95% CI, 0.23-0.67), and the mean right ventricle EF was 0.46 (95% CI 0.26-0.66).

Recently, *our group determined fetal CO from datasets acquired with 4D echocardiography, using STIC and VOCAL™ technology. From VOCAL™, the sub-feature "Contour Finder/Trace" was utilized, which employs a sophisticated algorithm that helps to find the contour of the ventricle as the mouse is moved along the ventricular wall. There were 102 fetuses evaluated cross-sectionally between 19-39 weeks of gestation, and results were grouped according to gestational age quartiles (19-24, 25-28, 29-32, and 33-39 weeks). Ventricular volumes were determined from both ventricles in systole and diastole. Left and right ventricular SV were calculated, and then used to compute CO (ml/min). CO was also expressed as a function of estimated fetal weight (ml/min/kg) (Table **11**).

Table 11: Mean (± SD) left and right cardiac output (also expressed as a function of estimated fetal weight) in relation to gestational age

Gestational age (weeks)	Left ventricle CO (ml/min)	Left ventricle CO (ml/min/kg)	Right ventricle CO (ml/min)	Right ventricle CO (ml/min/kg)
19-24 (n=26)	47 ± 12	98 ± 21	46 ± 11	96 ± 19
25-28 (n=26)	81 ± 22	89 ± 25	97 ± 16	96 ± 16
29-32 (n=26)	133 ± 28	89 ± 17	126 ± 35	84 ± 22
33-39 (n=25)	210 ± 39	85 ± 16	236 ± 41	95 ± 14

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The following observations were made: 1) left and right ventricular CO increased with gestational age (Spearman rho = 0.8, $P < 0.001$); 2) however, CO expressed as a function of estimated fetal weight did not change with advancing gestational age (LV CO: $r_s = -0.06$, $P = 0.5$; RV CO: $r_s = -0.03$; $P = 0.8$); and 3) intra- and inter-observer coefficients of variation were 2.3% and 4.0%, respectively. Therefore, we found that fetal CO can be reproducibly estimated with 4D echocardiography, using STIC and VOCAL™ techniques.

An area of future investigation that may be applicable to the fetus was studied by Pemberton *et al.* in 50 pediatric/adult patients (Pemberton JASE 2005) [40]. Pemberton *et al.* investigated the use of real-time color Doppler 3D echocardiography (xMatrix™ probe) to calculate SV, and compared this to 2D pulsed Doppler measurements. Using 3D Doppler data, flow volumes were calculated using specially designed computer software. There was excellent correlation between the SV obtained from live 3D echocardiography and 2D Doppler ($r^2 = 0.90$; $p < 0.001$, standard error of the estimate = 6.98 mL).

CONCLUSIONS

Quantifying ventricular volume and calculating cardiac function parameters reliably is important in assessing cardiac function, as well as the severity and prognosis of cardiac disease. Both two- and three-dimensional echocardiography have been used for these purposes, but have limitations. Four-dimensional echocardiography appears to overcome most of the pitfalls of traditional methods. It avoids geometric assumptions, is less susceptible to measurement errors, and has proven to be a feasible method to assess fetal cardiac function. Moreover, the performance of 4D STIC is uncomplicated and rapid. Using this technology, normal reference ranges of ventricular volume (end-diastolic and end-systolic), stroke volume, cardiac output, and ejection fraction throughout gestation have been generated. STIC promises to be part of the methodology used to assess fetal cardiac function. Further studies, however, are needed to determine the behavior of fetuses with abnormal cardiac function, and to correlate this with adverse perinatal outcome.

REFERENCES

[1] Esh-Broder E, Ushakov FB, Imbar T, Yagel S. Application of free-hand three-dimensional echocardiography in the evaluation of fetal cardiac ejection fraction: a preliminary study. Ultrasound Obstet Gynecol 2004; 23(6): 546-51.

[2] Simpson JM, Cook A. Repeatability of echocardiographic measurements in the human fetus. Ultrasound Obstet Gynecol 2002; 20(4):332-9.

[3] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 1976; 37(1):7-11.

[4] Bhat AH, Corbett V, Carpenter N, *et al*. Fetal ventricular mass determination on three-dimensional echocardiography: studies in normal fetuses and validation experiments. Circulation 2004; 110(9):1054-60.

