The Fetal Heart

Critical appraisal of three-dimensional echocardiography

Lukas Bastiaan Uittenbogaard

The studies described in this thesis were performed at the Division of Prenatal Medicine and Screening, Department of Obstetrics and Gynecology, VU University medical Center, Amsterdam, the Netherlands.

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CONTENT

APPENDICES

Chapter 1

General introduction

GENERAL INTRODUCTION

Ultrasound technology has fundamentally changed the view on intrauterine life. Until a few decades ago, knowledge of intrauterine life was relatively limited. Currently, prenatal sonographic assessment is a clinical standard in modern obstetric care. Both ultrasound technology and computing power have developed rapidly, providing sonographers detailed images of the growing human fetus. Targeted ultrasound examinations provide the possibility to diagnose a wide variety of structural malformations, genetic syndromes and diseases from early-stage pregnancy onward. Following other countries, the Netherlands adopted the twenty week anomaly scan as a standard component of obstetric care in 2007. The main aim of routine screening is the early detection of fetal anomalies. Sonographic findings may lead to changes in obstetric management increasing fetal or maternal prognosis. In case of major structural malformation it may, after thorough counselling, eventually lead to termination of pregnancy if this is requested by the parents[1].

The accuracy and success of prenatal screening programs, as they have been performed in many countries for a number of years, depend strongly on the skills of the sonographer. This especially holds true for the detection of congenital heart disease (CHD)[2, 3]. Although prenatal detection rate of most fetal malformations has increased over the years, CHD still comprises the most commonly overlooked lesions in prenatal screening programs[2, 14-16]. This is a valid cause for concern because CHD represents a common congenital anomaly. Approximately 4 to 8 out of 1000 babies are born with CHD[4]. Moreover, CHD is a leading cause of infant mortality in the first year of life[5]. It is known that early and accurate prenatal diagnosis reduces the neonatal morbidity and mortality rates by selecting of the appropriate institution for delivery[6-9]. In conventional screening protocols that are aimed at detecting CHD, the four-chamber view of the fetal heart has been the standard approach. A four-chamber view is a transverse image through the four 'chambers' of the heart. A standard four-chamber view displays both atria, both ventricles and a number of other cardiac structures that can be identified to rule out CHD. The additional identification of the right and left outflow tract, however, has been shown to markedly improve the detection rate of CHD[10-12]. Recent ultrasound guidelines have therefore adopted this as the extended basic cardiac examination used in current screening programs[13].

The application of new technologies has contributed to the improvements in sono-graphy during recent years. The resolution of the current high-end scanners is striking when compared to scanners built ten years ago. The early attempts to image the fetal heart in three dimensions reported the use of cumbersome acquisition systems employing elec-

tromagnetic position sensors attached to the standard transducers to compose three-dimensional (3D) cardiac images[17]. Improvements of both computing power and scanning technology have made it possible for the current advanced scanners to obtain a 3D cardiac volume in a matter of seconds. Improvements in image analysis according to their spatial and temporal domain added the time dimension to 3D echocardiography, referred to as four-dimensional (4D) ultrasound. Spatiotemporal image correlation (STIC) is a technology that creates this 4D volume of a fetal heart cycle[15]. STIC is an automated volume acquisition in which a computer analysis creates a moving image (cine-loop) of optimally rearranged two-dimensional images. The transducer performs a slow sweep, recording a 3D data set over a 7.5–15 second time period. This volume of interest is acquired at a sweep angle of approximately 20–40 degrees (depending on the size of the fetus) and frame rate of about 150 frames per second. If a volume acquisition time of 10 seconds and sweep area of 25 degrees are used, up to 1500 images are stored. During this acquisition the fetal heart beats 20–25 times, which means that within these 1500 images there are 20–25 images showing systolic peaks. This rhythmic movement is used to calculate the fetal heart rate. As a result, 40 consecutive volumes are used to reconstruct a complete heart cycle that is displayed in an endless loop. This cine-loop of a beating fetal heart can be manipulated to display any acquired scanning plane at any stage in the cardiac cycle.

Obstetricians and sonographers might benefit from the introduction of 3D echocardiography in several ways. This modality allows volume acquisition of the fetal heart, which enables the examiner to navigate within the heart, re-slice, and produce all of the standard image planes. This avoids the necessity of tilting, angling and rotation of the ultrasound probe, which is exactly what makes fetal echocardiography one of the most challenging areas for sonographers to master. The possibility to standardize sonographic assessments by using automated retrieval of all relevant anatomic cardiac planes could make fetal cardiac screening less dependent on individual scanning skills.

Additionally, in cases of suspected decreased fetal condition, timing of the delivery is commonly based on fetal haemodynamics. Fetal heart rate monitoring and the measurement of pulsatility indices of fetal arteries and veins are commonly used modalities. In case of CHD, hydrops fetalis, fetal diabetic cardiomyopathy, fetal infection, intrauterine growth restriction or twin-to-twin transfusion syndrome, accurate and reliable measurement of fetal cardiac function could be valuable for fetal surveillance and possibly aid in management decisions. Therefore, additional methods to give insight in the actual fetal condition could be very useful. Cardiac function, in both adults and children, is commonly expressed in ejection fraction and stroke volume. Both indices can be calculated from end-systolic and end-diastolic ventricle volumes. To obtain these volumes,

a common method in two-dimensional ultrasound is to divide the ventricle into parallel slices. The circumferences are then traced and summed using Simpson's rule[18]. This method is, however, based upon the assumption that the ventricle has a perfect cylindrical shape, which is known not to be accurate. Furthermore, these measurements are not very reliable[19,20]. Another well known conventional method to estimate fetal cardiac function uses pulsed Doppler to estimate fetal blood flow through the valve orifices[21, 22]. The clinical usefulness of this method is, however, also limited because of its inaccuracy[20, 23]. The dynamic and 3D nature of STIC volumes enables the reconstruction of 3D rendered images that contain depth and volume which may provide additional information that is not available from the thin multiplanar image slices. Volume measurements could provide volumetric information of both ventricles at different moments in the cardiac cycle without the need to make any geometrical assumptions about the shape of the heart. This new technique could therefore provide an improved alternative for the evaluation of cardiac function.

AIM OF THIS THESIS

The aim of this thesis was to evaluate the clinical use of 3D echocardiography in the human fetus. The studies described in this thesis aimed to answer the following questions:

- Can sonographers successfully implement the use of 3D ultrasound imaging and STIC in their routine ultrasound practice?
- Is automated cardiac screening using 3D ultrasound volumes feasible?
- How accurate are volumetric measurements made using 3D ultrasound imaging software and STIC in the small volume range of fetal cardiac ventricles?
- What measurement method should be used for such measurements?
- How reliable are these 3D volumetric measurements and what factors influence their reliability?
- What are normal values and reference ranges for fetal ventricle volumes throughout gestation?
- Can we establish normal values for fetal heart stroke volume and ejection fraction as parameters of the fetal cardiac function?

OUTLINE OF THE THESIS

In the first chapters we sought to evaluate the feasibility of 3D ultrasound in clinical practice. In **Chapter 2** the feasibility of an automated 3D software tool for an automated

Figure 1 *Accuracy and precision. left: High accuracy but low precision; right: High precision but low accuracy.*

extended basic cardiac screening is assessed in routine practice. This chapter describes a prospective study in which four sonographers incorporated the acquisition of 3D ultrasound volumes in their routine examinations during a 2-month period. All volumes were assessed on image quality and on the possibilities of automated retrieval of relevant cardiac imaging planes necessary for extended basic cardiac screening. In **Chapter 3** the feasibility of acquisition of STIC volumes is evaluated. In the prospective study described, STIC volumes were acquired by four sonographers with different levels of experience. The volumes were assessed on acquisition conditions, image quality, and the rendering abilities during postprocessing. Furthermore, possible learning effects and the influence of experience on STIC volume acquisition were studied.

In the next chapters we assessed STIC as a method to evaluate parameters of fetal cardiac function. **Chapter 4** describes an in vitro study. In this study a miniature balloon model was used to study the small volume range comparable to fetal cardiac heart chambers. In this study the feasibility and accuracy (or validity) of volumetric measurements from STIC volumes was evaluated (Figure 1). The STIC volumes were frozen in maximal and minimal distension of the balloon for volumetric measurements. Three different techniques of volumetric measurements were compared.
Because in the evaluation of a method, both accuracy and precision are of equal importance (Figure 1) we explored the precision (or reliability) of volumetric measurements from STIC volumes in **Chapter 5**. Three observers with different levels of experience performed a series of measurements based on manual tracing and summation of multiple slices. Both fetal cardiac chamber volumes and in-vitro balloon volumes were used to study intra-observer and interobserver reliability. The volumetric data were used to provide two indices of function, cardiac output and stroke volume. These were also assessed on reliability. **Chapter 6** describes an encountered limitation of four-dimensional ultrasound using STIC. In **Chapter 7** STIC volume acquisition and the studied volumetric mea-

surement method are assessed in a clinical setting. In this prospective longitudinal study STIC volumes were acquired periodically in 63 fetuses from 12 weeks of gestation onward. Volumetric data were measured by manual tracing of multiple slices of STIC volumes frozen in end-systole and end-diastole. These ventricle volumes were used to calculate indices of fetal cardiac function. This study provides reference values for left and right fetal heart chamber volumes and indices for cardiac function plotted against gestational age as well as estimated fetal weight. **Chapter 8** provides an overview of different non-invasive modalities currently available for the assessment the fetal cardiac function. Pulsed-wave Doppler and other ultrasound derived modalities such as tissue Doppler, myocardial strain and strain rate imaging are discussed. Further, the fetal electocardiogram and the newest imaging techniques as three and four-dimensional ultrasound and magneto resonance imaging (MRI) are also evaluated. This thesis ends with a general discussion and future prospects in **Chapter 9**. A summary of the thesis is given in **Chapter 10**.

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Chapter 2

A systematic analysis of the feasibility of four-dimensional ultrasound imaging using spatiotemporal image correlation in routine fetal echocardiography

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Adapted from *Ultrasound Obstet Gynecol* 2008; 31: 625–632

ABSTRACT

Objectives

To investigate the feasibility of incorporating spatiotemporal image correlation (STIC) into a tertiary fetal echocardiography program.

Methods

During the study period all pregnant women fitting our inclusion criteria were enrolled consecutively. Four sonographers participated in the study, one of whom had substantial previous experience of STIC volume acquisition and three of whom did not. STIC volumes were acquired within the time slot allocated for the usual examination and all attempts were recorded. STIC volumes were assessed on acquisition conditions, the quality (as defined by a checklist of cardiac structures that could be visualized), and the rendering abilities. Furthermore, possible learning effects and the influence of experience with STIC on volume acquisition were studied.

Results

STIC volume acquisition was successful in 75.7% (112/148) of cases in which it was attempted. The more experienced sonographer had a higher success rate in STIC volume acquisition (experienced vs. less experienced 88.4% vs. 70.5%, P=0.02). Of all analysed STIC volumes, 64.8% were of high or sufficient quality. STIC volume quality and rendering ability correlate strongly with the acquisition conditions. High quality STIC volumes successfully rendered the intracardiac septa in 84.6% of cases. The coronal AV plane was rendered in 12/26 cases (46.2%).

Conclusions

This study shows that incorporation of STIC volume acquisition into the daily practice of a tertiary fetal echocardiography program is feasible. Sonographers do not have to be specifically experienced in three- or four-dimensional ultrasound imaging to acquire high-quality STIC volumes. For successful STIC acquisition and subsequent successful analysis, correct acquisition conditions are of major importance. Finally, our results demonstrate that STIC is as susceptible as conventional two-dimensional ultrasound to individual variations and limitations in scanning windows.

INTRODUCTION

Advances in prenatal ultrasonography have improved prenatal care in recent decades and increased the detection of a large number of congenital malformations. The prenatal detection rate of congenital heart disease (CHD), however, has not shown the same increase as the detection of malformations in other fetal systems[1-3]. CHDs are the most commonly overlooked lesions in prenatal screenings programs[3, 4]. This causes concern as CHD represents the most common congenital malformation and is the leading cause of infant mortality in the first year of life[5]. It has been estimated that the incidence of CHD is 4-8 per 1000 neonates[6, 7].

Early detection and accurate prenatal diagnosis of CHD reduces neonatal morbidity and mortality rates by allowing provision of adequate prenatal and postnatal care[8, 9]. Before viability is reached, parents can be counselled on the diagnosis, severity, and prognosis. Furthermore, it provides parents with the opportunity to make informed decisions on the further course of pregnancy. The four-chamber view has become the standard approach to screen for CHD. The identification of the right and left outflow tracts markedly improves the detection rate of CHD[3, 10-13].

The myriad of fetal positions and the different maternal factors that influence the examination make fetal echocardiography one of the more difficult tasks for sonographers. To help improve and standardize fetal cardiac examinations, Yagel *et al.* and Yoo *et al.* described a method in which five cardiac planes are visualized for a complete examination of the fetal heart[14, 15]. Additionally, three and four dimensional (3D and 4D) ultrasound imaging can be a valuable tool in fetal echocardiography[1, 9, 10, 16-19].

Spatiotemporal image correlation (STIC) is a technique that allows examination of the fetal heart within a real-time 3D (i.e. 4D) volume, displayed in a cineloop. It has been proposed that fetal echocardiography using STIC has the potential to increase the detection rates for CHD because it decreases the dependency on sonographer skill[16, 20]. It allows the examination of cardiac planes, such as the lateral view of the interventricular septum (IVS plane) and the anteroposterior view of the atrioventricular annuli (CAV plane), that are technically very difficult to image using conventional two-dimensional (2D) echocardiography[17]. However, to be an effective tool in fetal echocardiography, STIC volume acquisition must be feasible in routine clinical practice. A number of reports on these new technologies have emphasized the requirement of a substantial learning curve for the use of 4D ultrasound imaging[10, 16]. This may explain the delay of a widespread introduction of this new technique in first- and second-level ultrasound departments, and even in tertiary referral centers. The aim of this study was to investigate the

Table 1 *Acquisition Condition score.*

Score	Movements	ROI setting	Acquisition angle	Apex position	Shadowing
0	Frequent	Too small	Too narrow	4-8 o'clock	Extensive
1	Rare	Too large	Too wide	8-10 or 2-4 o'clock	Moderate
2	Absent	Sufficient	Sufficient	11-2 o'clock	Absent

ROI, region of interest.

feasibility to incorporate STIC volume acquisition in a tertiary routine fetal echocardiography program of second-trimester fetuses.

METHODS

During a 2-months period (February and March 2007) all pregnant women who presented to the Department of Prenatal Medicine for a second trimester ultrasound examination, and who fitted our inclusion criteria, were enrolled consecutively. All women had an increased risk of fetal congenital malformations based on their history. Fetuses referred for suspected congenital malformations were excluded from the study. A 2D fetal echocardiographic examination is routinely incorporated into the ultrasound examination, using the five planes technique as described by Yagel *et al.* and Yoo *et al.*[14, 15].

Four experienced third-level sonographers participated in the study. In the 2-month study period, all sonographers incorporated STIC volume acquisition into their ultrasound examination of the fetal heart. All sonographers received brief training in the use of 3D and 4D ultrasound imaging, before the start of the study. One of the sonographers (L.U.) had already used STIC for more than a year in a dedicated fetal echocardiography program. This sonographer will be referred to as 'the STIC expert' and the other experienced sonographers as 'STIC beginners'. For this study all sonographers received the same instructions regarding the optimal conditions for STIC volume acquisition, as described by Gonçalves *et al.*, concerning fetal position, region of interest (ROI), acquisition angle, and acquisition time (detailed in Appendix)[9.] All women where asked to hold their breath during the STIC acquisition. Possible factors that would influence 4D ultrasound image quality were documented: body mass index (BMI), parity, gestational age, history of abdominal surgery, oligohydramnios, interfering fetal activity, fetal position and location of the placenta. The medical ethics committee approved to the study and all patients gave informed consent before ultrasound examination.

Table 2 *Spatiotemporal image correlation (STIC) volume quality according to visibility of cardiac structures.*

Displayed	High Quality	Sufficient Quality	Insufficient Quality
Four-chamber view*	√	√	-
Left outflow tract	√	√	-
Right outflow tract	√	√	-
Bifurcation of pulmonary artery	√	-	-
Pulmonary venous return	√	-	-
Systemic venous return	√	-	-
Entire aortic arch	√	-	-

*The minimum required cardiac structures visualized for each category of STIC volume quality are marked with √. *A four-chamber view is a symmetrical transverse plane through the fetal heart displaying two atria and two ventricles, the moderator band, the intracardiac septa, crux cordis and both atrioventricular valves.*

Volume acquisition, quality and rendering abilities

The sonographers were instructed not to make more than four attempts to acquire a STIC volume during each ultrasound examination. No additional time was added to the examination schedule of 30-minute slots, which included documentation. When a sonographer was not able to acquire a STIC volume within these limits, the attempt was documented as a failed acquisition. Every attempt was documented so that learning effects in STIC volume acquisition could be investigated. To acquire the STIC volumes, a 4D ultrasound system with integrated STIC software (Voluson E8, GE Medical Systems, Kretz, Austria) and a motorized 4-8 MHz curved-array transducer was used. After a successful acquisition the STIC volumes were stored and transported to a personal computer. The volumes were examined with Voluson 4D View 6.0 postprocessing software by one examiner (L.U.).

To examine the acquisition conditions, STIC volumes were assessed for movement artefacts, ROI setting, acquisition angle, fetal position and shadowing artefacts, using the score system shown in Table 1. Summation of the scores would determine the acquisition condition score (AC) score, which had a maximum of 10.
All successfully acquired STIC volumes were assessed with respect to their quality. STIC volumes were considered to be of insufficient, sufficient or high quality based on their ability to display different cardiac structures (Table 2). A number of different methods have been described for the examination of STIC volumes[1, 9, 10]. In this study we used the 'spin technique' as described by DeVore *et al.* in 2004, for the identification of the four-chamber view, the left and right outflow tracts, bifurcation of the pulmonary artery, pulmonary venous return, systemic venous return, ductal and aortic arches and arch vessels[10]. Figure 1 shows a STIC volume of high quality.

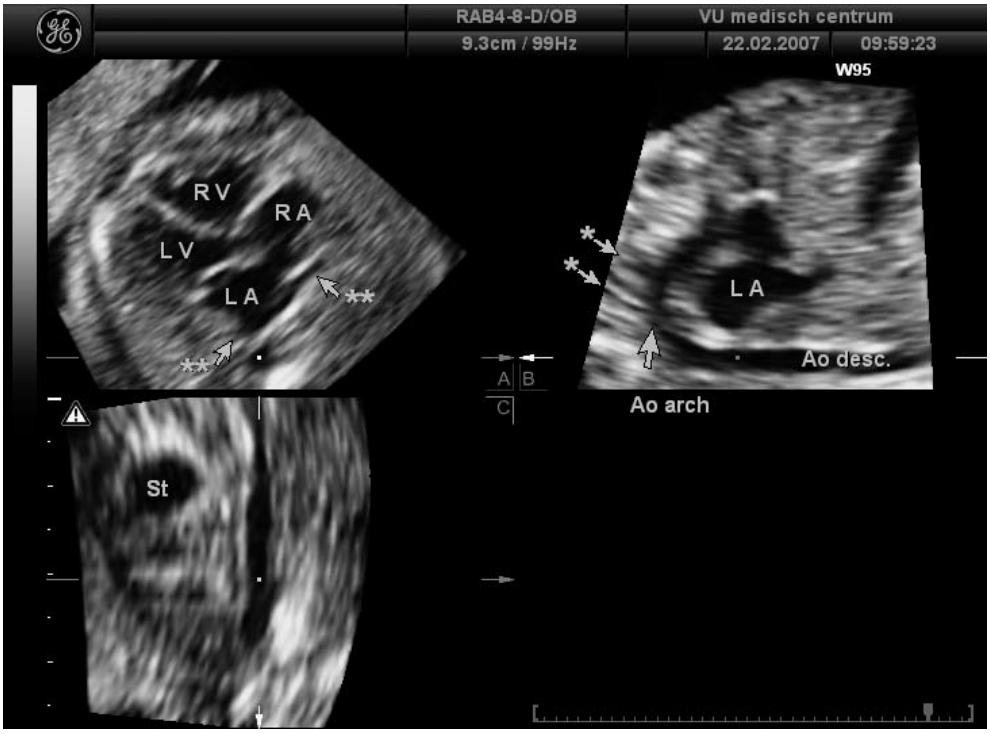

Figure 1 *A spatiotemporal image correlation (STIC) volume of a second-trimester (20 + 4 weeks) fetus in multiplanar view. The region of interest is set around the fetal heart and aorta, and the acquisition angle is set wide enough to fit the entire aortic arch and stomach. The apex of the fetal heart is pointing upwards in the original transverse plane (upper left panel), and there are no movement artefacts or shadowing artefacts visible. This STIC volume was considered to be of high quality. The four-chamber view is visible in the upper left panel with pulmonary venous return (**) to the left atrium (LA). The upper right panel is a longitudinal view of the aortic arch (Ao arch), with the neck and arm arteries (*) and descending aorta (Ao desc.) visible. LV, left ventricle; RA, right atrium; RV, right ventricle; St, stomach.*

In 2006, Yagel *et al.* described a method of rendering STIC volumes to display the IVS plane and the CAV plane[17]. All STIC volumes were assessed for their ability to display these virtual cardiac planes. For rendering to be scored as 'successful', the lateral view on the IVS plane had to display the entire interventricular septum and the foramen ovale, seen from the left ventricle (Figure 2). Rendering artefacts were reported. For the CAV plane to be scored as 'successful', the rendered image had to clearly display all four cardiac annuli. During the routine 2D fetal echocardiographic examination the intracardiac septa were imaged from different angles combined with color Doppler to exclude septal defects.

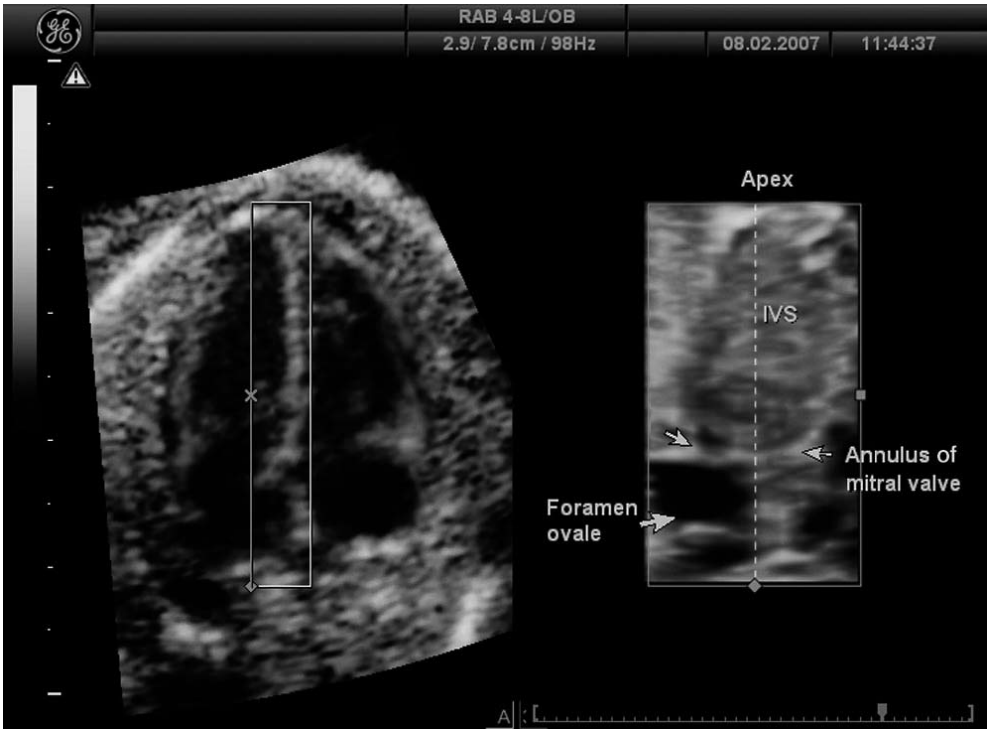

Figure 2 *Rendering of a spatiotemporal image correlation volume of a second-trimester fetus. The left panel shows a transverse plane through the four-chamber view with the rendering box placed around the intracardiac septa. The side of the rendering box marked with a green line is 'active' and determines whether the septa are imaged from the left ventricle to the right, or the reverse. The right panel shows rendering of the complete intracardiac septa with opened foramen ovale. The small arrows show the annulus of the mitral valve. The rendered image shows no rendering artefacts. IVS, interventricular septum.*

All STIC volumes were numbered consecutively, allowing analysis of the correlation between volume number, AC-score and STIC volume quality to examine learning effects. The results of the STIC expert were compared with those of the STIC beginners, with respect to ability to acquire STIC volumes, AC scores and STIC volume quality.

Statistical analysis

Possible exclusion bias was assessed among cases in which STIC acquisition was not attempted. BMI, parity, gestational age, placental location and fetal position in these cases were compared with those in which STIC acquisition was performed. In cases in which STIC acquisition was performed, the successful examinations were compared with those with acquisition failure. Possible differences in BMI, parity, gestational age, placental location, fetal position, interfering fetal activity, history of abdominal surgery and sonographer experience were tested for. In the successful acquisitions, differences between

STIC volumes of different quality were examined. Again, BMI, parity, gestational age, placental location, fetal position, interfering fetal activity, history of abdominal surgery, sonographer experience and AC scores were compared. Furthermore, the influence of experience in STIC volume acquisition was assessed. The success rate of STIC acquisition, the AC-score of STIC volumes and the quality of STIC volumes were compared between the STIC expert and the STIC beginners. Finally, the success of rendering of STIC volumes was studied by comparing differences in acquisition characteristics and rendering quality.

A *t*-test was performed for comparison of means between two groups. ANOVA with Bonferroni *post-hoc* correction was used for comparison of means between three or more groups. Pearson Chi-square test was applied when comparing two categorical variables. To investigate a possible learning effects, STIC volumes were numbered consecutively. The relationships between STIC volume numbers and AC scores, acquisition success rate and STIC volume quality were studied by Pearson correlation to assess the effect of learning. Significance was defined as $P < 0.05$. Standard statistical software was used for all the statistical analysis (SPSS 12.0.1 for Windows, Chicago, IL, USA)

RESULTS

Acquisition success rate

During the study period 165 women presented to the Department of Prenatal Medicine (Figure 3). No cardiac anomalies were diagnosed during the study period. In 148/165 women (89.7%), an attempt was made to acquire a STIC volume. STIC acquisition was not attempted in 17 women. This occurred mainly in the beginning of the project and we assume that acquisition was just forgotten in these 17 cases. There were no significant differences between cases in which STIC volume acquisition was performed and those in which it was not attempted. BMI was 25.2 kg/m^2 vs. 23.5 kg/m^2 (P=0.22), gestational age 21+0 weeks vs. 21+3 weeks (P=0.81) and parity 0.92 vs. 1.12 (P=0.42). The overall mean parity was 0.95 (range 0-6), the mean BMI was 25.2 (range 17.6-38.3)kg/m^2.

In 112/148 women (75,7%) STIC volume acquisition was successful and in 36/148 women (24.3%) was attempted but failed. No significant difference in BMI, gestational age, parity, interfering fetal activity, placental position, fetal position or history of abdominal surgery were found between the group with successful and that with failed acquisition (Table 3).

Quality analysis

During the first phase of the study, 7/112 successfully acquired STIC volumes, as reported on the data sheets, were not stored correctly. In the 105 remaining successful acquisiti-

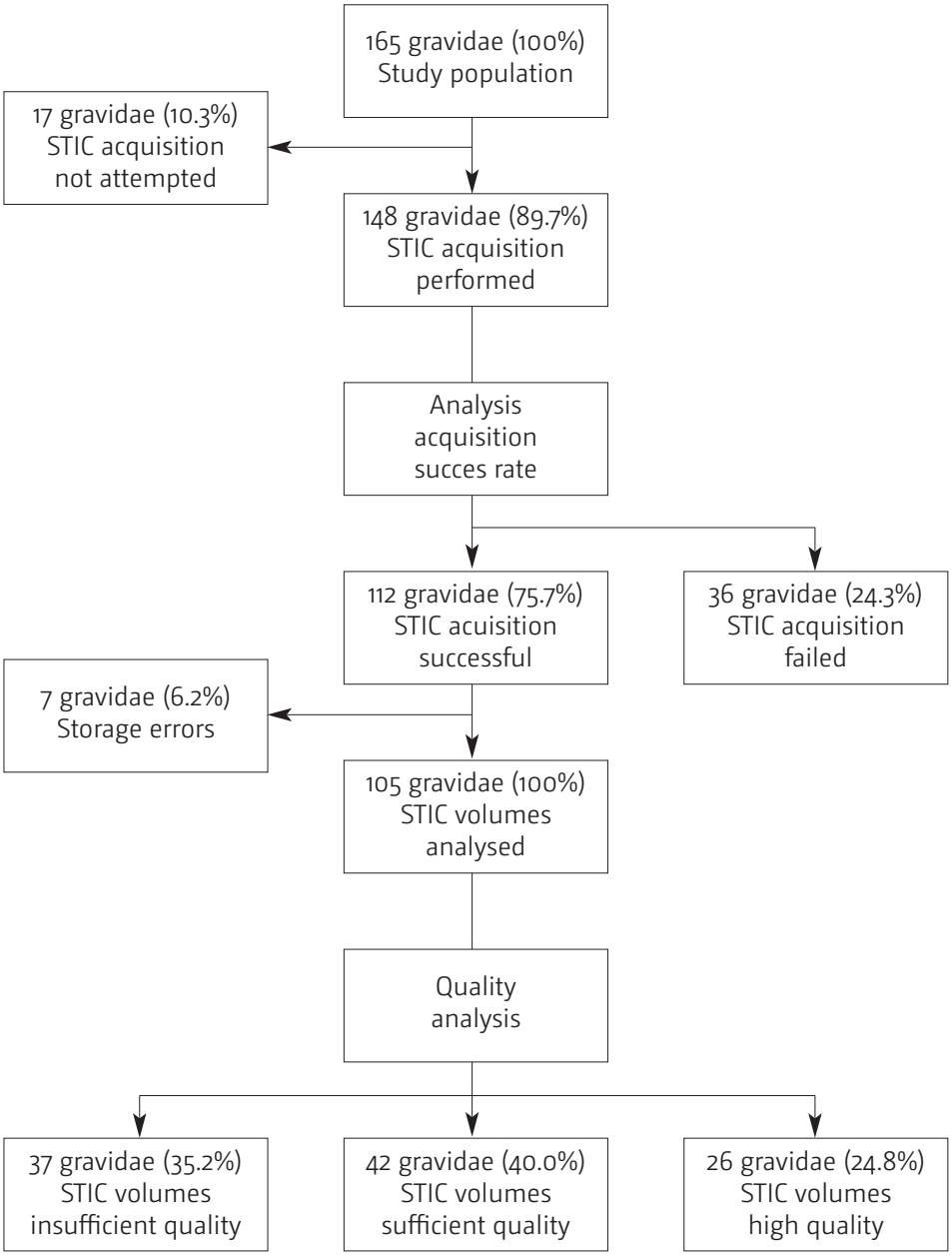

Figure 3 *Flow chart of the study population.*

Table 3 *Comparisons of means and percentages for different factors (failed vs. successful STIC volume acquisition attempts).*

Factor	STIC acquisition		p
	Failed (n=36)	Successful (n=112)	
Mean parity	0.71	0.99	0.21
Mean BMI (kg/m²)	25.9	24.9	0.30
Mean gestational age (weeks)	20 + 2	21 + 1	0.15
Fetal head position, caudal caput (%)	50	61.9	0.57
History of abdominal surgery (%)	11.4	21.4	0.19
Interfering fetal activity (%)	59.4	43.2	0.11
Posterior placental position (%)	65	47.4	0.11

ons, 26 (24,8%) were of high quality, 42 (40,0%) were of sufficient quality and 37 (35,2%) were of insufficient quality (Figure 3). The BMI was significantly lower in cases in which the STIC volumes were of high quality than in those in which STIC volumes were of insufficient quality (mean 23.8 kg/m^2 vs. 26.4 kg/m^2, P=0.04). The placenta was positioned on the posterior wall of the uterus significantly more often in the cases in which STIC volumes were of high quality than in those in which they were of insufficient quality (30.3% vs. 56.0%, P=0.05). No significant differences were found in gestational age, parity, fetal position, sonographer experience, interfering fetal activity or history of abdominal surgery between the three quality groups. To investigate the feasibility of performing offline fetal echocardiographic examination in a STIC volume, the visibility of the cardiac structures was analysed. STIC volumes of high quality were more likely to displayany given cardiac anatomical structure than were STIC volumes of sufficient quality (Table 4), including, of course, those structures used to define volume quality (Table 2).

A significant relation between AC score and quality of STIC volume was found in the three quality groups. The higher the AC score, the better the quality of the STIC volume. STIC volumes of insufficient quality had lower AC scores than those of sufficient quality (mean, 5.9 vs. 6.8, P < 0.05) and STIC volumes of sufficient quality had lower AC scores than those of high quality (mean, 6.8 vs. 8.4, P < 0.05). Figures 4 and 5 illustrate STIC volumes with suboptimal AC scores.

Rendering abilities

In STIC volumes of high quality, the IVS plane could be rendered in 22/26 cases (84,6%), with rendering artefacts in 9/22 cases (40,9%) (Figure 6). The CAV plane could be rendered in 12/26 cases (46,2%). When assessing the STIC volumes of sufficient quality, the lateral IVS plane could be rendered in 20/42 cases (47,6%), with rendering artefacts in 5/20

Table 4 *Visibility of cardiac structures in spatiotemporal image correlation volumes of sufficient and high quality.*

Structure	Sufficient Quality Visibility (n=42)	High Quality Visibility (n=26)
SVC and IVC	32 (76)	25 (96)
Pulmonary veins	28 (67)	26 (100)
Bifurcation of the pulmonary trunk	20 (48)	18 (69)
Entire aortic arch + head/arm arteries	1 (2)	16 (62)
Ductal arch	6 (14)	20 (77)
Rendered IVS plane	20 (48)	22 (85)
Rendered CAV plane	7 (17)	12 (46)

Values are n (%). CAV plane, anteroposterior view of the atrioventricular annuli; IVC, inferior vena cava; IVS plane, lateral view of the interventricular septum; SVC, superior vena cava.

cases (25%). The CAV plane could be rendered in 7/42 cases (16,7%). When the STIC volumes were of insufficient quality, rendering of the IVS plane was successful in 7/37 cases (18,9%). In 5/7 cases (71,4%) there were, however, rendering artefacts visible in the IVS plane. Rendering of the CAV plane in STIC volumes of insufficient quality only resulted in images of insufficient quality; in none of the 39 cases could we clearly visualize all four cardiac annuli.

There were significant higher AC scores in cases in which the rendered planes could be visualized (insufficient rendering vs. good rendering, IVS plane: mean, 5.7 vs. 7.8, $P < 0.05$; CAV plane: 6.4 vs. 8.3, $P < 0.05$). Associations between the rendering quality and each of the individual factors included in the calculation of AC score were tested. The most important limiting factors for rendering were movement artefacts (insufficient rendering vs. good rendering, 43.9% vs. 16.3%, $P = 0.01$) and the presence of shadows (insufficient vs. good rendering, 41.5% vs. 2.0%, $P < 0.01$) within the STIC volume. Limited rendering of the CAV plane was most often due to shadow artefacts (insufficient vs. good rendering, 26.2% vs. 0%, $P = 0.01$). Movement artefacts within a STIC volume played a limiting role although less strongly (insufficient vs. good rendering, 37.7% vs. 5.3%, $P = 0.08$). For the other factors determining the AC score no significant correlations were found.

Experience and learning

In a subanalysis of the sonographers, the STIC expert had a significantly higher success rate of STIC volume acquisition than the other sonographers (88.4% vs. 70.5%, P=0.02). No significant differences in maternal factors were found between the women examined

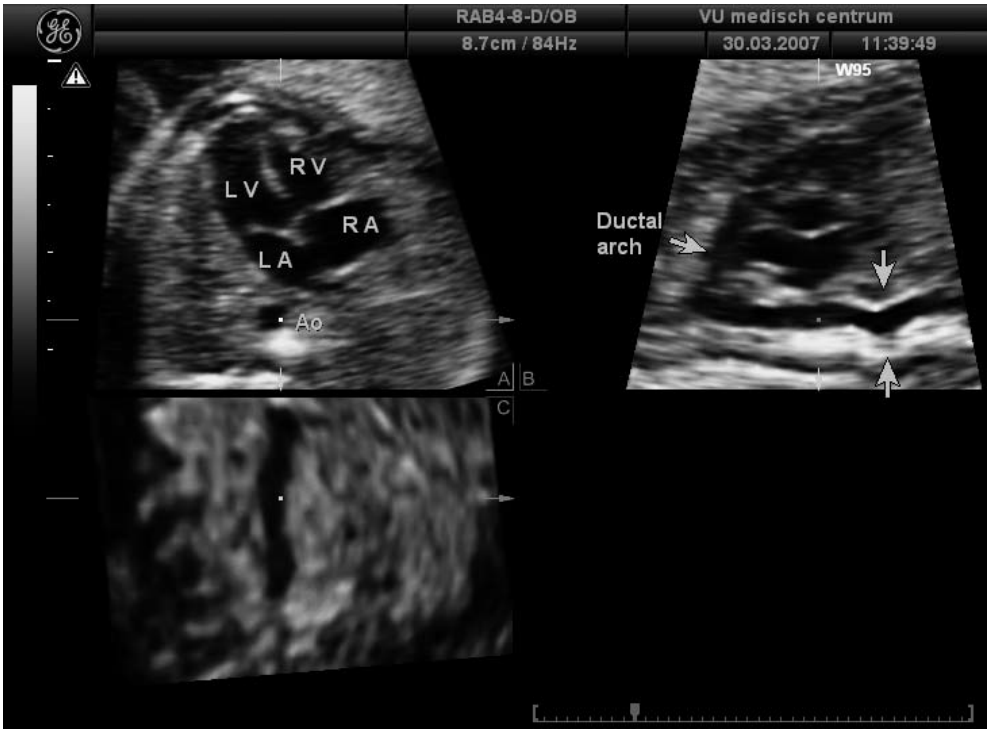

Figure 4 *A spatiotemporal image correlation volume of a second-trimester fetus displayed in multiplanar view. The upper left panel shows a transverse plane through the fetal heart. The upper right panel shows a longitudinal plane through the ductal arch and descending aorta; here a movement artifact is clearly visible (arrows). Ao, aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.*

by the STIC expert and those examined by the STIC beginners. All sonographers performed approximately the same number of STIC volume acquisitions (n^1=45, n^2=37, n^3=44, n^4=39). There were no significant differences in BMI, parity, fetal and placental position between the groups of women examined by the STIC expert and the STIC beginners. However, the gestational age did differ between the groups of women examined (STIC expert vs. STIC beginners, mean, 20 + 1 weeks vs. 21 + 5 weeks, $P < 0.05$). There was also a significant difference in reported fetal movements interfering with STIC volume acquisition (STIC expert vs. STIC beginners, 12% vs. 62%, $P < 0.05$). Comparison of STIC volumes showed that fewer movements artefacts were observed in volumes acquired by the STIC expert (STIC expert vs. STIC beginners, 16% vs. 42%, $P < 0.05$).

The AC scores of all STIC volumes showed an increase with volume number (ordered consecutively in time) ($r= 0.0299$, $P < 0.05$). Our data did not show significant differences in AC score between the different sonographers (range of means, 6.5-7.3, $P = 0.25$) or

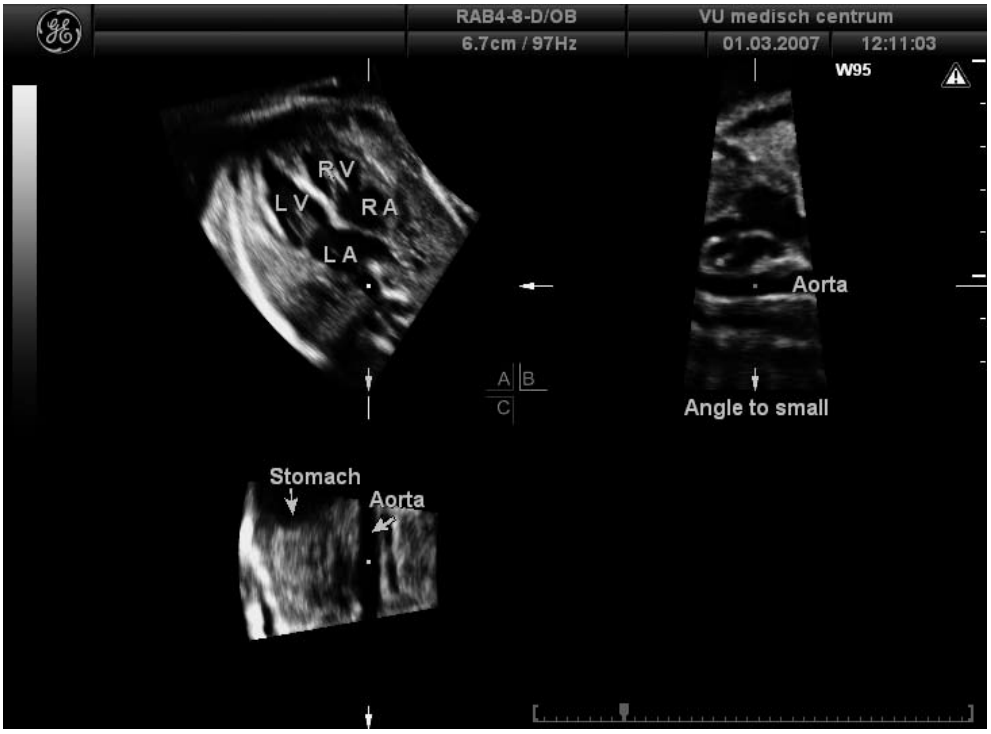

Figure 5 *A spatiotemporal image correlation (STIC) volume acquisition of a second-trimester fetus displayed in multiplanar view. The upper left panel shows a transverse plane through the fetal chest. The upper right panel shows a computer-constructed longitudinal view of the fetal heart. Here the descending aorta and part of the aortic arch are visible. In this STIC volume the angle is set too small to incorporate the entire aortic arch in the volume. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle*

between the STIC expert and the STIC beginners (STIC expert vs. STIC beginners, 7.3 vs. 6.7, P = 0.24). Although a positive correlation between AC score and STIC volume quality was found, we did not observe an increase in STIC volume quality with volume number. No significant difference in STIC volume quality between the STIC expert and STIC beginners was found (high quality, 34.2% vs. 19.4%, P = 0.24) .

DISCUSSION

4D fetal echocardiography using STIC has been described as having the potential to decrease the dependency on fetal position and sonographer skill because it provides a volume dataset that can be used to display the desired images[1, 10, 16]. Through surface rendering of a STIC volume, an operator has the ability to display virtual planes that are

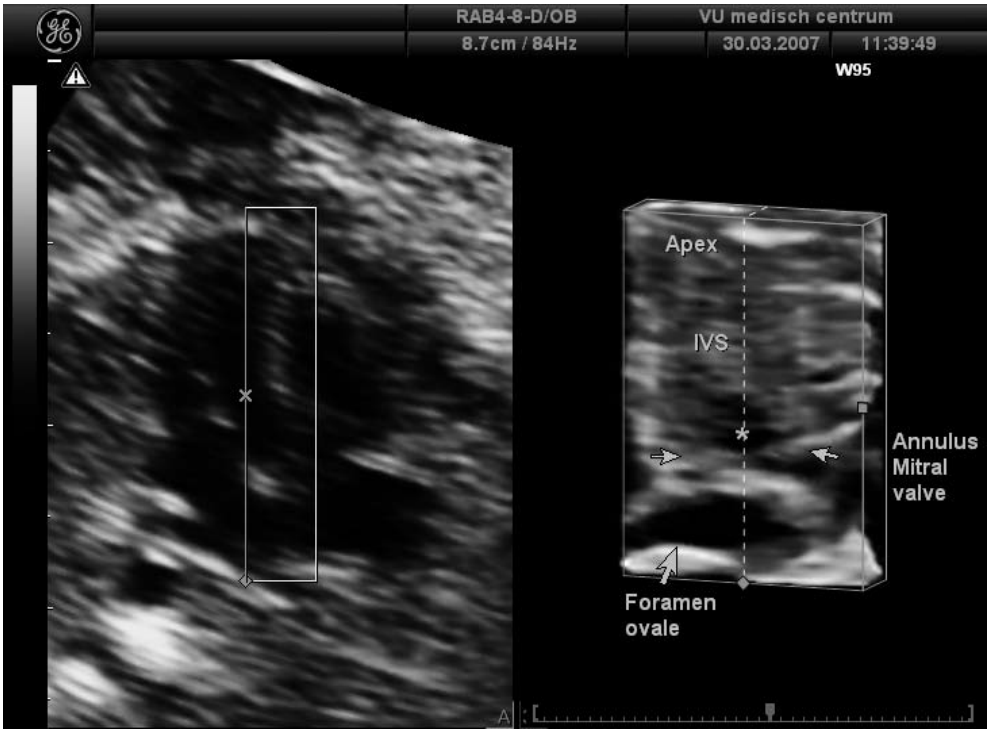

Figure 6 *Rendering of a spatiotemporal image correlation volume of a second-trimester fetus with a rendering artifact. The left panel shows a transverse plane through the four-chamber view with the rendering box placed around the intracardiac septa. The side of the rendering box marked with a green line is 'active' and determines whether the septum is imaged from the left ventricle to the right, or the reverse. The right panel shows rendering of the complete intracardiac septa with opened foramen ovale. The small arrows show the annulus of the mitral valve. The rendered image shows a rendering artifact (*) in the center of the interventricular septum (IVS). No actual ventricular septal defect was present.*

very difficult to display using conventional 2D ultrasound[17]. The IVS plane gives the operator a virtual lateral view on the intracardiac septa for the evaluation and detection of atrial and ventricular septum defects. The CAV plane displays all four annuli, and may be helpful in the evaluation of the atrioventricular valves and the alignment of the great vessels.

Because STIC uses a digital volume, most of the analysis can be performed after the initial acquisition. An operator can send the STIC volume to an expert for a second opinion, use images for educational purposes, or use the STIC volume for offline calculations or measurements. However, to be an effective new tool in fetal echocardiology, STIC volume acquisition has to be feasible clinically. Some reports on these new technologies have emphasized the requirement of a substantial learning curve for the use of 4D ultrasound

scanning[10, 16]. This may explain the delay in widespread introduction of this new technique in routine practice.