[5] Wyatt HL, Meerbaum S, Heng MK, Gueret P, Corday E. Cross-sectional echocardiography. III. Analysis of mathematic models for quantifying volume of symmetric and asymmetric left ventricles. Am Heart J 1980; 100(6 Pt 1):821-8.

[6] Sedmera D, Pexieder T, Vuillemin M, Thompson RP, Anderson RH. Developmental patterning of the myocardium. Anat Rec 2000; 258(4): 319-337.

[7] Simpson J. Echocardiographic evaluation of cardiac function in the fetus. Prenat Diagn 2004; 24 (13): 1081-91.

[8] Bhat AH, Corbett VN, Liu R, *et al.* Validation of volume and mass assessments for human fetal heart imaging by 4-dimensional spatiotemporal image correlation echocardiography: in vitro balloon model experiments. J Ultrasound Med 2004; 23(9)1151-

[9] Espinoza J, Gotsch F, Kusanovic, JP, *et al.* Changes in fetal cardiac geometry with gestation: implications for 3- and 4-dimensional fetal echocardiography. J Ultrasound Med 2007; 26(4): 427-43.

[10] Levental M, Pretorius DH, Sklansky MS, Budorick NE, Nelson TR, Lou K. Three-dimensional ultrasonography of normal fetal heart: comparison with two-dimensional imaging. J Ultrasound Med 1998; 17(6):341-8.

[11] Meyer-Wittkopf M, Cole A, Cooper SG, Schmidt S, Sholler GF. Three-dimensional quantitative echocardiographic assessment of ventricular volume in healthy human fetuses and in fetuses with congenital heart disease. J Ultrasound Med 2001; 20(4):317-27.

[12] Tutschek B, Sahn DJ. Semi-automatic segmentation of fetal cardiac cavities: progress towards an automated fetal echocardiogram. Ultrasound Obstet Gynecol 2008; 32(2):176-80.

[13] Uittenbogaard LB, Haak MC, Spreeuwenberg MD, VanVugt JMG. A systematic analysis of the feasibility of four-dimensional ultrasound imaging using spatiotemporal image correlation in routine fetal echocardiography. Ultrasound Obstet Gynecol 2008; 31(6): 625- 32.

[14] Huhta J. Congestive heart failure in the fetus. In Fetal Cardiology (2nd edition), Yagel S, Silverman NH, Gembruch U (eds.). Informa Healthcare USA, Inc: New York, NY, 2009; 561-78.

[15] Messing B, Cohen SM, Valsky DV, *et al*. Fetal cardiac ventricle volumetry in the second half of gestation assessed by 4D ultrasound using STIC combined with inversion mode. Ultrasound Obstet Gynecol 2007; 30(2):142-51.

[16] Molina FS, Faro C, Sotiriadis A, Dagklis T, Nicolaides KH. Heart stroke volume and cardiac output by four-dimensional ultrasound in normal fetuses. Ultrasound Obstet Gynecol 2008; 32(2):181-7.

[17] Rizzo G, Capponi A, Cavicchioni O, Vendola M, Arduini D. Fetal cardiac stroke volume determination by four-dimensional ultrasound with spatio-temporal image correlation compared with two-dimensional and Doppler ultrasonography. Prenat Diagn 2007; 27(12): 1147-50.

[18] Uittenbogaard LB, Haak MC, Spreeuwenberg MD, VanVugt JMG. Fetal cardiac function assessed with four-dimensional ultrasound imaging using spatiotemporal image correlation. Ultrasound Obstet Gynecol 2009; 33(3):272-81.

[19] DeVore GR, Falkensammer P, Sklansky MS, Platt LD. Spatio-temporal image correlation (STIC): new technology for evaluation of the fetal heart. Ultrasound Obstet Gynecol 2003; 22(4):380-7.

20] Gonçalves LF, Lee W, Espinoza J, Romero R. Examination of the fetal heart by four-dimensional (4D) ultrasound with spatio-temporal image correlation (STIC). Ultrasound Obstet Gynecol 2006; 27(3):336-48.

[21] Nelson TR, Pretorius DH, Sklansky M, Hagen-Ansert S. Three-dimensional echocardiographic evaluation of fetal heart anatomy and function: acquisition, analysis, and display. J Ultrasound Med 1996; 15(1):1-9.