The main aim of this study was to asses the clinical feasibility of STIC volume acquisition and the STIC volume quality and not to compare the diagnostic capabilities of STIC to conventional 2D ultrasound imaging. This study shows that it is feasible to incorporate STIC volume acquisition into a tertiary routine prenatal ultrasound program carried out by experienced sonographers. Second, the results show that sonographers do not necessarily have to be experienced specifically in the use of 3D and 4D ultrasound imaging to acquire high-quality STIC volumes. In this study no extra time was scheduled for the incorporation of volume acquisition. During the 2-month study period, the sonographers made successful STIC volume acquisitions in 75,7% of all examinations, 64,8% of the analysed STIC volumes were of high or sufficient quality for the examination of the fetal heart, even in the absence of a change in operating schedules.
These results, however, differ from those described by Vinals *et al.* in 2003[19], in which a complete cardiac examination, using multiplanar assessment of STIC volumes, was successful in 96,2% of examinations. The higher success rate can be explained by excessive pre-selection to inclusion, as in 30/130 acquisitions the 2D four-chamber view was not considered well visualized and so the patient was excluded. Furthermore, Vinals performed his study in the absence of time limits, which is illustrated by the only 4% fetuses with a 'continued anterior spine position'. In our opinion our results reflect a more realistic evaluation of the effectiveness of fetal echocardiography using STIC, when it is introduced into a department with experienced sonographers.
Our study also demonstrates that postprocess rendering abilities depend strongly on STIC volume quality. The results described by Yagel *et al.* in 2006 (IVS plane and CAV plane, 96.3% and 93.4%, respectively[17]) were, however, not achieved. In the acquired STIC volumes of high quality, the IVS and CAV planes were visualised in 84% and 48% respectively. The lower percentages of successful rendering in our study can be explained by the strict criteria for the visualization of the rendered planes. Yagel *et al.* also did not report assessment of rendering artefacts. In this study the presence of rendering artefacts was an important limiting factor, found in 41% of the rendered images. The artefacts were mainly caused by shadows over the interventricular septum. Also of importance is the angle of insonation, as ultrasound beams parallel to the interventricular septum can cause small artefacts in the 2D images and subsequently in the rendered images (Figure 6). Furthermore, in the study of Yagel *et al.* the examinations where not bound to strict time limits. The high success rates and the lack of an analysis of the unsuccessful STIC volume acquisitions suggests some form of selection bias. Finally, in the study by Yagel *et al.*, one highly dedicated sonographer examined all of the women included. Because the present study aimed to investigate the incorporation of STIC volume acquisition in

a routine third-level ultrasound program, a group of sonographers with different levels of experience in the use of 3D/4D sonography participated. Yagel *et al.* described excessive fetal activity and fetal breathing movements as major limitations in STIC volume acquisition. Our data show similar results, as movement artefacts and shadowing were the two limiting conditions for rendering in STIC volumes. The fact that the IVS plane could more often be visualised than the CAV plane is consistent with the results reported by Yagel *et al.*

This study shows that STIC volume quality correlates significantly with the acquisition conditions described by Goncalves *et al*[9]. Although our results show an increase in AC score with time, an increase in STIC volume quality in this study period was not observed. This could be explained by the fact that the sonographers were very experienced in 2D fetal ultrasound. Training in correct acquisition can probably minimize artefacts and could eventually lead to improvement of STIC volume quality. The positive influence of training and proper timing of acquisition, is illustrated by the fact that the STIC expert was less limited by interfering fetal movements during STIC volume acquisition and achieved a higher success rate at a lower gestational age.

This study has several limiting factors. First, because it is a feasibility study, all examined fetuses had normal cardiac anatomy. Therefore, this study is not conclusive with regard to the diagnostic capabilities of STIC cases of cardiac malformations. Further research is needed to compare the diagnostic capabilities of STIC with conventional 2D fetal echocardiography. Second, subjective explanations for all unsuccessful attempts were not reported. This might have given a different perspective on the factors that determine whether a STIC volume can be acquired successfully or not. Furthermore, the results of this study are based on the skills of only four sonographers. Our results may not be exactly replicated by other sonographers with various backgrounds and training. When interpreting these results, the fact that this group of sonographers is highly experienced in 2D fetal ultrasound imaging and echocardiography has to be taken into account.

We conclude that STIC volume acquisition can be incorporated with little difficulty into daily practice in a third-level ultrasound department. Some of the sophisticated features offered by this new technique were, however, not successful in all examined women. In our opinion, training in the acquisition of STIC volumes could eventually lead to higher success rates. Our results also indicate that STIC and subsequently all postprocess options are as susceptible as is conventional two-dimensional ultrasound imaging to individual variations and limitations in scanning windows.

APPENDIX

Instructions for spatiotemporal image correlation (STIC) volume acquisition based on Goncalves et al.[9]

Fetal position

The original plane of acquisition was always a transverse plane through the fetal thorax. The optimal fetal position is a position in which the fetus is lying on its back (the spine positioned between 5 and 7 o'clock) and the cardiac apex pointing upward (between 10 and 2 o'clock). This way the examiner is least frequently compromised by acoustic shadowing from the ribs.

Region of interest

By setting the region of interest (ROI) the examiner determines the width and height of the volume dataset. Using a large ROI will decrease the frame rate and may have a negative effect on the quality. An optimal ROI is set as narrow as possible around the fetal heart, including the descending aorta.

Acquisition angle

By setting the acquisition angle the examiner determines the depth of the volume dataset. For a complete examination of the fetal heart it is important that the acquisition angle is set large enough to include the fetal stomach, heart and great vessels in the volume dataset. For fetuses with gestational ages ranging from 18 to 22 weeks, acquisition angles of 20° and 25° are usually sufficient.

Acquisition time

By setting the acquisition time the examiner determines the time it takes for the ultrasound machine to record the volume dataset, ranging from 7.5 to 15 seconds. The longer the acquisition time, the higher the spatial resolution of the volume dataset. As with all settings the examiner should pay attention to the variable fetal conditions. Confronted with a very active fetus a fast acquisition will reduce motion artefacts as much as possible. For the best spatial resolution, STIC volumes should be acquired using the longest possible acquisition time.

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Chapter 3

Feasibility of automated three-dimensional fetal cardiac screening in routine ultrasound practice

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ABSTRACT

Objective

The purpose of this study to prospectively assess the clinical feasibility of an automated three-dimensional (3D) software tool, for the extended basic cardiac screening in routine ultrasound practice.

Methods

During the 2-month study period all gravidas fitting our inclusion criteria were consecutively included. Cardiac 3D volumes were acquired within the time slot allocated for the usual two-dimensional fetal examination. All volumes were assessed on their quality, based on the display of the 4-chamber view, and on the ability to sufficiently display diagnostic cardiac planes (left ventricle outflow tracts (LVOT) and right ventricle outflow tracts (RVOT) and stomach location) using Sonography-Based Volume Computer Aided Diagnosis software (SonoVCAD; GE Healthcare, Milwaukee, WI).

Results

Volume acquisition was successful in 107 cases of 126 (85%). For each sonographer over 70% the acquired cardiac volumes were of high or sufficient quality. Separately analysed, diagnostic planes of LVOT, RVOT and stomach location were visible in 62.1%, 81.6% and 92.2% respectively. An extended basic fetal cardiac examination based on the retrieval of all diagnostic cardiac planes form a single volume using SonoVCAD could be performed in 46.6% of the cases.

Conclusions

This study shows that cardiac volume acquisition can be incorporated in a routine ultrasound screening program without much difficulty. However, currently SonoVCAD software still lacks the consistency to be clinically feasible for cardiac screening purposes. Further advance in ultrasound technology and familiarization with 3D ultrasound might improve its performance.

INTRODUCTION

The success and quality of fetal echocardiography depend strongly on the manual skills of the sonographer[1, 2]. The anatomical complexity of the heart, combined with the myriad of fetal positions and maternal factors that influence any obstetric ultrasound examination, make fetal echocardiography one of the most challenging areas for sonographers to master. It is most likely that because of the difficulty of the examination, the overall performance of conventional obstetric sonography on the detection of fetal cardiac anomalies is suboptimal[3, 4]. Several studies have reported congenital heart disease (CHD) to be one of the most commonly overlooked lesions in prenatal screenings programs[1, 3, 4]. However, there is a wide variation in the estimated sensitivity of prenatal screening for CHD. Differing scanning skills, scanning equipment, and protocols and lack of standardization might account for the differing success in detecting CHD.

Early detection and accurate prenatal diagnosis of major CHD can reduce neonatal morbidity and mortality rates by providing the adequate prenatal and postnatal care[5]. In particular, lesions that are dependent on the patency of the arterial duct and those that require immediate surgical repair, have their outcomes improved by adequate prenatal management[1]. In conventional screening protocols, the 4-chamber view has been the standard approach to screen for CHD. Identification of the right and left outflow tracts, however, markedly improves the detection rate of CHD[6-8]. To help improve and standardize 2-dimensional ultrasound (2DUS) examinations, Yagel *et al.* and Yoo *et al.* described a method for a complete examination of the fetal heart[9, 10]. This method includes visualization of great vessels in the upper mediastinum in the 3-vessel view (3VV). Recent ultrasound guidelines have referred to this as the extended basic cardiac examination[11].

Additionally, sonographers might also benefit from more recent developments in fetal echocardiography which include 3-dimensional ultrasound (3DUS)[12, 13]. This modality allows volume acquisition of the fetal heart, and thus avoids the necessity of tilting, angling, and rotation of the ultrasound probe, which is considered one of the hardest parts of 2DUS for less experienced sonographers. Sonography-Based Volume Computer Aided Diagnosis (SonoVCAD; GE Healthcare, Milwaukee, WI) is a relatively new software tool for 3DUS that uses the relatively constant spatial relationship of the different cardiac planes, which are required for an anatomical evaluation of the fetal heart[14]. The software allows automated retrieval and display of the planes used in an extended basic fetal cardiac examination, thus making it less dependent on manual skills. To our best knowledge, no reports have been published that assessed the ability of this new software tool to display these cardiac planes in prospectively acquired volumes. Therefore, the aim of this study was to investigate the clinical feasibility of SonoVCAD for extended basic cardiac screening in volumes prospectively acquired in a routine ultrasound setting.

METHODS

During a 2-month period, all women visiting the Department of Prenatal Medicine for a second- trimester ultrasound examination between 18 and 23 weeks were consecutively included. They all had an increased risk for fetal congenital malformations based on their history. Exclusion criteria were suspected congenital malformations and unconfirmed gestational age (GA) by first trimester ultrasound. Assigned gestational weeks were rounded up at the fourth-day interval (ie, 18 weeks = 17 weeks 4 days to 18 weeks 3 days). The Medical Ethics Committee approved the study protocol and all patients gave informed consent before the ultrasound examinations.

Four third-level referral hospital-based sonographers participated in the study. In the 2-month study period, all sonographers incorporated a static 3-dimensional (3D) cardiac volume acquisition of the fetal heart in their ultrasound examinations. The static 3D cardiac volumes will henceforth be referred to as 'volumes'. One sonographer had already been using 3DUS in a specialized fetal echocardiography program for 3 years (L.U., sonographer 1). All other sonographers received a short training course in the use of 3DUS before the start of the study. This training consisted of 2 hands-on sessions in which the sonographers were taught how to operate the ultrasound machine to acquire volumes. To acquire the volumes, a motorized 4- to 8-MHz curved array transducer was used (Voluson E8, GE Medical Systems, Kretztechnik, Zipf, Austria). All volumes were acquired with a 4CHV as the initial reference plane. The acquisition angle was 35° to 45° in most volumes to ensure the inclusion of the fetal lower neck region and stomach. For acquisition of the volumes, the ultrasound machine used fetal cardiac settings with contrast resonance imaging (CRI) on and speckle reduction imaging (SRI) on level 6. The speed of the acquisition was set on intermediate speed to balance between image quality and minimization of motion artefacts. After a successful acquisition the volumes were stored and transported to a personal computer. All volumes were examined by 1 sonographer (L.U.) using Voluson 4D View 6.0 post processing software.

Sonography-Based Volume Computer-Aided Diagnosis

The volumes were standardized in the start plane. For uniformity of orientation, the software displays a fetal heart template of a 4CHV. Magnification settings were adjusted to make the 4CHV fit the template. Rotation along the z-axis was performed until the spine was at the 6-o'clock position. The reference point was placed at the septal insertion of the septal leaflet of the tricuspid valve (Figure 1). The setting of the reference point is an important step in the standardization because SonoVCAD includes rotation along the

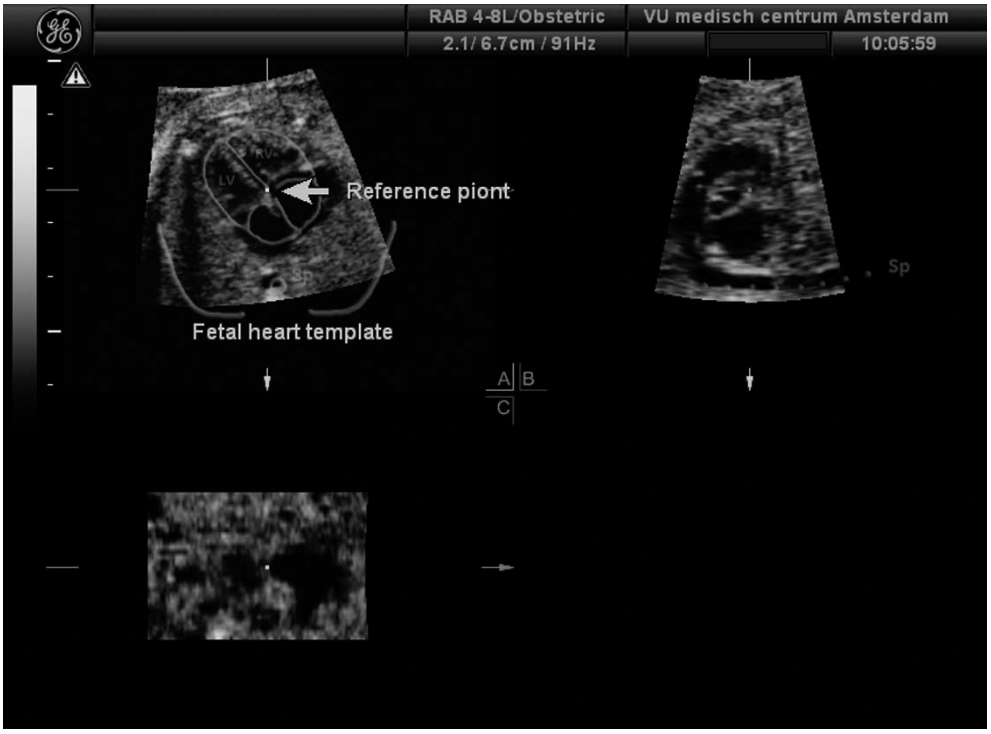

Figure 1 *Standardization of a 3D volume of a fetal heart. Plane A displays a 4CHV adjusted to fit a fetal heart template. The reference point is placed at the septal insertion of the tricuspid valve (arrow). Plane B and C are the corresponding orthogonal planes at the level of the reference point.*

axis through this point. For fetuses in a breech position the software has a button that automatically rotates the image 180° along the y-axis before applying the standardization as described above. After the standardization and setting of the starting plane, the software includes the option of displaying 3 diagnostic cardiac planes: cardiac 1 is a plane of the left ventricle outflow tract (LVOT); cardiac 2 is a plane of the right ventricle outflow tract (RVOT); and cardiac 3 is a plane that presents the abdomen and the position of the fetal stomach. The diagnostic cardiac planes were displayed with tomographic ultrasound imaging (TUI). Each diagnostic cardiac plane was displayed as 7 TUI images with different interslice distances: cardiac 1, 0.6 mm; cardiac 2, 1 mm; and cardiac 3, 2 mm.

To asses the feasibility for screening purposes, the different diagnostic planes were defined to meet the following criteria: for the starting plane (4CHV), a symmetric transverse plane through the fetal heart displaying 2 atria and 2 ventricles, the moderator band, the intracardiac septa, the crux cordis and both atrioventricular valves (Figure 2); for cardiac plane 1 (LVOT), clear continuity between the aorta and the ventricular septum at

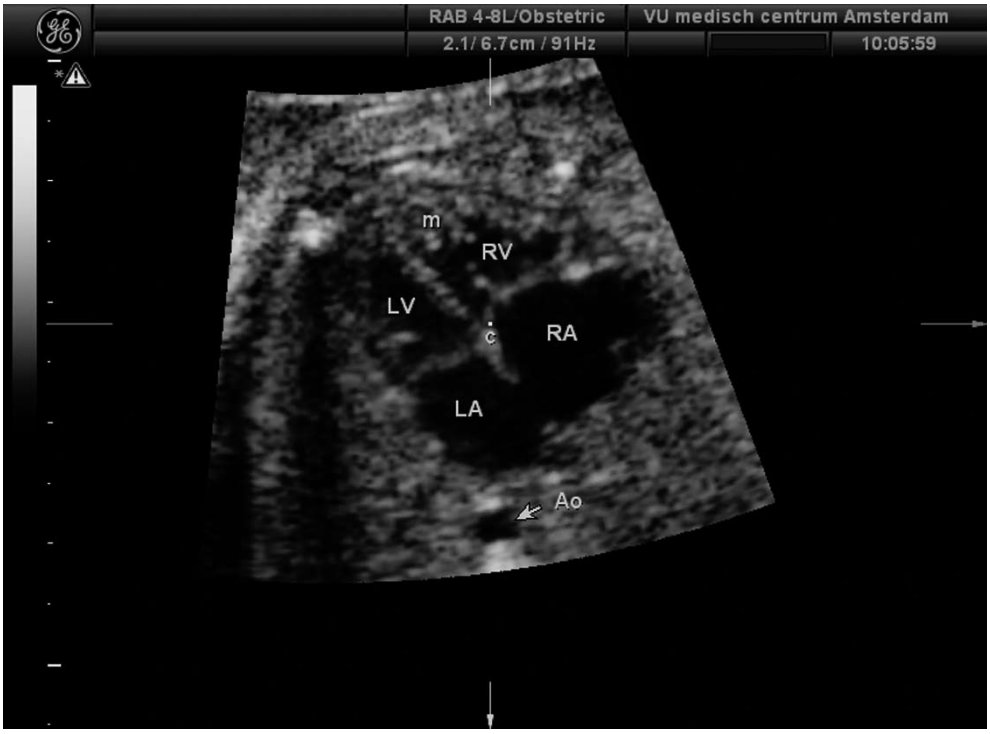

Figure 2 *Display of a 4CHV, a symmetrical transverse plane through the fetal heart, showing the right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), moderator band (m), intracardiac septa, crux cordis (c), and both atrioventricular valves. Ao indicates aorta.*

the longitudinal view of the outflow tract (Figure 3); for cardiac plane 2 (RVOT), clear display of the main pulmonary artery as it emerged from the right ventricle, clear display of a transverse image of the ascending aorta next to the pulmonary artery and clear display of a transverse image of the superior vena cava on the other side of the ascending aorta (3VV9; Figure 4); and for cardiac plane 3 (stomach position), display of a portion of the stomach on the left side in a transverse view of the abdomen (Figure 5). To asses the overall quality of the volumes the starting plane (4CHV) was scored. Volumes were considered of sufficient quality when 2 atria, 2 ventricles, the intracardiac septa, and the crux cordis could be recognized. Volumes were considered to be of high quality when all structures mentioned above were clearly visible. Furthermore, all the volumes were analyzed to determine whether the target diagnostic cardiac planes 1–3 were identified correctly in 1 or more of the 7 TUI planes. The number of times needed to adjust or readjust the starting plane to get the optimal results was scored, as was the total time needed for postprocess analysis.

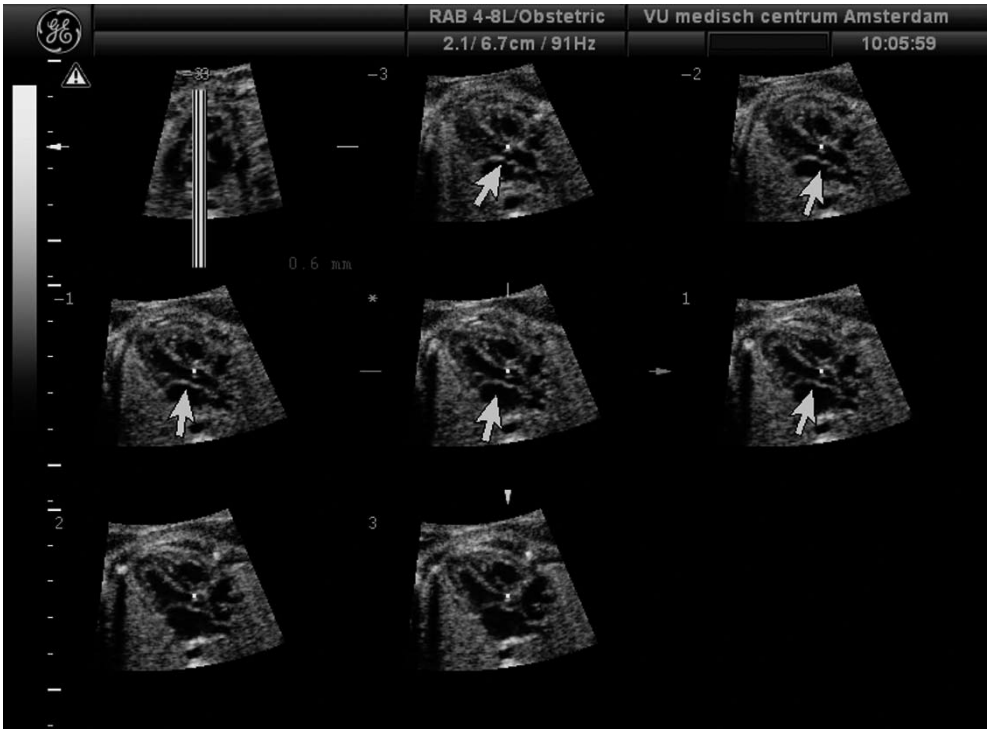

Figure 3 *Sonography-Based Volume Computer-Aided Diagnosis display of cardiac plane 1 (LVOT, and aorta) showing clear continuity between the aorta and the ventricular septum on a longitudinal view of the outflow tract. The LVOT is clearly visible in several TUI planes in this volume (arrows).*

Statistical analysis

A possible exclusion bias was examined for the cases in which 3D acquisition was not attempted. The body mass index (BMI) and GA in these cases were compared with those in the cases in which 3D acquisition was performed. In the cases in which 3D acquisition was performed, the successful examinations were compared to the unsuccessful examinations. Possible differences in BMI, GA, placental location, and fetal position were studied. In the successful acquisitions, differences between volumes of different quality were examined. Again, the BMI, GA, placental location, and fetal position were compared. For the comparison of means between 2 groups, a *t*-test was used; for comparison of means between 3 or more groups, an analysis of variance with Bonferroni post hoc correction was used; and for comparison of 2 categorical variables, a Pearson χ^2 test was used. Significance was defined as $P< 0.05$. For all the statistical analysis standard statistical software was used (SPSS 15.0.1 for Windows; SPSS Inc, Chicago, IL).

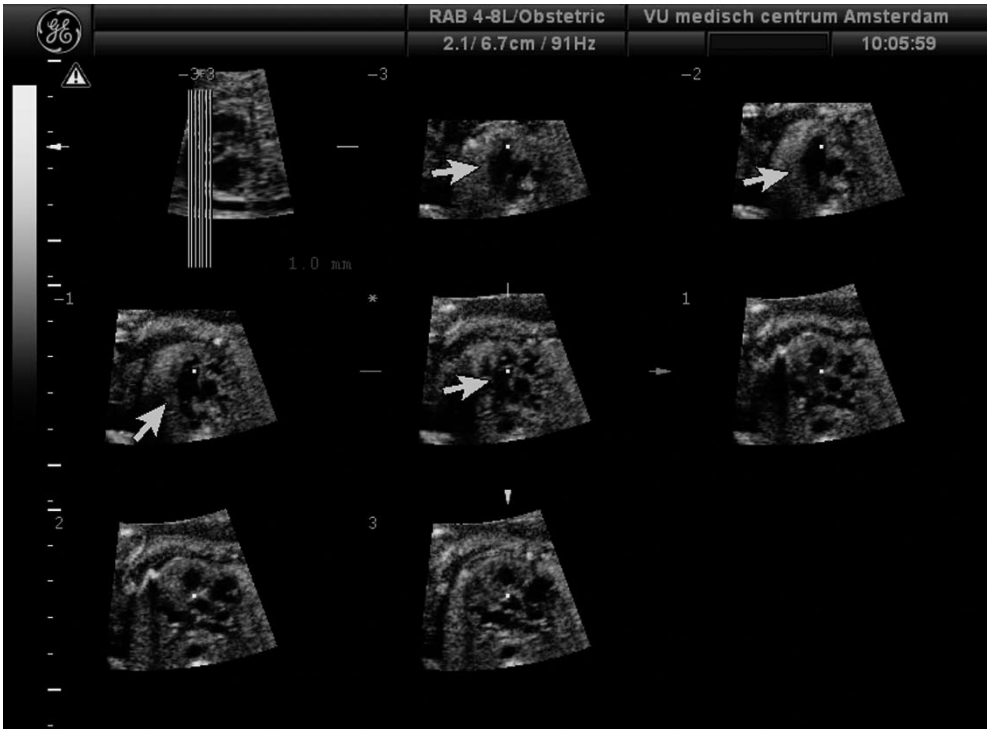

Figure 4 *Sonography-Based Volume Computer-Aided Diagnosis display of cardiac plane 2 (RVOT and pulmonary artery, and 3VV) showing a clear display of the main pulmonary artery as it emerged from the right ventricle, a transverse image of the ascending aorta and a transverse image of the superior vena cava on the other side of the ascending aorta (3VV). RVOT is visible in 4 images in this volume (arrows).*

RESULTS

Acquisition Success Rate

During the study period, 141 women met our inclusion criteria. All were consecutively included. No cardiac anomalies were diagnosed during the study period. In 126 of 141 women (89.4%), an attempt was made to acquire a volume. In 15 women, 3D acquisition was not attempted. This occurred mainly at the beginning of the project, and we assume that acquisition was just forgotten in these 15 cases. The BMI, GA, placental location (anterior or posterior) and fetal position (cephalic or breech) were not significantly different in the cases in which volume acquisition was attempted compared with the cases in which it was not attempted (BMI: 25.2 versus 23.4, P=0.30; GA: 20 weeks 1 day versus 20 weeks 2 days, P=0.61; placenta: posterior versus anterior, P=0.13; fetal position: cephalic versus breech, P=0.82).

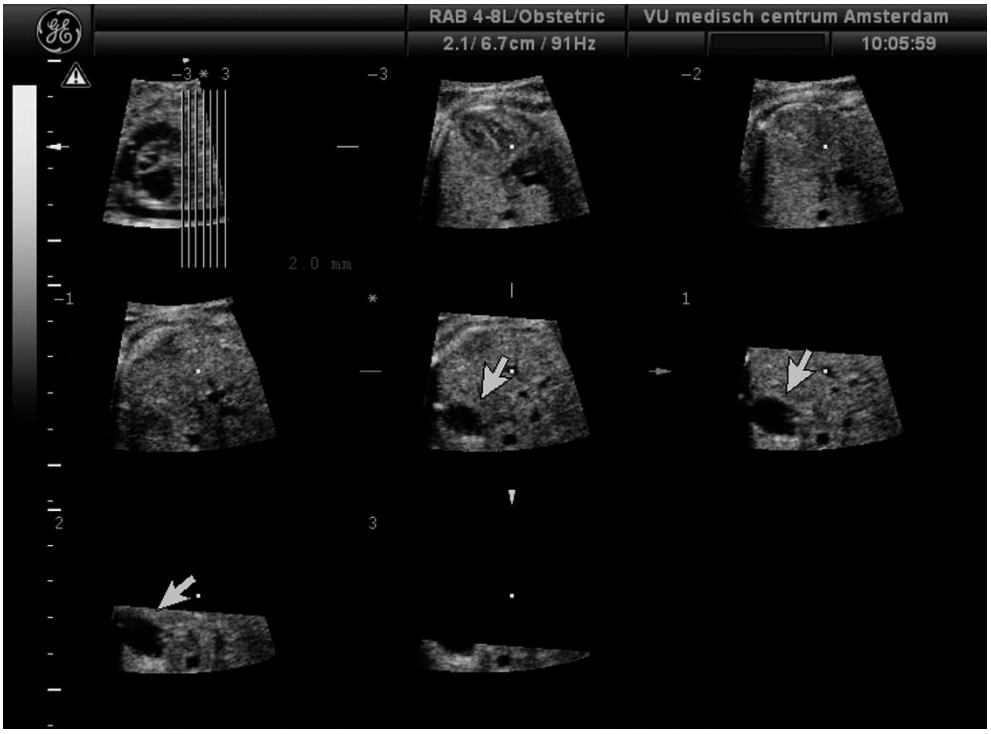

Figure 5 *Sonography-Based Volume Computer-Aided Diagnosis display of cardiac plane 3 (stomach position) showing of a portion of the stomach in a transverse view of the abdomen (arrows). A clear display of a left-sided stomach is shown in 3 images in this volume (arrows).*

In 107 of 126 women (84.9%) volume acquisition was successful. In the 19 of 126 women (15.1%) in which volume acquisition was attempted but failed, different conditions that could have been the cause for the failure were studied. No significant difference in BMI, GA, placental position, and fetal position were found between the cases with successful versus unsuccessful acquisitions. Although we did not record why our sonographers were not able to acquire a volume in 15.1% of the cases, we presume this was caused by extensive fetal movement, abundant acoustic shadowing due to a superior spine position, or a lack of sufficient time within the allocated time slot. Comparing the sonographers, there was a significant difference in the success rate between sonographer 1 and the other sonographers grouped together (100% versus 79.3); however, sonographer 4 also achieved a very high success rate of 96.9% (Table 1).

Quality Analysis

During the first phase of the study, 4 of 107 successful acquired volumes, as reported on the data sheets, were not stored correctly as reported on the data sheets. In the 103 re-

Table 1 *Success rate of 3D volume acquisition by four operators.*

Operator	Examinations, n (%)	Not atempted, n (%)	Unsuccessful, n (%)	Successful, n (%)
1	35	1 (2.9)	0 (0.0)	34 (97.1)
2	35	10 (28.6)	8 (22.9)	17 (48.6)
3	37	2 (5.4)	10 (27.0)	25 (67.6)
4	34	2 (5.9)	1 (2.9)	31 (91.2)
Total	141	15 (10.6)	19 (13.5)	107 (75.9)

maining successful acquisitions, the volumes displayed the 4CHV in high quality in 44 of 103 (42.7%), sufficient in 38 of 103 (36.9%), and insufficient in 21 of 103 (20.4%) (Table 2). Effects that caused insufficient quality included abundant movement artefacts and acoustic shadowing artefacts. No significant differences were found for BMI (P=0.13), GA (P=0.59), placental position (P=0.25), and fetal position (P=0.87) between the 3 quality groups. Analysis of the different sonographers showed no significant differences in quality of the acquired volumes. Furthermore, the data showed that the volumes were of sufficient or high quality in more than 70% for each sonographer (Table 2). The average time needed for postprocess analysis was 2 minutes (120.8 seconds; range, 64-226 seconds). There was a small but significant difference in the time needed to analyze volumes with a sufficient-quality 4CHV compared to a high-quality 4CHV (129 versus 111 seconds; P=0.05). The average number of times that were needed to adjust the starting image to get optimal results was 0.7 (range 0-3). There were no significant differences between the different quality groups (insufficient versus sufficient versus high, 1 versus 0.76 versus 0.5).

Visibility of Cardiac Planes

When the data were analyzed for all 103 volumes grouped together, SonoVCAD displayed a sufficiently visible or clearly visible cardiac plane 1 (LVOT) in at least 1 TUI plane in 64 of 103 volumes(62.1%), cardiac plane 2 (RVOT) in at least 1 TUI plane in 84 of 103 volumes (81.6%), and cardiac plane 3 (stomach) in at least 1 TUI plane in 95 of 103 volumes (92.2%). Table 3 gives an overview of the quality and visibility of the cardiac diagnostic planes.
When the volumes were analyzed for the number of output planes visible, cardiac plane 1 was visible in 3 or more TUI planes in 48 of 64 volumes (75.0%), cardiac plane 2 was visible on 3 or more TUI planes in 49 of 84 volumes (58.3%), and cardiac plane 3 was visible in 3 or more TUI planes in 65 out of 95 volumes (68.4%).

Feasibility for Cardiac Screening

During the fetal screening using a freehand 2DUS sweep, all four cardiac planes needed for an extended basic cardiac examination could be visualised in all patients (100%). Of

Table 2 *Quality of the successfully acquired volumes.*

Operator	Examinations, n (%)	Insufficient, n (%)	Sufficient, n (%)	High, n (%)
1	32	5 (15.6)	13 (40.6)	14 (43.8)
2	16	4 (25.0)	4 (25.0)	8 (50.0)
3	25	7 (28.0)	11 (44.0)	7 (28.0)
4	30	5 (16.7)	10 (33.3)	15 (50.0)
Total	103	21 (20.4)	38 (36.9)	44 (42.7)
Errors	4			

Table 3 *An overview of the quality and visibility of different diagnostic cardiac planes. The four-chamber view (4CHV), the left ventricle outflow tract (LVOT), right ventricle outflow tract (RVOT) and intra-abdominal stomach position (Stomach). Ex. refers to Examinations.*

4CHV Quality	Ex. n (%)	LVOT, n (%)			RVOT, n (%)			Stomach, n (%)		
		not seen	visible	clearly	not seen	visible	clearly	not seen	visible	clearly
Insufficient	21	12 (57,1)	5 (23,8)	4 (19,0)	12 (57,1)	4 (19,0)	5 (23,8)	3 (14,3)	7 (33,3)	11 (52,4)
Sufficient	38	18 (47,4)	6 (15,8)	14 (36,8)	3 (7,9)	13 (34,2)	22 (57,9)	3 (7,9)	6 (15,8)	29 (76,3)
High	44	9 (20,5)	10 (22,7)	25 (56,8)	4 (9,1)	11 (25,0)	29 (65,9)	2 (4,5)	7 (15,9)	35 (79,5)
Total	103	39 (37,9)	21 (20,4)	43 (41,7)	19 (18,4)	28 (27,2)	56 (54,4)	8 (7,8)	20 (19,4)	75 (72,8

a total of 103 volumes, 82 displayed the 4CHV sufficiently or clearly. Within these volumes, 77 also displayed cardiac plane 3 (Stomach) sufficiently or better. Of these volumes, which displayed both the 4CHV and correct stomach position, 71 also displayed cardiac plane 2 (RVOT) sufficiently or better. Finally, in 48 of these 71 volumes cardiac plane 1 (LVOT) was also displayed sufficiently or better. As a result, in 48 of all 103 volumes (46.6%), all 4 cardiac planes necessary for a basic cardiac examination (4CHV, LVOT, RVOT and stomach) were sufficiently or clearly visible. Subanalysis of the different sonographers showed no significant differences between the sonographers for the percentage of acquired volumes in which all standardized cardiac planes could be obtained (sonographers 1-4: 52%, 42%, 55%, and 76%, respectively).

DISCUSSION

Three-dimensional ultrasound adds new possibilities to conventional 2DUS. The technical advantages of having a 3D data set available include offline rotation, reslicing and multiple postprocess possibilities, including surface rendering and inversion mode. With

the ongoing improvements in ultrasound technology and the increasing availability of high-end ultrasound systems, more and more sonographers will have the possibility to use 3DUS in routine practice. In reports on fetal echocardiography, 3DUS has been described as having the potential to decrease dependency on sonographer skills[14, 15]. Because the fetal heart is the most difficult area for sonographers to master, all tools that have the potential to decrease dependency on sonographer skills and experience, might be very useful in clinical practice, especially for less experienced sonographers who might have difficulties with the tilting, angling and rotation necessary during an extended basic fetal cardiac examination. Automated postprocess retrieval of diagnostic cardiac planes (SonoVCAD) could be a first step towards automation of fetal sonography. The main aim of this study was to asses the clinical feasibility of SonoVCAD as a new software tool for automated retrieval of the 4 diagnostic planes necessary for extended basic cardiac screening from a volume. To our knowledge, a study evaluating this new commercially available software tool prospectively in a routine clinical setting has not been reported previously. As a consequence, the image quality of the volumes used in this study differed. It is our opinion that the results of this study therefore give a more realistic view on the software's clinical performance than the results of earlier published reports[14]. This study focussed on GAs of 18 to 23 weeks because the software was designed for screening purposes that usually take place within this window. Data for other GAs, however, showed similar software performance (data not shown).

The results of this study show, first, that incorporation of volume acquisition in a tertiary routine prenatal ultrasound program with sonographers experienced in fetal screening is feasible. After a short training session in the principles of 3DUS, our sonographers were able to acquire volumes in 85% of the studied population. Further research is needed, however, to asses whether sonographers with less experience and training in 2DUS will achieve similar results. Also, it must be taken into consideration that the 3DUS training received consisted of 2 hands-on sessions only, which might be insufficient for some, especially for using 3DUS on the fetal heart.
Earlier reports on 3DUS have emphasized a substantial learning curve for the use of 3DUS[15, 16]. A steep learning curve might also explain the delay of widespread introduction of these new technologies in routine practice.

Although most of our sonographers were not experienced in the use of 3DUS, approximately 80% of all acquired volumes was of sufficient or high quality. However, despite no obvious effect of BMI, placental location, and fetal position, only 42% of the acquired volumes was of high quality on the basis of the 4CHV at the reference plane. In addition, although some of our sonographers would presumably benefit from more training and experience, other sonographers showed success and quality rates comparable with the

sonographer most experienced in 3DUS. This was supported by the fact that the trained sonographer was able to achieve a 100% success rate versus 79% for the other sonographers. In addition, acknowledging that the results of this study were based on only 4 sonographers and may not be exactly replicated by others, they imply strong individual differences in learning curves for 3DUS. A limitation of this study might relate to the fact that there was no mechanism to compare the acquired 2DUS images with 3DUS volumes with regard to quality.

Nevertheless, in contrast to the 100% successful extended basic fetal screening rate for conventional 2DUS, with SonoVCAD, only 46,6% of all volumes could sufficiently display all 4 diagnostic cardiac planes. These findings differ from the findings of Abuhamad *et al.*[14], in which 72 high-quality 3D volumes were randomly selected from a digital database. The database itself, however, was in our opinion prone to a selection bias because only high-quality volumes were stored[14]. Furthermore, in their study, cardiac planes were retrieved separately in more than 90% of the volumes. It remains unclear, however, what percentage of the volumes could display all 4 diagnostic cardiac planes in the same volume. This information seems relevant, because for an extended basic examination of the fetal heart in a screening setting, all 4 cardiac planes have to be retrieved from a single volume.

In conclusion, it is possible to incorporate volume acquisition in a routine ultrasound practice. Nevertheless, this study implies that the software assessed still lacks the consistency and has too many limitations to be clinically feasible in prenatal screening programs.

The data from this study show that volume quality plays a critical role in the performance of the SonoVCAD software. Therefore, further advances in ultrasound technology, including matrix transducers, enhanced computing power, and, importantly, increasing familiarization and training of sonographers in the use of 3DUS, are likely to improve its performance. Finally, although not yet clinically feasible, the concept of automated sonography with further standardization of the approach to acquire and display volumes, has the potential to be advantageous in prenatal ultrasound.

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Chapter 4

Validation of volume measurements for fetal echocardiography using four-dimensional ultrasound imaging and spatiotemporal image correlation

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ABSTRACT

Objective

To asses the accuracy and reliability of four-dimensional (4D) ultrasound imaging using spatiotemporal image correlation (STIC) employing three different techniques to measure volumes in vitro.

Methods

Customized miniature balloons attached to a pump system were used to mimic fetal cardiac chambers. After the balloon model had been immersed in a bath filled with viscous gel, 4D datasets were acquired and three methods were used for volume analysis: three dimensional (3D) slice method, Virtual Organ Computer-aided AnaLysis (VOCAL™) and VOCAL combined with inversion mode. Accuracy and measurement error were measured as the difference between the volume measurements and the actual volumes. Intraobserver reliability was assessed by computing coefficient of variation (CV) and intraclass correlation (ICC)).

Results

Measurement of 76 different volumes, ranging from 0.30 to 4.95 mL, resulted in a total of 912 measurements. The 3D slice method had a mean error of -3.3%, the inversion method underestimated the volumes with a mean error of -6.1%, and VOCAL had a mean error of -2.9%. The 3D slice method had best agreement (95% limits of agreement (LOA -11.2 to 4.7%), followed by VOCAL (95% LOA -14.1 to 8.3%); the inversion mode demonstrated the worst agreement (95% LOA -21.4 to 9.2%). All three methods were reliable with CV <10% and ICC >0.95.

Conclusions

4D ultrasonography with STIC is a feasible and accurate method for calculating volumes of 0.30 mL upwards. In an in-vitro model the 3D slice method proved accurate, was the least time consuming, had the best reliability and the smallest LOA. This method may prove useful when applied to in-vivo investigations.

INTRODUCTION

Conventional two-dimensional (2D) ultrasound techniques, including pulsed Doppler and M-mode, have been found to be of limited value for the evaluation of fetal cardiac function[1-3]. The sources of error to which these methods are prey are well described in literature[4]. Ejection fraction and stroke volume are both indices of systolic cardiac function[2, 5-7]. Both indexes can be calculated from systolic and end-diastolic ventricle volumes. Prenatal quantitative measurements of ventricular volumes using 2D ultrasound are, however, inaccurate owing to the necessary use of geometric assumptions about the ventricular shape[1]. Spatiotemporal image correlation (STIC) is a technique that creates a four-dimensional (4D) ultrasound volume of the fetal heart[8]. Because STIC gives the investigator the opportunity to freeze a 4D cardiac loop in end-diastole and end-systole, volumetric measurements to calculate fetal stroke volume and cardiac output can be performed.

A number of studies have assessed the possibilities of cardiac volume and mass measurements using 3D/4D ultrasound imaging[1, 9, 10]. Normal values of fetal ventricular volumes, stroke volume and cardiac output have been reported[5, 11]. A recent study by our group corroborates these results[6]. Interestingly, compared with the conventional 'Doppler' studies on fetal stroke volume, all the studies using 4D ultrasonography report remarkably smaller volumes[5, 6, 11-16]. Indeed, the difference can be as much as four fold, as is shown in Table 1. In an earlier study, Bhat *et al.* conducted a series of measurements in volumes ranging from 5 to 10 mL with stroke volumes of 2.5 mL and 5 mL[17]. To our knowledge, no validation has been preformed for actual volumetric measurements using

Table 1 *Combined left and right stroke volumes in previous studies using both two- and four-dimensional ultrasound imaging.*

Reference	Method of measurement	Combined stroke volume (mL)		
		20 weeks	24 weeks	30 weeks
Kenny *et al.* (1986)[13]	Vessel area and TVI	1.93	2.74	4.63
Allan *et al.* (1987)[12]	Vessel area and TVI	1.16	2.13	4.49
Rasanen *et al.* (1996)[16]	Vessel area and TVI	1.18	2.89	6.28
Mielke & Benda (2001)[15]	Vessel area and TVI	0.85*	2.06*	4.56*
Messing *et al.* (2007)[11]	STIC and inversion method	0.41	1.26	2.95
Molina *et al.* (2008)[5]	STIC and VOCAL	0.55	1.27	2.69
Uittenbogaard *et al.* (2009)[6]	STIC and 3D slice method	0.72	1.72	2.63

**Extracted from figures. 3D, three dimensional; STIC, spatiotemporal image correlation; TVI, time velocity integral; VOCAL, Virtual Organ Computer-aided AnaLysis.*

STIC in volumes smaller than 5 mL. This is remarkable because all recently published studies show fetal ventricle volumes to be smaller than 5 mL during most of gestation[5, 6, 11].

In various published reports on 4D ultrasound imaging, several methods have been used to obtain the volumetric measurements[5, 6, 11, 18-21]. Virtual Organ Computer-aided AnaLysis (VOCAL™), a rotational technique, is utilized most frequently[5, 18, 20-22]. Another relatively new method uses an inversion mode algorithm to display and measure fluid-filled structures[11]. A third method is 3D slice method, which uses a more conventional technique by obtaining volumetric measurements from equally spaced parallel slices of the 3D volume[6].

The primary goal of this study was to asses the accuracy and reliability of volumetric measurements using STIC in volumes comparable to fetal cardiac ventricle volumes in the mid-second and third trimester using three different techniques.

METHODS

To validate the volumetric measurements a modification of a balloon model previously described by Bhat *et al.* was used[17]. Customized miniature balloons simulated fetal car-

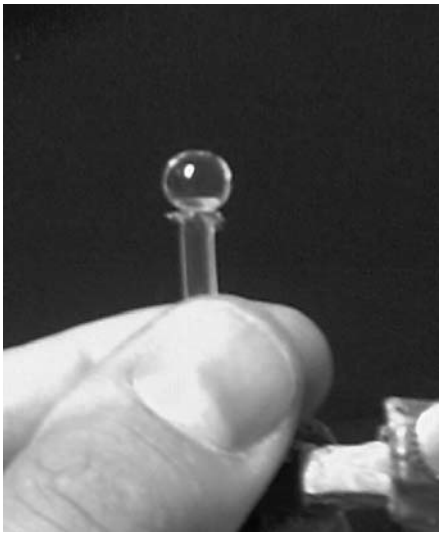


Figure 1 *Customized balloon made of latex fixed on top of a rigid catheter. The catheter was attached to a pump system to simulate the fetal cardiac phases.*

diac ventricle volumes (Figure 1). The balloons were made of small pieces of ultrasound probe covers, comprised of a single layer of latex and fixed on top of a small rigid plastic catheter with a diameter of 5 mm and a lumen of 3 mm. This catheter was attached to a custom-made pump system, capable of generating variable low stroke volumes at high frequencies to mimic fetal cardiac phases. The stroke volumes were created through the movement of the plunger within a syringe tube containing maximum of 5 mL. An electromotor rotated a wheel that was attached to the plunger by a connecting rod. By manually positioning the connecting rod slightly off the centre of the wheel, the operators were able to adjust accurately movement of the plunger and thus of the ejected stroke volume. The 'systolic' volumes were set using an accurate 1-mL syringe.

The pulsatile balloons were immersed in a customized acrylic (Perspex) bath constructed in our laboratory which was filled with ultrasound gel mixed with talcum powder. A small window was cut out in one side of the water bath and replaced by a latex film, to serve as the scanning window and here the transducer was applied for the volume acquisition. The distance between the balloon and transducer was approximately 5 cm in order to obtain the highest possible image quality. The systolic balloon volumes ranged from 0.3 to 3.3 mL, which is comparable with ventricle volumes in the early second to third trimester. For each systolic balloon volume, the system was set to produce three different stroke volumes based on 0.25, 0.50 and 0.75 times the systolic balloon volume. The stroke volumes ranged from 0.08 to 1.65 mL and, consequently diastolic volumes ranged from 0.38 to 4.95 mL. For each systolic-stroke volume combination, four STIC volumes were acquired (Table 2). The pump system was set at a frequency of 130/min to simulate a fetal heart rate.

System calibration

To test the accuracy and repeatability of the system, the amount of water ejected by the syringe was repeatedly weighed using an accurate balance. The accuracy of the injection of 'systolic' volumes was also assessed by repeatedly weighing the amount of injected water.