[22] Gonçalves LF, Espinoza J, Lee W, Mazor M, Romero R. Three- and four-dimensional reconstruction of the aortic and ductal arches using inversion mode: a new rendering algorithm for visualization of fluid-filled anatomical structures. Ultrasound Obstet Gynecol 2004; 24(6):696-98.

[23] National Institute of Standards and Technology. Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results. http://emtoolbox.nist.gov/Publications/NISTTechnicalNote1297s.pdf (July 13 2009).

[24] Bartlett JW, Frost C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. Ultrasound Obstet Gynecol 2008; 31(4): 466-75.

[25] Hamill N, Romero R, Hassan S, *et al.* The repeatability and reproducibility of fetal cardiac ventricular volume calculations utilizing Spatio-Temporal Image Correlation (STIC) and Virtual Organ Computer-aided AnaLysis (VOCAL™). J Ultrasound Med, 2009; 28:1301-1311.

26] Mielke G, Benda N. Cardiac output and central distribution of blood flow in the human fetus. Circulation 2001; 103(12)1662-8.

[27] Stewart WJ, Jiang L, Mich R, Pandian N, Guerrero JL, Weyman AE. Variable effects of changes in flow rate through the aortic, pulmonary and mitral valves on valve area and flow velocity: impact on quantitative Doppler flow calculations. J Am Coll Cardiol 1985; 6(3):653-62.

[28] Eik-Nes S, Marsal K, Kristofferson K. Methodology and basic problems related to blood flow studies in the human fetus. Ultrasound Med Biol 1984; 10(3):329-37.

[29] Chaoui R, Heling KS, Taddei F, Bollmann R. Doppler echocardiographic analysis of blood flow through the fetal aorta and pulmonary valve in the second half of pregnancy. Geburtshilfe Frauneheilkd 1995; 55(4): 207-17.

[30] Rasanen J, Wood DC, Weiner S, Ludomirski A, Huhta JC. Role of the pulmonary circulation in the distribution of human fetal cardiac output during the second half of pregnancy. Circulation 1996; 94(5): 1068-1073.

[31] Thornburg KL, Reller MD. Coronary flow regulation in the fetal sheep. Am J Physiol 1999; 277(5 Pt 2): R1249-60.

[32] Schmidt KG, Silverman NH, Hoffman JIE. Determination of ventricular volumes in human fetal hearts by two-dimensional echocardiography. Am J Cardiol 1995; 76(17):1313-16.

[33] Allan LD, Chita SK, Al-Ghazali W, Crawford DC, Tynan M. Doppler echocardiographic evaluation of the normal human fetal heart. Br Heart J 1987; 57(6):528-33.

34] De Smedt MC, Visser GH, Meijboom EJ. Fetal cardiac output estimated by Doppler echocardiography during mid- and late gestation. Am J Cardiol 1987; 60(4):338-42.

[35] Kenny JF, Plappert T, Doubilet P, *et al.* Changes in intracardiac blood flow velocities and right and left ventricular stroke volumes with gestational age in the normal human fetus: a prospective Doppler echocardiographic study. Circulation 1986; 74(6):1208-16.

[36] Veille JC, Sivakoff M, Nemeth M. Evaluation of the human fetal cardiac size and function. Am J Perinatol 1990; 7(1):54-9.

[37] Anderson PAW. Myocardial development. In Fetal and Neonatal Cardiology, Long W (ed.). W.B. Saunders: Philadelphia, PA, 1990; 17-38.

[38] Teitel DF. Physiologic Development of the Cardiovascular System in the Fetus. In Fetal and Neonatal Physiology (3rd edition), Polin RA, Fox WW, Abman H (eds.). W.B. Saunders: Philadelphia, PA, 2003; 609-19.

[39] Winberg P, Jansson M, Marions L, Lundell BP. Left ventricular output during postnatal circulatory adaptation in healthy infants born at full term. Arch Dis Child 1989; 64:1374-78.

[40] Pemberton J, Li X, Kenny A, Davies CH, Minette MS, Sahn DJ. Real-time 3-dimensional Doppler echocardiography for the assessment of stroke volume: an in vivo human study compared with standard 2-dimensional echocardiography. J Am Soc Echocardiogr 2005; 18(10):1030-36.