Volume acquisition and measurement

The balloons were imaged using a Voluson E8 (GE Medical Systems, Kretz, Austria) with a motorized curved array 6-12 MHz transvaginal 3D/4D transducer. To avoid bias owing to image enhancement software, Speckle Reduction Imaging (SRI) or Cross XBeam (CRI) were not used. The impact of post process gain or contrast changes on the 3D volume measurements was evaluated by measuring 30 volumes using different settings in gray-scale curve. For STIC, the angle of acquisition was set as small as possible to obtain the highest frame rate. To conceal the true volumes for the observer during the post-process

Table 2 *Specifications of the 76 acquired spatiotemporal image correlation volumes.*

Systolic volume (mL)	Stroke volume (mL)	Diastolic volume (mL)	Number of volumes acquired
0.30	0.08	0.38	3*
0.30	0.15	0.45	4
0.30	0.23	0.53	4
0.80	0.20	1.00	4
0.80	0.40	1.20	4
0.80	0.60	1.40	4
1.30	0.33	1.63	4
1.30	0.65	1.95	4
1.30	0.98	2.28	4
1.80	0.45	2.25	3*
1.80	0.90	2.70	4
1.80	1.35	3.15	4
2.30	0.58	2.88	4
2.30	1.15	3.45	4
2.30	1.73	4.03	4
2.80	0.70	3.50	4
2.80	1.40	4.20	4
2.80	2.10	4.90	3*
3.30	0.83	4.13	3*
3.30	1.65	4.95	4
3.30	2.48	5.78	†

**One volume excluded owing to insufficient image quality. †Volumes could not be obtained because of limitations in system set-up.*

measurements, all STIC volumes were stored under a code assigned by a second observer. The STIC volumes were saved to a personal computer for off-line analysis. All STIC volume acquisitions and measurements were done by one operator (L.U.). To allow assessment of reliability, all measurements were performed twice by the same operator in two separate series of measurements. A software package was used for off-line examination (4D View™ Version 6.0, GE Medical Systems). The volumes were visualized in multiplanar display. Manual adjustment of the cine sequence provided the minimal and maximal balloon expansion, as the equivalent of end-systole and end-diastole.

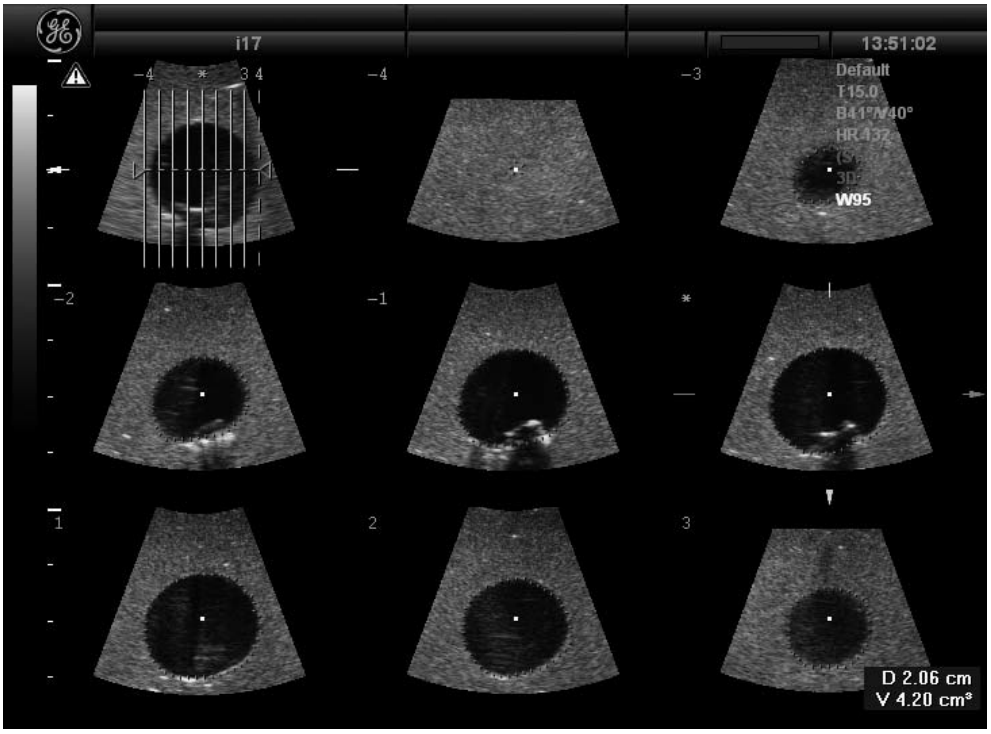


Figure 2 *Three-dimensional slice method; nine parallel slices at equal distances were traced manually to measure the balloon volume.*

Three-dimensional slice method

3D slice method is a more conventional technique for 3D volume measurements and is based on Simpson's rule (Figure 2)[23]. This method consists of slicing a 3D volume into a series of parallel slices that are then traced manually. In this study, all balloon volumes were sliced at equal distance using the Fractional Limb Volume software tool. This tool allowed manual delineation of the balloon contours after selecting the primary area, which was subsequently divided into nine parallel slices. The volume was calculated by summation of the traced area and multiplied by the interslice distance. The interslice distance ranged from 1.0 to 2.2 mm.

Virtual Organ Computer-aided AnaLysis

Currently this is one of the most frequently used methods to obtain volume measurements. VOCAL rotates the selected images around a fixed vertical axis in steps of 6-30° for manual or automated volume measurement. Given the regular shape of the measured volumes used, and based on the results of a previous report, in this study rotational steps of 30° were used[21]. In the measurement process VOCAL contour definition

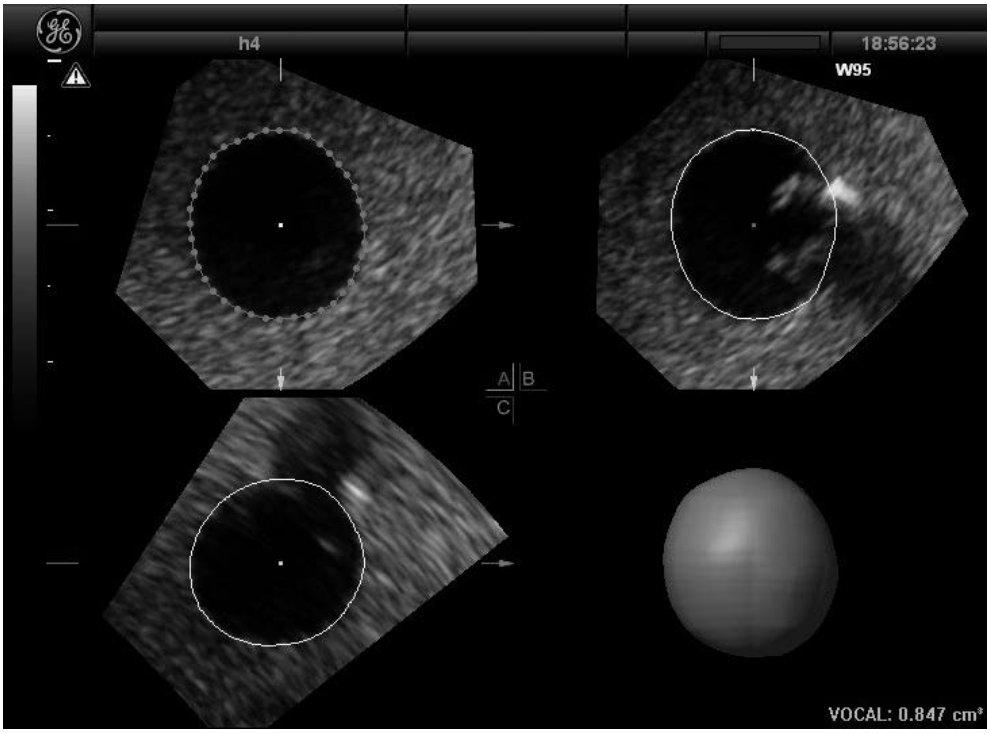


Figure 3 *Virtual Organ Computer-aided AnaLysis (VOCAL) method rotates the selected image around a fixed vertical axis. Semiautomated tracing is used to measure the volume.*

was set to automated sphere contour; the diameter of the sphere was set by the operator and followed by a fine-tuning process of manual adjustment to exactly fit the balloon contour in each of the rotational planes (Figure 3). By reviewing the whole volume in three perpendicular planes, the adequacy of the rotational area measurement was checked.

Inversion method

Inversion mode is an algorithm that identifies echolucent voxels which can be used for volume measurements. Volumetric measurements are possible because the dimension of each voxel within the volume dataset is known[24]. The method consists of an automated sphere contour mode using VOCAL, which was set widely around the outline of the balloon image. Small adjustments were made to the sphere contour to prevent shadowing artefacts interfering with an automated volume measurement using inversion mode (Figure 4). After this, the inversion mode threshold was set. During thresholding all measured voxels color brightly, allowing careful threshold setting and thus avoiding measurement errors. In this way care was taken to only measure the true echolucent volume of the fluid filled balloon.

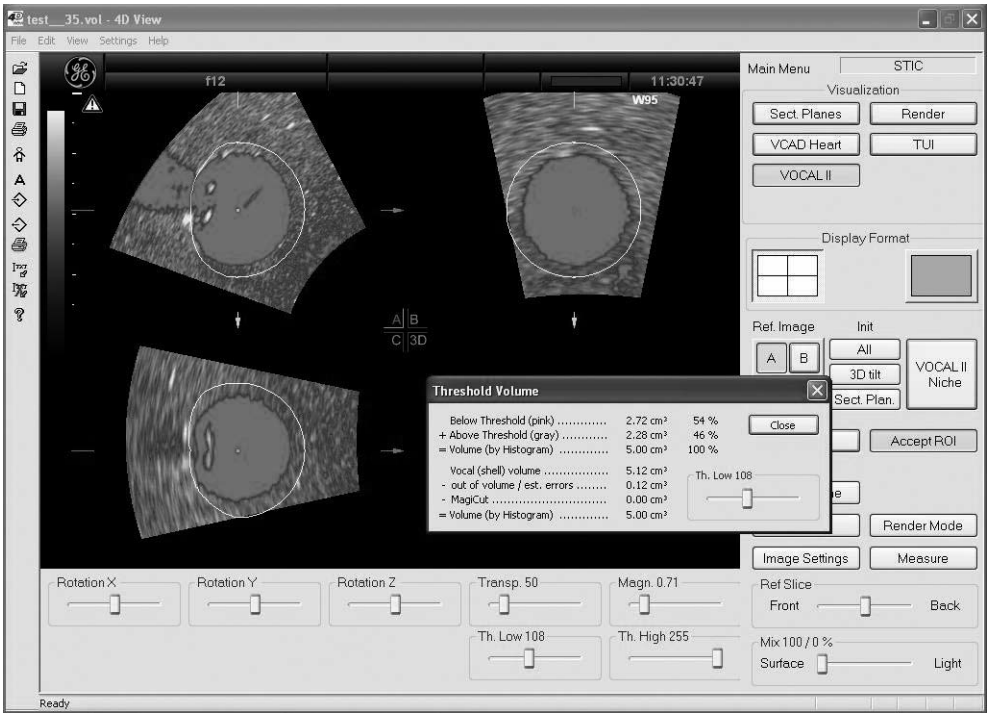


Figure 4 *The inversion method measures the volume of the echolucent voxels only, adjustable through threshold setting, within a selected area defined by an automated contour using Virtual Organ Computer-aided AnaLysis (VOCAL) software.*

To avoid measuring the end of the catheter inside the balloon as being 'liquid' volume, the catheter tip volume was calculated and subtracted from the measurements done with the 3D slice method and VOCAL method. Further, the time needed to perform a volumetric measurement was assessed by timing measurements of a series of 10 randomly chosen volumes using each method.

Statistical analysis

The accuracy of the three methods was assessed by calculating the differences between the first series of volume measurements and the true volumes. A positive error indicated an overestimation, whereas a negative error indicated an underestimation. Because differences between the measurements and the true volumes increased as the size of the measured volume increased, an attempt was made to normalize the variance by expressing the volumetric difference as a percent of the measured volume[25]. The SD of the difference was used to calculate the 95% limits of agreement (LOA) (mean difference +/- 1.96*SD). Accuracy was defined as a mean percentage error of <5%. Stroke volumes and injection fractions were calculated from the observed volumes. Stroke volume was de-

fined as end-diastolic volume minus end-systolic volume, the injection fraction was defined as stroke volume divided by end-systolic volume. Intraobserver reliability was assessed by calculating the intraclass correlation (ICC)[25, 26] and coefficients of variation (CV), which was defined as the SD of the within subject differences expressed as a percentage of the mean as described by Bland and Altman[27]. ICC values of >0.95 and CV <5% were regarded to reflect good reliability and agreement respectively. CV <10% was regarded to reflect acceptable agreement. SPSS 16.0 was used for all statistical analysis (SPSS Inc, Chicago, IL, USA). Significance levels were set at $p < 0.05$.

RESULTS

A total of 80 STIC volumes were acquired from balloons with varying systolic, stroke and diastolic volumes (Table 2). Four volumes had to be excluded from further analysis due to insufficient image quality and echo dispersion caused by small amounts of air trapped in the gel. As all 76 volumes were measured twice in both systolic and diastolic phase, using the 3D slice, VOCAL and Inversion method, a total of 912 volumes were measured. The frame rate in all STIC volumes was around 89 Hz.

System calibration

The balloon model proved to be valid with good accuracy as assessed by the 95% LOA. Manual injection of 0.8 mL fluid was subtracted from the weight of the fluid and showed a mean difference of -0.01 mL (95% LOA -0.02 - -0.01; n=10) and manual injection of 1.5 mL showed a mean difference of -0.02 mL (95% LOA -0.03 - -0.02; n=10). Further, measurement outcome was not significantly influenced by post-process gray-scale curve variation as assessed by the 95% LOA (mean difference (darker subtracted from original), 0.03 mL (95% LOA -0.02- 0.08; P=0.12); mean difference (lighter subtracted from original), 0.04 mL (95% LOA -0.01 - 0.08; P=0.08)).

Three-dimensional slice method

The average time to perform a measurement using the 3D slice method was 118 (range 95-134) s (n=10). The actual systolic volumes and corresponding observed systolic volumes with error analysis are shown in Table 3. In Figure 5a, the systolic and diastolic percentage error are plotted against the true volumes. The 3D slice method showed a mean percentage error of -3.3% for volumes between 0.3 and 5.0 mL (95% LOA -11.2 to 4.7%; n=152). The calculated stroke volume showed a mean bias of -2.3% (95% LOA -29.8 to 25.1%; n=72). An actual injection fraction of 25% was calculated as 24.5% (95% LOA 16.3 - 32.6%) and an injection fraction of 50% was calculated as 50.2% (95% LOA 32.3 – 68.1%) whereas an injection fraction of 75% was calculated as 73.4% (95% LOA 62.6 – 84.2%).

Table 3 *Actual and observed systolic volumes with error analysis for all three methods.*

Method	Actual systolic volume (mL)	n	Mean ± SD observed volume (mL)	Mean ± SD error (%)
3D Slice	0.30	11	0.30 ± 0.01	-6.61 ± 3.44
VOCAL	0.30	11	0.31 ± 0.02	-3.93 ± 5.08
Inversion	0.30	11	0.27 ± 0.04	-10.00 ± 14.76
3D Slice	0.80	12	0.78 ± 0.04	-5.17 ± 4.33
VOCAL	0.80	12	0.79 ± 0.03	-4.83 ± 3.09
Inversion	0.80	12	0.76 ± 0.03	-5.52 ± 4.28
3D Slice	1.30	12	1.30 ± 0.06	-2.09 ± 4.26
VOCAL	1.30	12	1.34 ± 0.06	1.29 ± 4.64
Inversion	1.30	12	1.39 ± 0.07	6.60 ± 5.43
3D Slice	1.80	11	1.75 ± 0.07	-4.02 ± 3.64
VOCAL	1.80	11	1.77 ± 0.11	-2.81 ± 6.18
Inversion	1.80	11	1.69 ± 0.13	-6.26 ± 7.30
3D Slice	2.30	12	2.21 ± 0.07	-4.84 ± 3.05
VOCAL	2.30	12	2.09 ± 0.07	-10.16 ± 3.20
Inversion	2.30	12	2.01 ± 0.09	-12.43 ± 3.83
3D Slice	2.80	11	2.80 ± 0.06	-1.02 ± 1.95
VOCAL	2.80	11	2.80 ± 0.19	-1.02 ± 6.79
Inversion	2.80	11	2.62 ± 0.08	-6.33 ± 2.73
3D Slice	3.30	7	3.37 ± 0.07	1.39 ± 2.14
VOCAL	3.30	7	3.45 ± 0.05	3.69 ± 1.40
Inversion	3.30	7	3.29 ± 0.15	-0.22 ± 4.64

3D, three dimensional; VOCAL, Virtual Organ Computer-aided AnaLysis.

Virtual Organ Computer-aided AnaLysis

The average time to perform a complete volume measurement using VOCAL was 179 (range 137-225) s (n=10). The differences between the mean observed volumes and actual systolic volumes are shown in Table 3. The percentage errors of both systolic and diastolic volumes are plotted against the true volumes in Figure 5b. VOCAL showed a mean error of -2.9% for volumes between 0.3 and 5.0 mL (95% LOA -14.1 to -8.3%; n=152). The mean percentage error for stroke volumes was -2.2% (95% LOA -27.7 to 23.4%; n=76). An actual injection fraction of 25% was calculated as 25.7% (95% LOA 15.4 - 36.0%), an injection fraction of 50% was calculated as 47.3% (95% LOA 37.7 - 57.0) and an injection fraction of 75% was calculated as 74.2% (95% LOA 63.1 - 85.4).

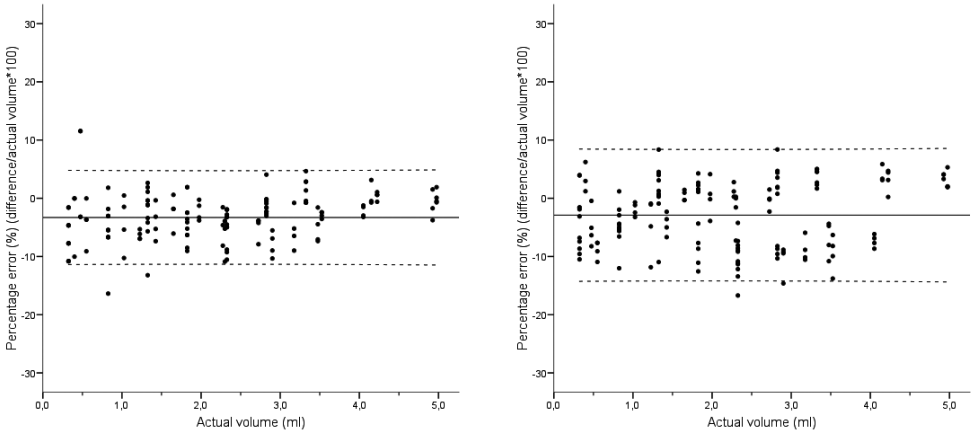


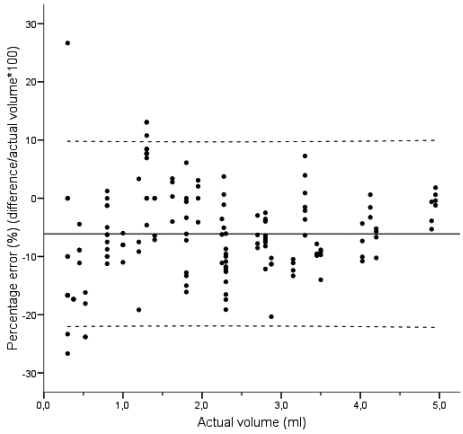
Figure 5 *Percentage errors (difference/actual volume ×100) of systolic and diastolic balloon volumes with limits of agreement (- - - -) plotted against the actual volume for the three-dimensional slice method (a), the Virtual Organ Computer-aided AnaLysis (VOCAL)(b) method and the inversion method (c).*

Inversion method

The average time to perform a complete volume measurement using the Inversion method was 134 (range 99-181) s (n=10). The different actual systolic volumes and corresponding observed systolic volumes using Inversion mode are shown in Table 3. Figure 5c shows the measured differences and percentage errors of the observed systolic and diastolic volumes. Mean percentage error for volumes ranging from 0.3 to 5.0 mL was -6.1% (95% LOA -21.4 to 9.2%; n=152). The mean difference for stroke volume was -12.1% (95% LOA -44.2 to 19.9%; n=76). An actual injection fraction of 25% was calculated as 23.6% (95% LOA 13.5 - 33.7%), an injection fraction of 50% was calculated as 44.4% (95% LOA 19.6 - 69.2) and an injection fraction of 75% was calculated as 75.1% (95% LOA 53.8 - 96.3).

Intraobserver reliability

Intraobserver reliability was assessed for each method using all 152 systolic and diastolic measurements. CVs for 3D slice method, VOCAL and Inversion method were 2.9%, 4.0% and 7.3% respectively. ICCs were 0.999 (95% LOA 0.998-0.999), 0.997 (95% LOA 0.996-0.998) and 0.993 (95% LOA 0.990-0.995) respectively.



DISCUSSION

The introduction of 3D ultrasound has made volume measurements more accurate than those made by two-dimensional ultrasound[20, 28-30]. In the literature, different methods have been described to obtain volumetric measurements from 3D and 4D volumes[5, 6, 11, 18-21]. To our knowledge this is the first study to validate 3D volume measurements obtained from a 4D dataset in the very small volume range comparable to cardiac ventricular volumes from approximately 20 weeks' gestation onwards[6]. Therefore, we can compare our results only with those of Bhat and colleagues, who measured systolic volumes ranging from 5, 7.5 and 10 mL using VOCAL and STIC[17]. In their paper they report a mean underestimation of 5% for these systolic volumes, which is comparable to our results.

This study demonstrates that three different techniques can be used to provide volumetric measurements in this low volume range from 3D datasets. All measurement techniques, however, underestimated the actual volumes. VOCAL and 3D slice method were most accurate, with a mean underestimation of approximately 3% in the range 0.3 to 5.0 mL. The inversion method underestimated the volumes to the greatest extent (approximately 6%). The 3D slice method had the narrowest LOA and the inversion method the widest. All methods showed good reliability (ICC > 0.95); the 3D slice and VOCAL methods also showed good agreement (CV < 5%) whereas the inversion mode showed acceptable agreement (CV < 10%). Calculated stroke volume was also underestimated, and the 3D slice and VOCAL methods were more accurate than the inversion method. The large range in the mean percentage error for stroke volume for each method can be explained partly by the fact that two estimates were used to calculate stroke volume,

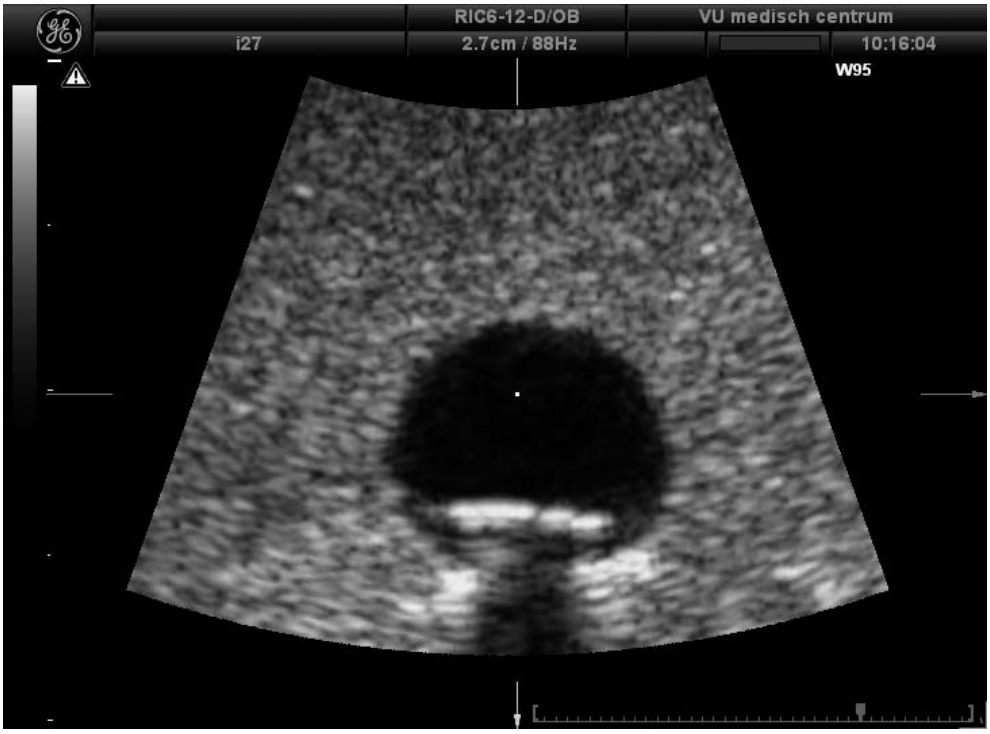
which increases variance. Furthermore, as the calculation of the injection fractions was based on three volume estimates (diastolic minus systolic volume, divided by systolic volume), the variance of these estimates was further enlarged. This resulted in rather large LOA. Therefore, with regard to *in-vivo* measurements, it remains to be determined whether fetal ejection fraction is a more useful parameter than stroke volume for evaluating fetal cardiac function.

In clinical 4D echocardiography, the endocardial borders in parallel slices of the four-chamber view are generally of better image quality in parallel planes than the computer-created rotational images used in VOCAL software. We therefore hypothesise that the 3D slice method will prove more useful in clinical practice. This method was accurate, showed the narrowest LOA, was the least time consuming, and was highly repeatable.

We acknowledge that a limitation of this study relates to the complexity of the shape of fetal ventricles. No conclusions can be drawn from this study regarding which method would best address the complex contour and geometric shape of the fetal ventricle *in-vivo*. In our opinion, however, it is likely that the 3D slice method will prove more useful compared to inversion or VOCAL methods as fetal volumetric measurements using VOCAL or inversion methods might be complicated by the following three factors: low resolution of computer-created rotational images, variability due to threshold settings and shadow artefacts, which are a frequent limiting factor in post-process analysis of STIC volumes[6].

The most obvious source of error in sonographic volumetric measurements is the delineation of the endoventricular border or, in this study, balloon edges. As histograms of the balloon images demonstrate, even hard-edged contrast balloon walls are not displayed as hard-edged echolucent to echogenic grey-scale margins on screen (Figure 6). *In vivo*, ultrasonographic margins are often even less clearly demarcated and can further be complicated by acoustic shadowing and maternal obesity. Further, in a clinical setting, errors might be larger than found in this study, as a 6-12 MHz 4D transvaginal transducer was used to obtain images instead of an abdominal 5-9 MHz ultrasound probe, which is more commonly used for fetal scanning.

Although small errors in area measurement might seem acceptable in most cases, the influence of these errors is enlarged in the smallest volume ranges. As the magnitude of these errors will increase relative to the volume size, inaccuracies will be highest in the smallest volumes. Our own unpublished data do indeed point in this direction, as underestimation of volumes of 0.15 mL reached 40-50%. Therefore, one has to be careful in the interpretation of volume measurements smaller than 0.3 mL, which *in vivo* are



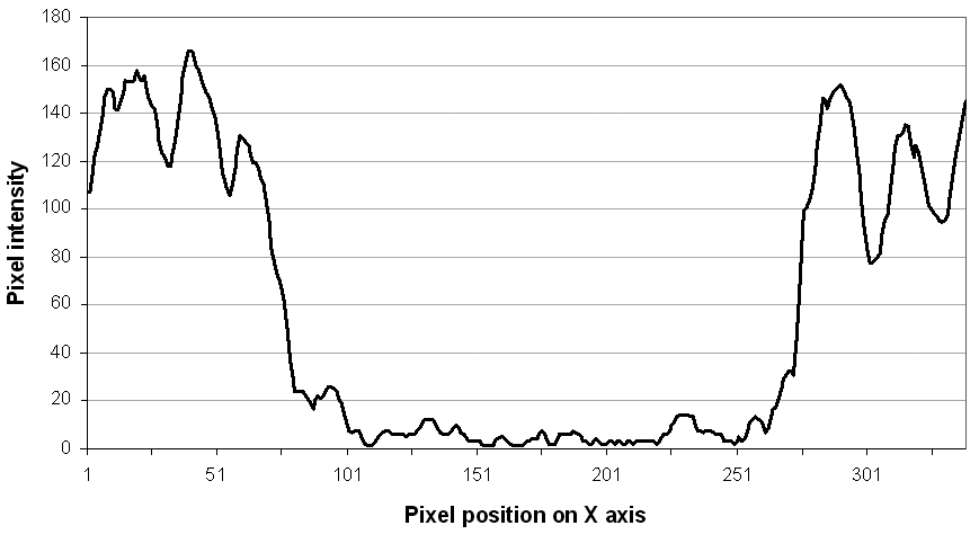


Figure 6 *a) Ultrasound image of a balloon model clearly illustrating that the hard-edged contrast balloon margins are displayed as a gradual transition in the pixel intensity. b) Histogram of an image showing pixel intensity against position on the x-axis through the reference point in Figure 6a.*

comparable to systolic fetal ventricle volumes before approximately 20 gestational weeks[5, 6].

Three-dimensional ultrasound is not real-time ultrasound. Therefore, the measurements were not obtained from real-time images. STIC volumes display a virtual cycle, computed from the spatial displacements within the raw 2D data. However, in this study the STIC volumes contained approximately 40 2D images per cardiac cycle (10 s, 90 Hz, 130 beat/min) which last less than 0.5 s. We therefore feel that 4D ultrasound using STIC allows accurate volumetric measurements.

Despite everything discussed above, the underestimation observed in this study may not entirely be a direct result of errors in area measurement as described because all volumes in this study were also measured using the inversion method, which counts voxels above a given grey-scale threshold. This suggests that image display and spatial resolution of the ultrasound scanner itself may play a role in measurement error. These errors are likely to be dependent on system settings including B-mode gain, grey-scale curve, and dynamic range setting of the ultrasound machine as post-process grey-scale variation did not significantly change the results. Accordingly, very low-volume measurements in the fetus in the very low volume ranges might be impossible currently without acceptance of high levels of uncertainty and inaccuracy. This study might draw attention to one of the biggest problems in fetal cardiac volume measurement early in pregnancy i.e. that the currently available ultrasound systems do not have the large penetration depth, high frequency and high resolution that are a needed for accurate measurements.

In conclusion, 4D ultrasonography with STIC is a feasible and accurate method for calculating volumes of 0.30 mL upwards. In an *in-vitro* model the 3D slice method proved the most accurate, the least time consuming, and had the best reliability and smallest LOA. This method may prove useful when applied to *in-vivo* investigations.

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Chapter 5

Reliability of fetal cardiac volumetry using spatiotemporal image correlation assessment of *in-vivo* and *in-vitro* measurements

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ABSTRACT

Objective

To assess the reliability of measurement of fetal cardiac ventricular volume, stroke volume, and ejection fraction with four-dimensional (4D) ultrasound using spatiotemporal image correlation (STIC).

Methods

Volume datasets were collected from two sources: 24 from fetuses over a range of gestational ages and 12 from a miniature balloon model. Datasets were analyzed by three observers, repeatedly in 12 fetal datasets and all balloon datasets. Volume calculations were obtained by manually tracing multiple parallel slices (three-dimensional (3D) slice method). Measurement error was assessed by calculating standard errors of measurement (SEM) and coefficients of variation (CV). Reliability was assessed by calculating interobserver and intraobserver intraclass correlation coefficients (ICC).

Results

Measurement errors of balloon volumes were small and reliability was good (SEM ≤ 0.07 mL, ICC 0.98-1.00). Fetal ventricle volume measurement error ranged from 0.09 to 0.20 ml and CVs from 14.6-28.3%. Ventricular volume reliabilities for intra- and interobserver comparisons were greater than or equal to 0.94 and 0.75 respectively. Fetal stroke volume measurement error (SEM 0.17 mL), CV (21.9%) and reliability were measured (intraobserver ICCs: left ventricle stroke volume (LVSV) 0.93, right ventricle stroke volume (RVSV) 0.88; interobserver ICCs: LVSV 0.75 vs. RVSV 0.86). The measurement error decreased with increasing operator experience. The reliability of ejection fraction calculations was poor (ICC < 0.7) for intra- and interobserver comparisons.

Conclusions

Volume measurements obtained with STIC and 3D slice methods using a balloon model were reliable. In the fetus, measurement errors decreased with operator experience and reliability was better for stroke volume than for ejection fraction.

INTRODUCTION

Valid and reliable indices of fetal cardiac (dys)function can be valuable in the diagnosis and follow-up of cases with CHD, hydrops fetalis, fetal diabetic cardiomyopathy, intrauterine growth restriction (IUGR) or twin-to-twin transfusion syndrome (TTTS). Conventionally, in the fetus these indices have been estimated using two-dimensional (2D) ultrasound measurement of valve orifices, combined with Doppler flow velocity tracings. This method is known to have limitations with regards to its reliability[1-4]. Spatiotemporal image correlation (STIC) is a technique that uses a four-dimensional (4D) data volume that can be used fo 3-dimensional (3D) volume estimations[5-7].

Reliability of a method is defined as the degree to which the measurement is free from measurement error and refers to the consistency or precision of the measurements[8]. Validity is defined as the degree to which an instrument truly measures the construct(s) it purports to measure and is an indication of accuracy. In assessing the utility of a fetal echocardiographic method of ventricular volume estimation, reliability of the volume estimations is as important as validity.

The validity of STIC for cardiac volumetry has been previously tested in studies performed by our group using a miniature in-vitro balloon model[9]. The model consisted of small balloons attached to a pump system immersed in a bath filled with viscous gel mimicking a cardiac cycle with a frequency of 130 beats per minute (bmp). The results of that study showed that 3D volume measurements from STIC volumes are valid from 0.5 mL onward. The magnitude of differences between repeated measurements in the same subject by the same observer and between different observers, however, must be defined before any quantitative technique can be used reliably to document 'real' changes in fetal heart volume or function over time. Different reports have been published on the reliability of 3D volumes[10-14], though reports published on the reliability of volume estimations using STIC for fetal echocardiography are limited[6, 15]. A recent study by Hamill *et al.* has shown good results using STIC and Virtual Organ Computer-Aided Analysis (VOCAL)[15]. No reports, however, have been published addressing the reliability of volume estimations with STIC using the 3D slice method. The aim of this study was to asses the reliability of fetal cardiac volume measurements and indices of fetal cardiac function within the same observer and between observers. Further, this study aimed to asses the influence of volume shape and observer experience on the reliability of volumetric measurements.

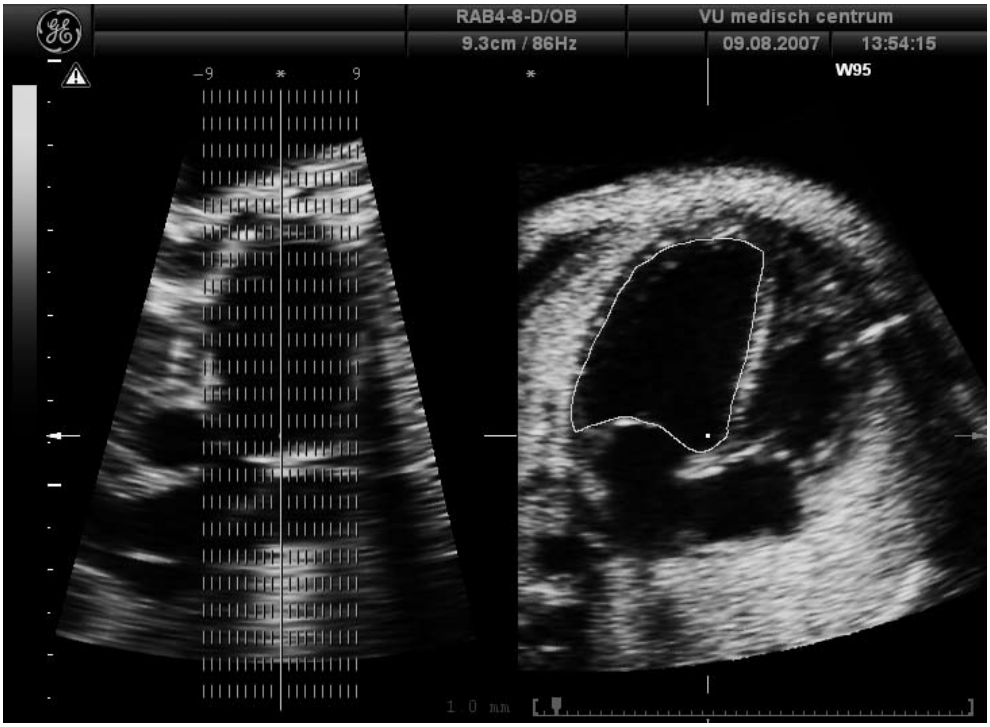

Figure 1 *The three-dimensional slice method applied to a frozen four-dimensional volume of the fetal heart in diastole, showing the manual trace used to outline the contour of the endocardium.*

METHODS

Digital cardiac volumes of 24 healthy fetuses with gestational ages between 16+4 and 30+3 weeks (mean 23+4) weeks were included in this study. All pregnancies were dated by last menstrual period and confirmed by first-trimester CRL measurement. Cardiac anatomy was normal, based on a 2D third-level echocardiographic cardiac examination and uneventful follow up after birth. All volumes were acquired by one experienced operator (L.U.) at our outpatient clinic and randomly selected. In the fetal cardiac volumes both left and right ventricles in end-systole and end-diastole were measured (Figure 1). Additionally, 12 volumes with a similar volume range were assessed using a customized miniature balloon model, as described earlier (Figure 2)[9].

All volumes were acquired by using a Voluson E8 with a 4-8 MHz abdominal transducer or a 6-12 MHz transvaginal transducer (GE Medical Systems, Kretz, Austria) and saved to a personal computer. For the off-line analysis a software package was used (4DView 6.0, GE Medical Systems). Of the three observers participating in this study, two were expe-

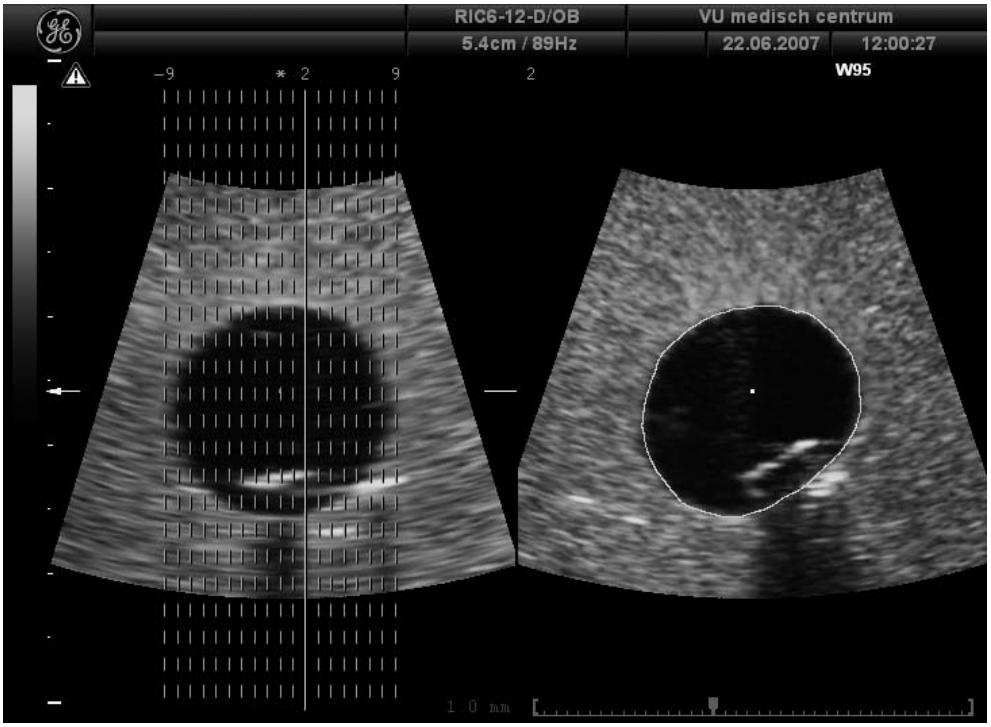

Figure 2 *The three-dimensional slice method applied to a static volume of a miniature balloon model, showing the manual trace used to outline the contour of the balloon.*

rienced in the use of post process software (Observers A & B). The third Observer (C) was an experienced sonographer, but was less specialized in echocardiography and unfamiliar with the use of 3D postprocessing software. This sonographer learned to use the 3D software primarily for the purpose of this study. Each observer first measured end-systolic and end-diastolic volumes of left and right ventricles in 24 fetal heart volumes. Secondly, the measurements were repeated twice for the first 12 hearts to produce three measurements per fetal ventricle for each observer. After the fetal cardiac measurements, the observers measured maximal and minimal distension in 12 balloon volumes. These measurements were also repeated twice. As such, three series of 96 measurements, six series of 48 measurements and three series of 72 balloon measurements resulted in a total 792 volumetric measurements available for analysis.

The volumes were reviewed in multiplanar mode to optimize image magnification and contrast settings. Static 3D volumes in end-systolic and end-diastolic phase were manually selected by scrolling through the dynamic 4D cardiac cycle. The end-diastolic phase was defined as starting with the first image obtained after the instant of closing

of the atrioventricular (AV) valves and the end-systolic phase was defined as starting with the first image obtained prior to the instant of opening of the AV valves. In the balloon model, images of maximal and minimal distension were selected by scrolling through the cineloop. For the volume measurement in both the fetal cardiac ventricles and the balloons the 3D Slice method was used as previously described[7]. This method avoids the use of digitally constructed images in rotational methods like VOCAL. The 3D slice method was found to be valid and had smaller coefficient of variation than VOCAL and Inversion mode[9]. To minimize measurement error, the manual trace was used to outline the contour of the endocardium. Based on these tracings of multiple slices of the four-chamber view, measured areas are multiplied by the slice thickness and summed (Figures 1 and 2). The minimal interslice distance was 1.0 mm. Tracing of the endocardium or balloon contours was performed on the white (endogenic) side of the black-white boundary. Landmarks for left and right ventricle measurements were the AV valves and the aortic and pulmonary valves, respectively. In the fetal hearts the papillary muscles were considered part of the ventricular cavity in this analysis, in accordance to earlier studies[5]. The balloon volumes were measured in maximal and minimal distension based on visual selection of images within the volume. End-systolic and end-diastolic left and right fetal ventricular and balloon volumes were used to calculate stroke volumes *(stroke volume = diastolic volume - systolic volume)* and ejection fractions *(ejection fraction = (diastolic volume - systolic volume) / diastolic volume)*. This study was approved by the medical ethics committee and all patients signed informed consent prior to inclusion.

Reliability and measurement error

Reliability is inversely related to random error. As all measurements are prone to various sorts of error, the importance of measurement error depends upon the context in which the measurements are to be used. Therefore it is useful to interpret measurement error within the context of the mean and variation of the data. In this study two aspects of measurement error were assessed, the absolute standard error of measurement (SEM) and the relative measurement error (coefficient of variation (CV)). The SEM quantifies how close measurements made on the same object are and is a characteristic of the measurement method involved. The CV is a normalized measure of variation and is used to express the absolute measurement error relative to the mean as a ratio expressed as a percentage. Reliability (intraclass correlation coefficient (ICC)) relates the magnitude of the measurement error to the inherent variability of the measurements and examines how well measurements can be distinguished from each other, despite measurement error.

Statistical analysis

For the statistical analysis SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel for Windows were used. Variance components were assessed using SPSS VAR-

Table 1 *The overall mean, SD and minimal and maximal values (range) of the measurement dataset.*

Parameter	n*	Volume (mL)	
		Mean (range)	SD
Left ventricle ESV	144	0.79 (0.04–3.25)	0.61
Left ventricle EDV	144	1.67 (0.15–5.34)	1.13
Right ventricle ESV	144	0.68 (0.04–2.20)	0.40
Right ventricle EDV	144	1.54 (0.13–3.98)	0.84
Stoke volume left ventricle	144	0.87 (0.04–3.43)	0.61
Stoke volume right ventricle	144	0.86 (0.09–2.05)	0.50
Ejection fraction left ventricle	144	0.52 (0.15–0.82)	0.12
Ejection fraction right ventricle	144	0.56 (0.28–0.80)	0.10
Minimal balloon volume	108	1.90 (0.68–3.41)	0.82
Maximal balloon volume	108	2.86 (0.99–5.01)	1.22
Balloon stroke volume	108	0.95 (0.31–1.92)	0.41
Balloon ejection fraction	108	0.34 (0.28–0.46)	0.03

**Twelve fetal hearts and 12 balloons were measured three times by each of three observers (to give 108 measurements each), and 12 other fetal hearts were measured once by all three observers (to give 36 measurements). EDV, end-diastolic volume; ESV, end-systolic volume.*

COMP analysis. The following variance components were calculated: variance between hearts, variance between different observers, variance between different measurements made by the same observer, and (random) error variance. These variances were used to calculate reliability and measurement error[16]. Intraobserver and interobserver reliability were calculated using ICC (the exact formulas can be obtained from the authors). ICC > 0.70 are considered to reflect good reliability[17, 18]. Intraobserver reliability was also calculated for each of the observers separately to asses the differences between the experienced and inexperienced observer. Measurement error was calculated as SEM, calculated from the square root of the error variance of the ICC. CV were calculated in a separate analysis for each of the repeatedly measured volumes separately and averaged. CV were also calculated for experienced observers only to asses the influence of experience.

RESULTS

All fetal cardiac and balloon volumes were successfully measured and all 792 volumetric measurements were analyzed. The overall mean, standard deviation, minimal and maximal values of the measurement dataset are shown in Table 1. Data for intraobserver and

Table 2 *Intraobserver and interobserver reliability data.*

Parameter	Overall			Observer A		Observer B		Observer C	
	SEM (mL)	Inter-ICC	Intra-ICC	SEM (mL)	Intra-ICC	SEM (mL)	Intra-ICC	SEM (mL)	Intra-ICC
Left ventricle ESV	0.10	0.86	0.97	0.12	0.95	0.00*	0.99	0.07	0.99
Left ventricle EDV	0.15	0.91	0.98	0.00*	0.99	0.00*	1.00	0.20	0.97
Right ventricle ESV	0.09	0.75	0.94	0.11	0.95	0.08	0.93	0.11	0.92
Right ventricle EDV	0.20	0.87	0.94	0.16	0.97	0.07	0.99	0.30	0.87
Left stroke volume	0.16	0.75	0.93	0.12	0.96	0.10	0.97	0.23	0.91
Right stroke volume	0.17	0.86	0.88	0.13	0.93	0.09	0.96	0.24	0.80
Left ejection fraction	0.08	0.14	0.65	0.06	0.68	0.05	0.63	0.11	0.62
Right ejection fraction	0.06	0.33	0.48	0.06	0.46	0.05	0.48	0.08	0.47
Minimal balloon volume	0.07	0.98	0.99	0.10	0.99	0.04	1.00	0.04	1.00
Maximal balloon volume	0.05	0.99	1.00	0.07	1.00	0.05	1.00	0.04	1.00
Balloon stroke volume	0.06	0.97	0.98	0.10	0.95	0.04	0.99	0.02	1.00
Balloon ejection fraction	0.02	0.76	0.76	0.03	0.62	0.01	0.87	0.01	0.88

*Observers A and B were more experienced in fetal ultrasonography and in three-dimensional image processing than was Observer C. *Variance (error) estimate was negative and standard error of measurement (SEM) was set to zero. EDV, end-diastolic volume; ESV, end-systolic volume; ICC, intraclass correlation coefficient.*

interobserver reliability and SEM are given for all three observers and for each of the observers separately in Table 2. Average overall CVs and those for the experienced observers are given in Table 3.

In-vitro balloon measurements

For the balloon volume estimates and the stroke volumes, measurement error was small, illustrated by SEM ≤0.07 mL and small CVs. Reliability was high, demonstrated as both high intraobserver ICCs and high interobserver ICCs (ICCs 0.97-1.00). ICC values were lower for estimates of balloon ejection fraction (ICCs both 0.76).

Fetal cardiac volume measurements

The SEMs for ventricle volume estimates ranged from 0.09 to 0.20 mL. CVs were smaller for diastolic volumes than for systolic volumes and ranged from 10.6 to 20.3% for experienced observers. Both intra- and interobserver reliability values were high (ICC 0.75 - 0.97) with no apparent difference between the observers. No clear differences between left and right ventricle measurements were observed, but interobserver ICCs were slightly higher for diastolic measurements.

Table 3 *Average overall coefficients of variation (CVs) and those for the experienced observers.*

Parameter	All observers CV(%)	Experienced observers CV(%)
Left ventricle ESV	25.9	20.3
Left ventricle EDV	14.6	10.8
Right ventricle ESV	28.3	19.2
Right ventricle EDV	16.2	10.6
Left stroke volume	25.2	14.3
Right stroke volume	18.6	14.8
Left ejection fraction	20.6	13.6
Right ejection fraction	13.3	10.5
Minimal balloon volume	5.4	6.3
Maximal balloon volume	4.5	5.3
Balloon stroke volume	4.5	5.3
Balloon ejection fraction	4.4	3.9

CVs were calculated for all observers and for experienced observers separately, based on CVs of each heart/balloon and averaged. EDV, end-diastolic volume; ESV, end-systolic volume.

Calculated indices for cardiac function

The measurement error for fetal stroke volume was small, as shown by small SEMs (0.16 - 0.17 mL). For experienced observers CVs were around 14.5% for left and right ventricle stroke volume. High inter- and intraobserver ICCs (0.75 - 0.93) demonstrated high reliability. For the estimated fetal ejection fraction SEMs were 0.08 and 0.06 for left and right ventricles respectively. Intraobserver and interobserver ICCs were low and indicated poor reliability (0.14 – 0.65). This was mainly caused by relatively large variation between observers for the measured cardiac volumes.

Subgroup analysis showed differences between the experienced and inexperienced observers in both reliability and measurement error (Tables 2 and 3). For the balloon volume measurements, however, no clear differences between experienced and inexperienced observers were observed. For the ventricle volume measurements average CVs were clearly smaller when the measurements of experienced observers were analyzed. Also, for fetal stroke volume estimates the SEM of the inexperienced observer (C) was approximately double the size of the experienced observers (A & B). No clear differences were observed in intraobserver reliability.

DISCUSSION

The clinical use of measurements made using 3D ultrasound imaging has been studied in various research settings[10, 19-21]. The reliability for larger volumetric measurements has been reported to be good[10, 12, 13, 19]. Recent studies have evaluated 4D ultrasound imaging with STIC for very small fetal cardiac volume measurements in order to create normograms for fetal ventricle volumes and indices of fetal cardiac function during gestation[5-7]. Before the introduction of the 3D cardiac examination, the small volumes and indices were conventionally assessed using cross-sectional planimetered surface area measurements and 2D ultrasound measurements of valve orifices or outflow tracts combined with pulsed-wave Doppler flow velocity tracings[22]. The measurement error of these methods is quite large, with intra observer CVs of stroke volumes reported up to 16% and interobserver limits of agreement ratios 0.63 – 2.50[1]. The limitations of these methods are well described in literature[2-4]. In this study we aimed to assess reliability and measurement error of small volume measurements acquired from STIC volumes using the 3D slice method. This method avoids the use of digitally constructed images used in methods like VOCAL. To our knowledge no studies have been published on the 3D slice method examining the reliability of these measurements.

The results of this study show that reliability of volume measurements acquired using 4D ultrasound with STIC and measured using 3D slice method is good. However, reliability is influenced by volume shape and operator experience, because the results clearly show that reliability of volumetric measurements in the balloon model was better compared to measurements in fetal hearts. Also, the results of this study indicate that CVs decrease with increasing experience of the operator. Further, the differences observed in reliability between systolic and diastolic measurements may indicate a limit in the size to which volumes can be measured with acceptable levels of reliability and measurement error. Also, as expected, observed intraobserver reliability was better than interobserver reliability. Finally, the results demonstrate that as a parameter for cardiac function, fetal stroke volume is to be preferred over ejection fraction.

Sources of variability

The results of this study demonstrate that the reliability of *in-vitro* volumetric measurements is better than *in-vivo* volumetric measurements. This implies a strong dependency on volume shape, image quality and anatomical knowledge of the fetal heart. Contour definition in the balloon model did not require any anatomical knowledge, because the balloon is clearly imaged and regular shaped and was relatively easy to measure, independent on the level of experience of the observer. The excellent interobserver and intraobserver ICCs illustrate that different balloons with different stroke volumes

could be very well distinguished even within the small volume range used. Further, the differences in SEM, CV and intraobserver ICC between observers indicate that experience increases reliability and training is very useful for accustoming an observer to the scanning technique and viewing of the post processing modalities. Although CV were still quite large for the smallest volumes, it has to be taken into account that when the mean value is near zero, the CV is sensitive to small changes in the mean, thus limiting its usefulness for small volume measurements. Over the entire range of measured ventricle volumes, SEMs were around one tenth of a milliliter and one might not expect volumetric measurement errors to get much smaller. Moreover, as 3D image quality relies on 2D ultrasound image quality, further technical advances in ultrasound equipment with improvement of image resolution, standardization of acquisition and automated volume calculation software could further improve reliability[23].

Reliability and measurement error

As the interobserver reliability of any given measurement depends on the intraobserver reliability, variation between observers will therefore be partly explained by variation within an observer. Our data demonstrated this for both cardiac and balloon volumes. This finding supports the idea that in clinical situations where volumetric measurements are considered to be important, serial measurements should ideally be performed by the same observer. Further, as variation in measurements primarily occurred in the measurements of systolic and diastolic ventricle volumes, all calculations involving multiple measurements increased variability. This effect was clearly noticeable in the calculated ejection fractions, the formula for wich included three measurements *(ejection fractions = diastolic volume - systolic volume / diastolic volume)*.

Limitations

Clearly, reliability of STIC volume acquisition affects the ability to asses the reliability of contour definition. In this study all ultrasound examinations and STIC volume acquisitions were performed by one experienced third-level sonographer, while contour definition and volume measurements were also performed by two other observers. However, as a recent study done by our group showed, sonographers do not necessary have to be very experienced in the use of 3D ultrasound imaging to acquire high quality STIC volumes[24]. Thus, we believe variance in measurements mainly occurs in the post processing phase of fetal volumetry. Furthermore it should be mentioned that the measurements were not obtained from real-time images. STIC volumes display a virtual cycle, computed from the spatial displacements within the raw 2D data. In this study, STIC volumes contained approximately 40 2D images per cardiac cycle (10 sec, 90 Hz, 130 bpm) which allows accurate volumetric measurements.

We were not able to relate the SEMs and CVs to gestational age in this study because of the small number of subjects. Further, the results of this study are based on the skills of only three observers. Therefore, our results may not exactly reflect the general population of sonographers and may not be generalizable to other sonographers with various backgrounds and training.

In the evaluation of fetal cardiac function the operator has a number of methods and modalities available. The reliability of fetal cardiac volume measurements, acquired using 4D ultrasound imaging and STIC and measured with 3D slice method, is good. Measurement errors tend to decrease with operator experience. As a parameter for cardiac function, reliability of fetal stroke volume seems to be better than ejection fraction. However, further research is needed to assess the clinical applicability of 3D volumes measurements in this small volume range. It is our opinion that given the variety in clinical situations encountered during a cardiac evaluation, operators should use all available ultrasound modalities depending on the clinical setting.

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Chapter 6

STIC hallucinations and gating artefacts in an *in-vitro* model

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Submitted for publication

ABSTRACT

Introduction

Spatiotemporal image correlation (STIC) is frequently used in current fetal echocardiography. STIC provides the opportunity to measure ventricular volumes, which can be used to calculate stroke volume and cardiac output. An in-vitro setting aimed at testing the accuracy of these volumetric measurements yielded impressive image reconstruction artefacts, which we present here.

Methods

A latex miniature balloon was attached to a high frequency pump system to mimic fetal cardiac phases. This balloon was immerged in water. STIC recordings of the balloon were made, to assess the volume of the balloon by four-dimensional (4D) ultrasound to compare it with the actual volume.

Results

The 2D-images displayed a clear image of the round balloon as a thin echogenic ring in a translucent area. The 4D-constructed images of the balloon in the Y- and Z-plane showed, however, a severely distorted balloon. Furthermore, the wall of the balloon was thickened and blurred in some recordings. Several adjustments were made to the model, mainly by adding echogenic pixels in the volume. Eventually the artefacts disappeared when the surroundings of the balloons were made echogenic, mimicking the in-vivo setting.

Comment

The distorted shape, the thickened wall and the blurred image representation, in our opinion, were the result of gating artefacts. The software may have not had the capability to process enough voxels in these volume datasets to detect the temporal relationship properly. Therefore, the spatial relationship was not arranged correctly, resulting in the depiction of distorted and blurred balloons. This is the first report that systematically describes artefacts that can occur in STIC volumes. These experiments can be relevant for the understanding of the methodology of STIC and thus for the analysis of STIC volumes in daily practice.

INTRODUCTION

Three-dimensional (3D) ultrasound imaging is currently frequently used in prenatal medicine. 3D volumes allow the sonographer to freely visualize the human fetus from different angles, including planes that are not easily achievable in conventional sonography. Previously, 3D reconstruction of the fetal heart was hampered by technical problems caused by the moving heart within the volume. This has been overcome with the introduction of spatiotemporal image correlation (STIC)[1]. STIC is based on the analysis of cardiac motion within a recorded 3D volume. With STIC, the spatial information, which is acquired in a single slow sweep (7.5-15 seconds), is rearranged and synchronized to the heart beat, which is called 'cardiac gating'. This results in a volume dataset containing temporal information and three-dimensional spatial information, which allows reconstruction and the interactive display of cardiac morphology in any plane[2]. Another advantage over two-dimensional (2D) ultrasound is the possibility to measure volumes within a 3D dataset. This has proven to be a reliable method to estimate the actual volume of an organ or fluid filled space[3]. STIC has different postprosessing volumemeasurement tools. In the fetal heart, reliable volume measurements could potentially be a breakthrough because other attempts to develop a reliable instrument to measure cardiac performance have been proven not to be accurate[4-6]. With ventricular volume measurements, end-diastolic and end-systolic volumes can be measured and stroke volume and cardiac output can be calculated. To test the accuracy of measurements in STIC we developed an in-vitro balloon model. Accuracy tests of volumes in the range of 5-10 mL were earlier performed by Bhat *et al.*[7]. Because recently published studies showed that fetal ventricle volumes are smaller than 5 mL during most of gestation[8-10], we were interested in volumes smaller than 5 mL. The actual results were described earlier[3]. Here we present several problems we encountered in the development of the balloon model which can be relevant for the understanding of the methodology of STIC and thus for the analysis of STIC volumes in daily practice.

METHODS

To create a suitable balloon model to simulated fetal cardiac ventricle volumes, we used customized miniature balloons and modified a model previously described by Bhat *et al.*[7] (Figure 1). The balloons were made of small pieces of latex ultrasound covers and fixed on top of a small rigid plastic catheter with a diameter of 5 mm and a lumen of 3 mm. The catheter was attached to a custom made pump system, capable of generating variable low stroke volumes at high frequencies to mimic fetal cardiac phases. The stroke volumes were created through the movement of the plunger within a syringe tube con-

taining a maximum volume of 5 mL. An electromotor rotated a wheel which was attached to the plunger by a connecting rod. By manually positioning the connecting rod slightly out of center of the wheel, it was possible to adjust accurately the movement of the plunger and thus of the ejected stroke volume.

The pulsatile balloons were immersed in an acrylic (Perspex) bath which was initially filled with water. In one side of the water bath, a small window was cut out and replaced by a latex film, to serve as the scanning window. Here the transducer was applied for the STIC volume acquisition. The distance between the balloon and transducer was approximately 5 cm in order to obtain the highest possible image quality. The systolic balloon volumes ranged from 0.3 to 3.3 mL, which is comparable with early second to third trimester ventricle volumes. For each systolic balloon volume, the system was set to produce three different stroke volumes based on 0.25, 0.50 and 0.75 times the systolic balloon volume. The pump system was set at a frequency of 130/min to simulate a fetal heart rate.

Volume acquisition and measurement

The balloons were imaged using a Voluson E8 (GE Medical Systems, Kretz, Austria) with a motorized curved array 6-12 MHz transvaginal 3D/4D transducer. To avoid bias due to image enhancement software, Speckle Reduction Imaging (SRI) or Cross XBeam (CRI) were not used. During STIC acquisition the angle of acquisition was set as small as possible to obtain the highest frame rate. The STIC volumes were saved to a personal computer for off-line analysis.

RESULTS

The first recordings were of the balloons filled with water, immersed in a water filled bath. The 2D images displayed a clear image of the round balloon as a thin echogenic ring in a translucent area. However, when the STIC volumes were analysed, the reconstructed images of the balloon in the Y- and Z-plane showed a distorted balloon. The shape was not round, and showed deformation of the balloon as shown in Figure 2. The shape of the distortion differed within the 'heart' cycle, producing irregular shapes in the cine-loop. Several recordings were made in this setting with different volumes. The shape of the balloon in the Y- and Z-plane was not consistent in the different recordings, but variably distorted in each.

The initial hypothesis was that the image distortions were caused by gating problems within the STIC software algorithm. The first attempt to resolve this artefact was to in-

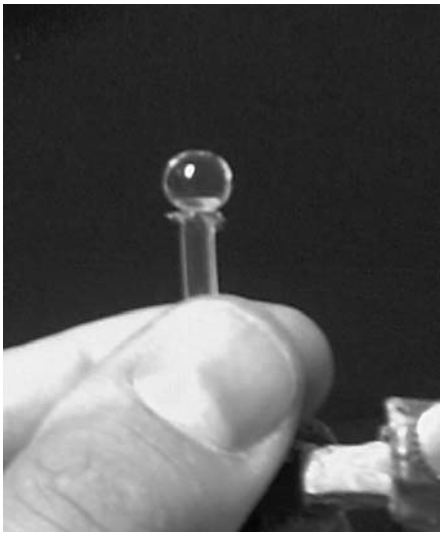

Figure 1 *Customized balloon made of latex fixed on top of a rigid catheter. The catheter was attached to a pump system to simulate the fetal cardiac phases.*

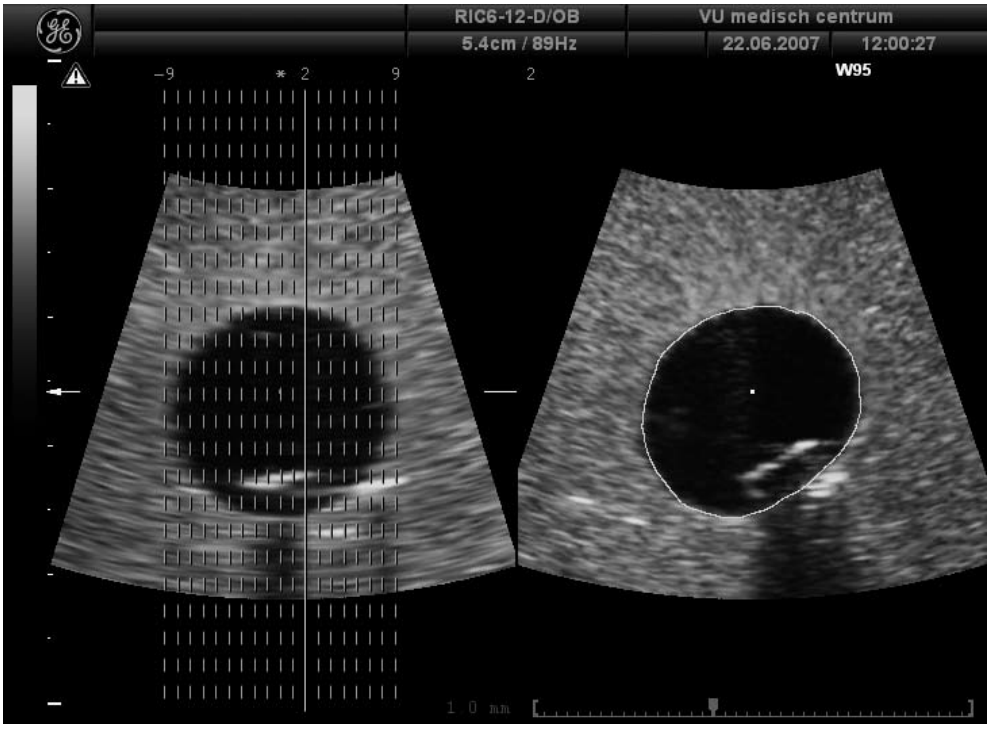

Figure 2 *Depiction of the balloon immerged in water in one STIC volume. The three rows are at 3 different moments within one heart cycle (beginning, middle and end). First row is the initial recording plane, the second row the Y plane, the third row the Z plane. Note oval distorted depicting in the Y plane and the thickened wall in the Z plane.*

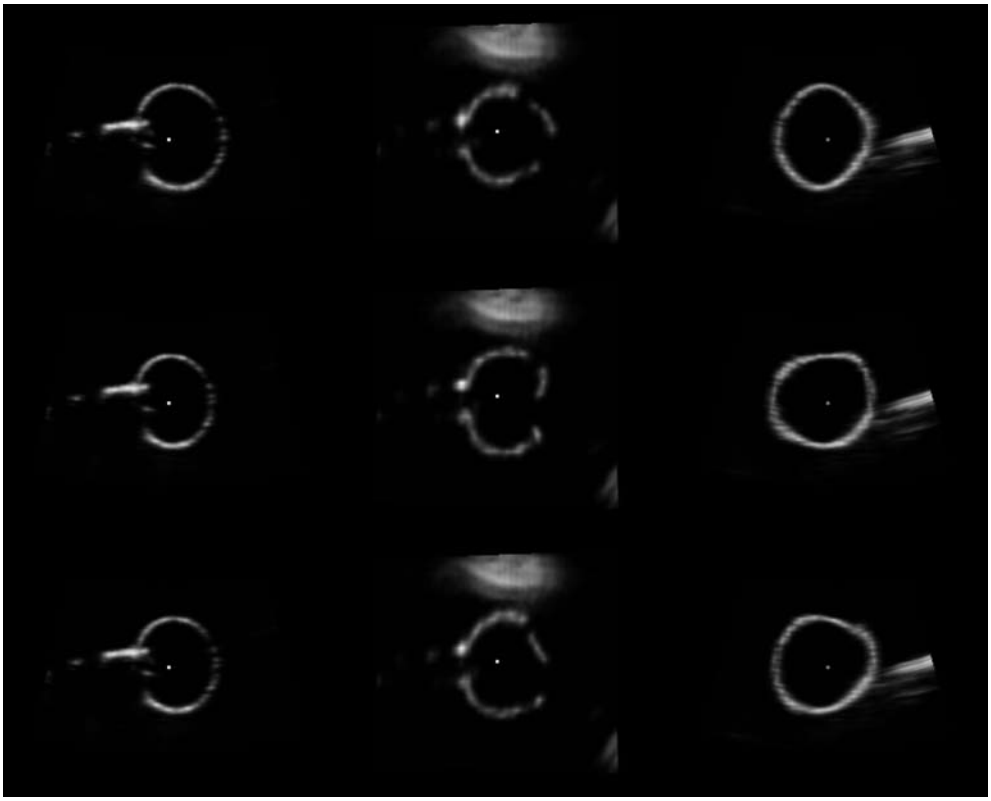

Figure 3 *Depiction of the balloon immerged in water in one STIC volume. The balloon was coated with talc powder. The three rows are at 3 different moments within one heart cycle (beginning, middle and end). First row is the initial recording plane, the second row the Y plane, the third row the Z plane. The Y plane is severely distorted and not fully depicted. The Z plane shows a mildly thickened wall in the Z plane. Furthermore some echogenic reflections occur.*

troduce more echogenic pixels in the volume by coating the balloon surface with talcum powder. In these recordings the distorted shape of the balloon was still present, but a second artefact was introduced. Besides the distortion, the wall of the balloon in the Y- and Z-plane was thicker than expected and blurred (Figure 3). The shape of the balloon in the X-plane image was good (round, sharp and thin) in all recordings.

The next attempt to introduce more echogenic pixels was to inject the balloon with ultrasound contrast, containing micro bubbles in one attempt and water enriched with toothpaste in the other 13 attempts. In these 14 attempts we had 5 recordings of good quality, but distorted balloons in 8. Furthermore, the distortion was subjectively present to a lesser extent. Furthermore the speckles within the balloons were blurred, whereas in the X-plane the depiction was very sharp.

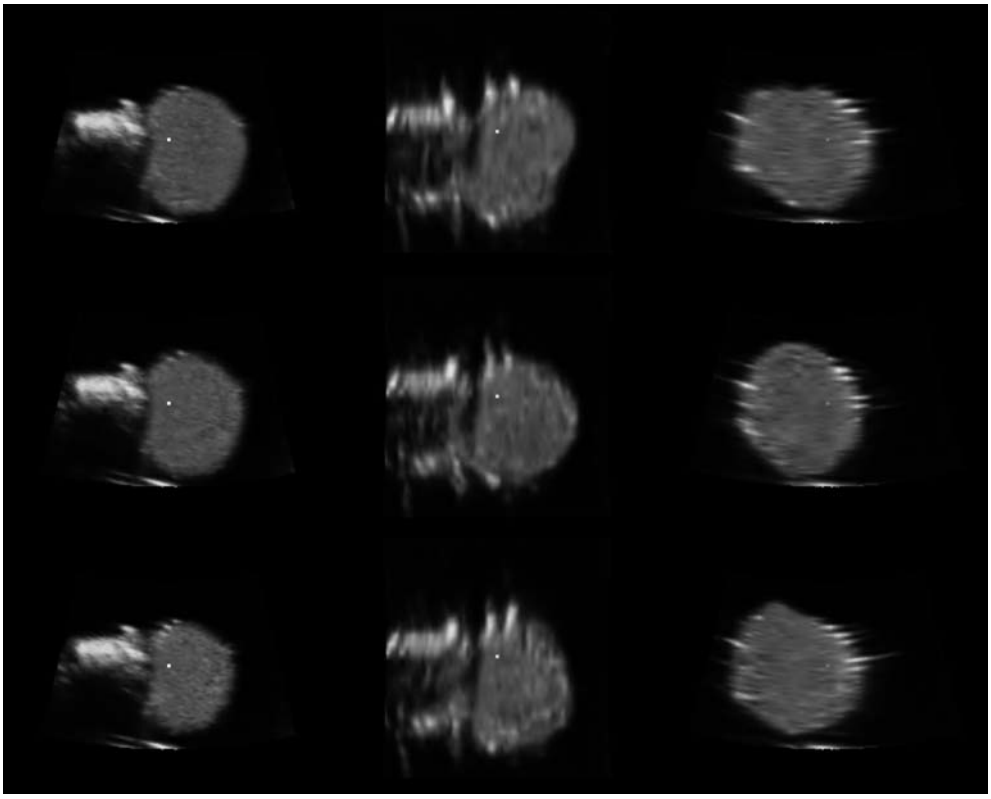

Figure 4 *Depiction of the balloon filled with in water enriched with toothpaste in one STIC volume. The three rows are at 3 different moments within one heart cycle (beginning, middle and end). First row is the initial recording plane, the second row the Y plane, the third row the Z plane. In this recording the balloon shows humps and the speckles are blurred.*

Because the introduction of an echogenic area within the volume box seemed to improve the STIC acquisitions, we tried to modulate the model more towards the in-vivo setting: echolucent content of the balloon in an echogenic surrounding. To create the echogenic surrounding, the ultrasound gel was mixed with talcum powder. This resulted in good image quality in the X, Y and Z plane (Figure 4). No distortion was present. This experimental setting resulted in good STIC acquisitions in which all our accuracy tests were performed, which have been published previously[3].

COMMENT

STIC is now applied worldwide in fetal echocardiography, both in clinical practice and in research settings. It is therefore increasingly important to understand its technical back-

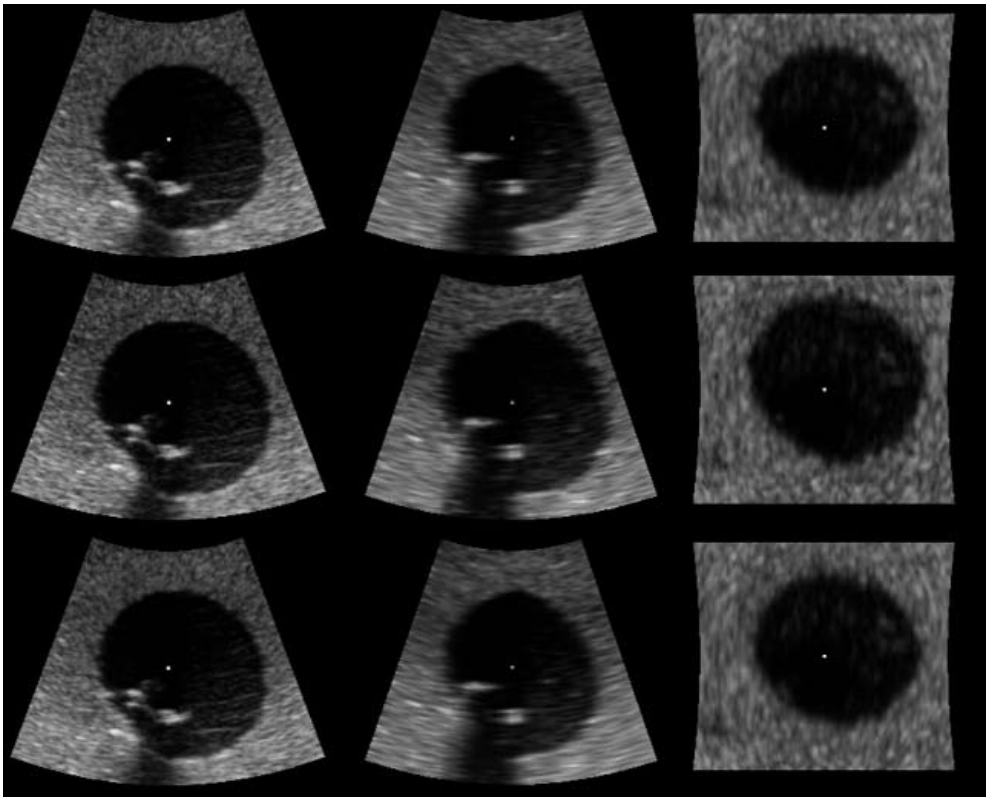

Figure 5 *STIC recording of a balloon filled with water in a echogenic surrounding. The described artefacts are not visible in this recording.*

ground and limitations. These experiments in which balloons are scanned, and image processing reassembles an interpretation of physical structure, reveal a limitation of the STIC technique. When round water-filled balloons expand, like the balloons in this experiment, the balloons are expected to remain round in all three dimensions by the laws of physics. In our setting, this was indeed optically confirmed during all acquisitions and also during the final acquisitions when the settings were optimalized. In our opinion the distorted shape, the thickened wall and the blurred speckles, were the result of gating artefacts. It may be the case that the software did not have enough echogenic voxels in these volume datasets to detect the temporal relationship properly. Therefore, the spatial relationship was not arranged correctly, resulting in the depiction of distorted and blurred balloons. This theory is supported by the fact that the artefacts resolved when the balloons were placed in a echogenic surrounding, giving more non-moving echogenic voxels within the volume dataset. Deng and Rodeck describe a very similar,

but slightly different artefact, called 'wavy artefact'[11]. This can frequently be observed in daily practice and is ascribed to the successive contraction of the myocardium.

Although the artefacts discussed in this paper were observed in an in-vitro balloon model, in our opinion, these observations are important for clinicians working in daily practice. It is not impossible that the conditions under which the artefacts occurred in this in-vitro model, could also occur in the acquisition volumes of a fetal heart, e.g. in case of hydrops, pericardial effusions, massive CCAMs or other echolucent tumors in the fetal thorax. The presence of fetal movements can also produce important artefacts. Goncalves describes a case in which a tetralogy of Fallot was misinterpreted as a double outlet right ventricle, due to excessive fetal movements[12]. In this case the spatial relation was not arranged correctly, similar to the artefacts in this experiment, in their case the temporal information was, however, disturbed by the general fetal movements. This illustrates the importance of initial acquisition conditions.

In conclusion, this experimental effort clearly shows that STIC, albeit a very promising technique, can produce several artefacts, just like conventional 2D ultrasound. This is important knowledge for sonographers working with four-dimensional ultrasound and STIC in routine clinical practice. In our opinion, the analysis of 4D volumes should always be accompanied by a full 2D cardiac examination.

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Chapter 7

Fetal cardiac function assessed with four-dimensional ultrasound imaging using spatiotemporal image correlation

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Adapted from *Ultrasound Obstet Gynecol* 2009; 33: 272-281

ABSTRACT

Objective

The goal of this study was to use spatiotemporal image correlation (STIC) to provide reference values for left and right ventricle volumes, and indices of fetal cardiac function.

Methods

In this prospective longitudinal study, STIC volumes were acquired periodically from 12 weeks of gestation onwards. The STIC volumes were frozen in end-systole and end-diastole, and volumetric data were measured by manual tracing and summation of multiple slices. These ventricle volumes were used to calculate stroke volume, ejection fraction and cardiac output.

Results

Some 202 STIC volumes of 63 fetuses were included in the analysis. Mean left and right ventricle stroke volume increased from 0.02 mL at 12 weeks to 1.41 mL and 1.46 mL, respectively, at 30 weeks, while mean right to left stroke volume ratio remained stable at 1.2. Mean left and right ventricle cardiac output increased from 2.40mL/min and 2.60 mL/min at 12 weeks to 197.74 mL/min and 204.81 mL/min, respectively, at 30 weeks. Both left and right mean ejection fraction remained constant at around 0.45 with advancing gestational age. Bland-Altman analysis showed a coefficient of variation for measured stroke volume of 13.7%.

Conclusions

This study establishes reference values for fetal cardiac volumes and indices for fetal cardiac function from 12 to 30 weeks of gestation using STIC. STIC seems to overcome many of the pitfalls of conventional ultrasound methods and has the potential to become the method of choice.

INTRODUCTION

Timing of the delivery in cases of suspected impaired fetal condition is commonly based on hemodynamic parameters such as fetal heart rate monitoring and the measurement of pulsatility indices of fetal arteries and veins. In a fetus with a congenital cardiac malformation, hydrops fetalis, fetal diabetic cardiomyopathy, intrauterine growth restriction (IUGR) or twin-twin transfusion syndrome, accurate and reliable measurement of fetal cardiac function could be valuable for making the diagnosis and for fetal surveillance. In spite of its widespread use, fetal heart rate monitoring is known to have its limitations[1]. When abnormalities in the fetal heart rate pattern eventually become apparent, the fetal cardiac function has probably been compromised for quite some time. In cases of IUGR, however, pulsatility indices can show abnormal values as a sign of abnormal hemodynamics long before a fetus shows signs of actual hypoxia. Therefore, additional methods to give obstetricians insight in the actual fetal condition might be useful.

Cardiac function, both in adults and children, is commonly expressed in ejection fraction (EF) and stroke volume (SV). Both indexes can be calculated from end-systolic and end-diastolic ventricle volumes (ESV and EDV, respectively). To obtain these volumes, a common method in two dimensional (2D) ultrasound imaging is to divide the ventricle into parallel slices. In each plane the circumference is obtained and used to calculate the cross-sectional area; these areas are multiplied by the slice thickness an then summed to estimate the total volume using Simpson's rule[2]. This method is, however, based upon the assumption that the ventricle has a perfect cylindrical shape. This may be more or less applicable to the left ventricle, but the right ventricle has a much more complex geometric shape. Furthermore, these measurements are not accurately repeatable[3].

Another well known conventional method for estimation of fetal cardiac function uses pulsed Doppler imaging to estimate fetal blood flow through the valve orifices[4-6]. The clinical usefulness of this method is, however, also known to be limited because of its inaccuracy[3, 7, 8].

Since the introduction of three-dimensional (3D) and four-dimensional (4D) ultrasound imaging, these new modalities have been assessed for their use in the evaluation of fetal cardiac function, as they could possibly overcome some of the limitations of 2D ultrasound[9-12]. Studies in adults have shown that 3D echocardiography is more accurate than 2D echocardiography for volume measurements[13]. Spatiotemporal image correlation (STIC) is a technique that adds a time component to 3D ultrasound imaging of the fetal heart[14-17], creating a virtual 4D cardiac loop. STIC gives the investigator the opportunity to freeze the displayed cardiac loop in end-diastolic and end-systolic phase. The

number of frames available to the STIC algorithm depends on fetal heart rate, scanning frequency and acquisition time. 3D measurements of both the left and the right ventricle can be used to calculate fetal heart SV and cardiac output (CO). This method has been found to be quantitatively accurate in validation studies, and has also been shown to be effective when applied to other fetal organs[18-20].

The use of STIC in the sonographic evaluation of the fetal cardiac SV and CO may give fetal and pediatric cardiologists and obstetricians more parameters with which to asses the fetal condition. This could also aid in our understanding of pathophysiological processes in fetuses in distress. The goal of this study was to use STIC to provide reference values for left and right ventricle volumes, fetal cardiac SV, left/right ventricle and SV ratios, and cardiac output CO.

METHODS

Sixty-three healthy women were included in this prospective longitudinal study at the ultrasound unit of the department of Prenatal Medicine of the VU University Medical Center in Amsterdam, The Netherlands. From these uncomplicated singleton pregnancies, with a low risk for cardiac anomalies, a total of 202 STIC volumes were acquired and included in the final analyses. The gestational age was confirmed by first-trimester ultrasound. Questionnaires concerning the newborn's health were filled in by the parents after birth in all cases. The medical ethics committee approved the study and all women gave informed consent.

All STIC volumes were acquired using 4D ultrasound imaging with STIC (Voluson 730 Expert and Voluson E8, GE Medical Systems, Kretz Austria), using motorized curved array transvaginal (6-12 MHz) and transabdominal (4-8 MHz and 5-9 MHz) 3D/4D transducers. If image resolution was insufficient using transabdominal ultrasound in early pregnancy, a transvaginal transducer was used. After an acquisition, the STIC volumes were stored and saved to a personal computer. Volume measurements required well performed STIC acquisitions without movement or shadow artefacts. The STIC acquisition time was 12.5 sec and the frequency was around 100 Hz. Given a fetal heart rate of 140 beats/min, the STIC volumes were based on approximately 43 frames per cardiac cycle. The volumes were all acquired by one examiner (L.U.). Measurements were performed with 4DView 6.0 post-processing software (GE Medical Systems).

Patients were recruited during a prenatal intake visit at our outpatient clinic. Fetal biometry and STIC volumes were acquired periodically from 12 weeks' gestational age on-

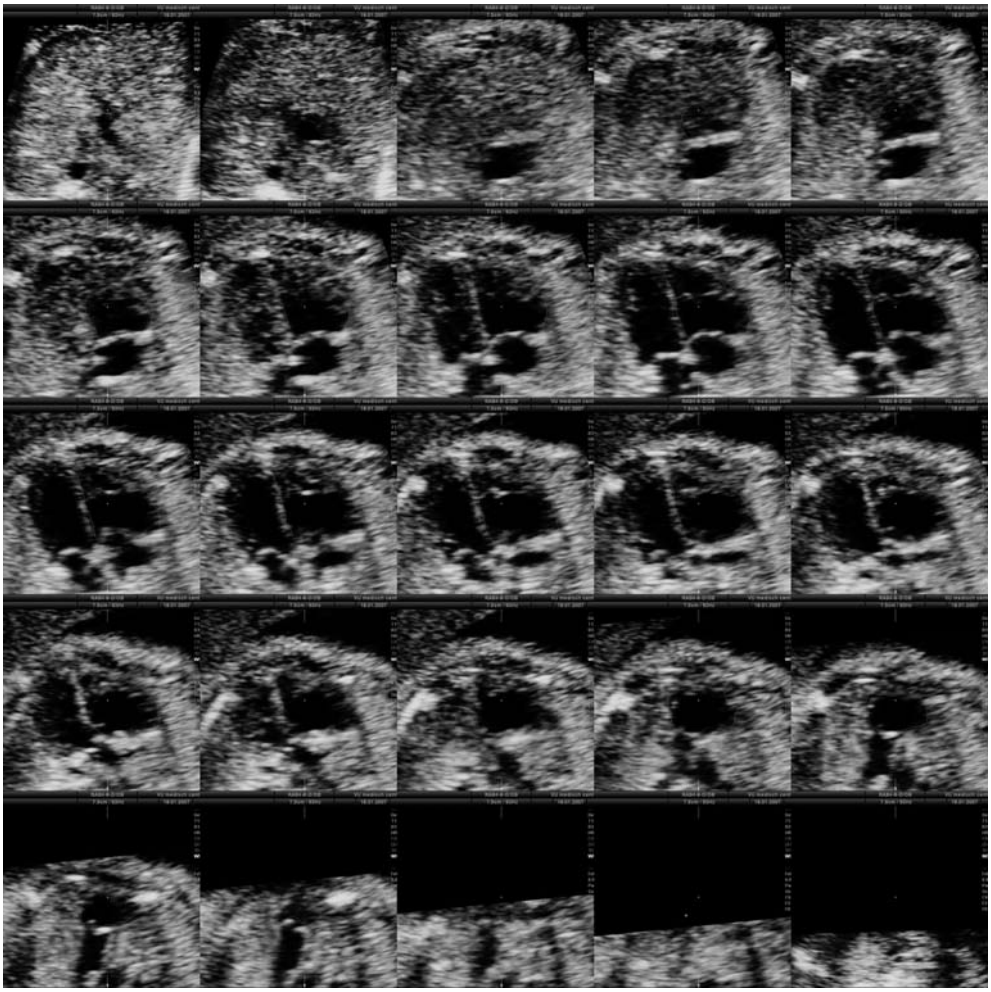

Figure 1 *Compilation of images 1mm apart obtained by multiplanar imaging of a spatiotemporal image correlation volume (3D slice method) frozen in end-diastole. The outlines of the right ventricle have been traced as indicated by a thin green line. The Figure demonstrates the complex geometric shape of the right ventricle.*

wards, with an interval of 3-4 weeks, as long as acquisition of high quality STIC volumes was possible. All STIC volumes were acquired without color Doppler information. 3D volume measurements started in a classic four-chamber view in multiplanar mode[15]. The images were optimized using magnification and contrast settings. The cardiac loop was frozen in end-systole or end-diastole by visually observing the moment of opening and closing of the atrioventricular (AV) valves. End-systole was defined as the frame just before to the opening of the AV valves. End-diastole was defined as the frame after the closing of the AV valves. Based on the results of a previous study, all 3D volume mea-

surements were done using the 3D slice method (Figure 1), which is based on Simpson's rule. Multiple slices of the four-chamber view were manually traced and the circumferences summed and the areas multiplied by the slice thickness and summed. Ventricular contours were manually traced on the echogenic side of the endocardial border. Papillary muscles were considered part of the ventricular cavity in our analysis. Left and right ESV and EDV were used to calculate both SV, EF and CO. In order to allow us to evaluate the relationship between the cardiac parameters and the estimated fetal weight (EFW), the bipartietal diameter, head circumference, abdominal circumference and femur length were measured, from which the EFW was derived using the 1985 Hadlock formula[21]. In 12 randomly selected cases, both left and right ventricle SV was measured by the same operator twice in order to compare the measurements and to calculate intraobserver agreement.

Statistical analysis

The relation of both the ventricular volumes and the indices for cardiac function with gestational age and EFW were analysed with Generalized Estimating Equations (GEE) analysis. GEE analysis is a technique that takes into account the fact that repeat measurements were performed on each fetus; the technique uses all of the available data, irrespectively of the number of repeated measurements, meaning that it is not compromised by missing data. Furthermore, GEE analysis is capable of dealing with irregular time intervals[22]. Within GEE analysis, correction for the dependency of observations is performed by adding a 'within subject correlation structure' to the regression model. In this study an exchangeable correlation structure was used, which means that correlations between subsequent measurements are assumed to be the same, irrespective of the time between the measurements. For each variable, separate GEE analyses were performed with gestational age and its higher order terms (powers of 2 and 3) as independent variables to account for the possibility of a non-linear relation. To assess the relationship of the different variables with EFW, GEE analyses were performed with EFW as independent variable. Based on the equations acquired for both the mean and the SD, population reference intervals for gestational age and EFW were estimated. Before analysis a logistic transformation was performed on the data when necessary. Bland-Altman analysis was used to compare the measurement agreement and bias for a single observer[23, 24]. A value of $P < 0.05$ was considered statistically significant. The data was analysed using Excel for Windows 2003 (Microsoft Corp., Redmond, WA, USA) and SPSS version 15.0. (SPSS Inc., Chicago, IL, USA).

Table 1 *Mean, 5th (p5) and 95th (p95) centiles of left and right systolic and diastolic ventricle volumes in relation to gestational age (GA).*

GA	Left ventricle ESV (mL)			Left ventricle EDV (mL)			Right ventricle ESV (mL)			Right ventricle EDV (mL)		
(weeks)	Mean	p5	p95	Mean	p5	p95	Mean	p5	p95	Mean	p5	p95
12	0,03	0,02	0,04	0,04	0,03	0,06	0,03	0,02	0,03	0,05	0,03	0,06
13	0,04	0,03	0,05	0,07	0,05	0,09	0,04	0,03	0,05	0,07	0,04	0,10
14	0,06	0,04	0,08	0,10	0,07	0,13	0,06	0,04	0,08	0,11	0,06	0,15
15	0,08	0,04	0,13	0,15	0,09	0,20	0,09	0,05	0,13	0,16	0,09	0,23
16	0,12	0,05	0,18	0,21	0,12	0,30	0,13	0,07	0,18	0,23	0,13	0,34
17	0,16	0,07	0,26	0,30	0,17	0,43	0,17	0,09	0,26	0,33	0,18	0,47
18	0,22	0,09	0,35	0,41	0,23	0,59	0,24	0,12	0,35	0,45	0,26	0,64
19	0,29	0,12	0,45	0,55	0,31	0,78	0,32	0,17	0,47	0,61	0,36	0,86
20	0,37	0,16	0,58	0,72	0,42	1,02	0,41	0,22	0,60	0,80	0,49	1,11
21	0,48	0,21	0,74	0,93	0,55	1,30	0,53	0,29	0,76	1,03	0,64	1,41
22	0,60	0,28	0,91	1,17	0,71	1,62	0,66	0,37	0,95	1,29	0,83	1,75
23	0,73	0,36	1,11	1,44	0,89	1,98	0,81	0,47	1,16	1,59	1,04	2,13
24	0,89	0,45	1,33	1,73	1,08	2,37	0,98	0,57	1,38	1,90	1,26	2,54
25	1,06	0,55	1,57	2,03	1,28	2,79	1,16	0,68	1,63	2,22	1,48	2,97
26	1,24	0,66	1,82	2,34	1,47	3,21	1,34	0,80	1,88	2,54	1,69	3,39
27	1,43	0,76	2,09	2,63	1,64	3,62	1,52	0,90	2,14	2,84	1,87	3,80
28	1,61	0,87	2,35	2,89	1,77	4,02	1,69	0,99	2,39	3,09	2,00	4,18
29	1,79	0,95	2,62	3,11	1,85	4,38	1,85	1,06	2,63	3,29	2,08	4,51
30	1,95	1,02	2,88	3,27	1,86	4,69	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.

RESULTS

The mean number of STIC volumes per fetus was 3 (range 1–6) and the mean time interval between the examinations was 25 (range 5–49) days. STIC acquisition was successful in 71% (205/287) of all examinations. Beyond 29 weeks of gestation the failure rate of STIC acquisition increased remarkably. This resulted in only three technically acceptable STIC volumes beyond 30 weeks of gestation, which had to be excluded to avoid bias due to small numbers. Limiting factors for STIC acquisition included: low image resolution at young gestational age, abundant fetal movement, numerous acoustic shadows at advanced gestational age and a persistent unfavourable fetal position.

The curves for best fit for mean right and left ventricle ESV and EDV against gestational age (weeks) as the independent variable, were quadratic regression equations after log

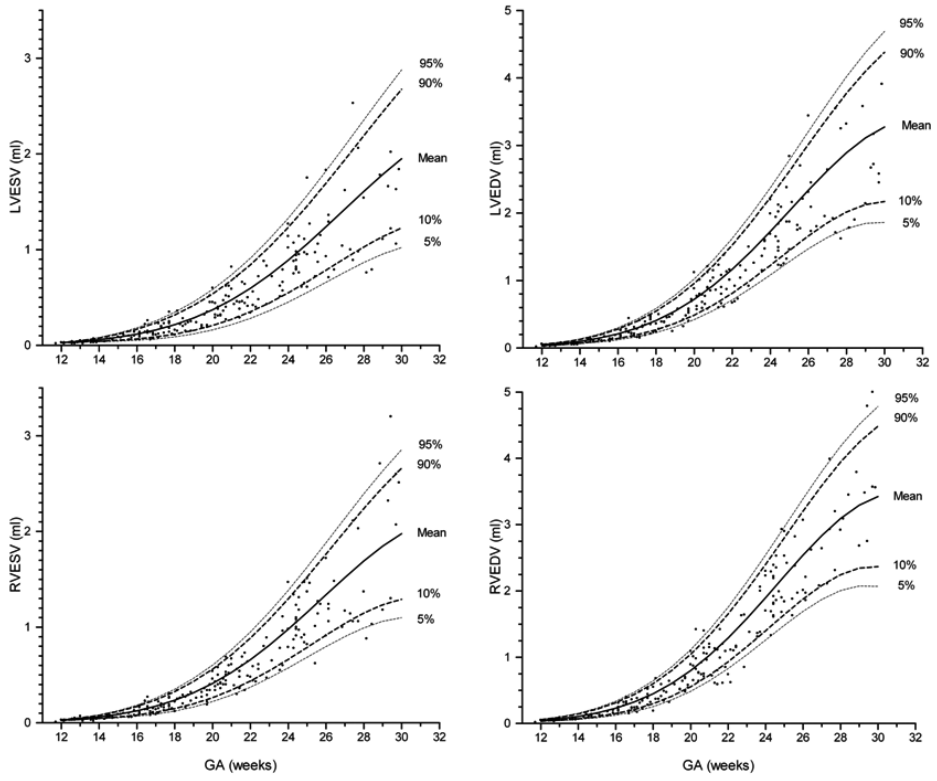

Figure 2 *Volume measurements of left and right ventricles plotted against gestational age (GA) in 202 fetuses. Left ventricle end-systolic volume (ESV) (ln(y)= -9,528 + 0,601 x GA - 0,009 x GA²) (a) and left ventricle end-diastolic volume (EDV) (ln(y)= -10,058 + 0,710 x GA - 0,011 x GA²) (b); right ventricle ESV (ln(y)= -9,972 + 0,653 x GA + 0,010 x GA²) (c) and EDV (ln(y)= -10,226 + 0,736 x GA - 0,012 x GA² (d). Mean values (——), 10th and 90th centiles (- - -), and 5th and 95th centiles (····) are shown.*

transformation (Figure 2 and Table 1). Based on the acquired equation the predicted mean left ventricle ESV ranged from 0.03 mL (95% CI, 0.02–0.04) at 12 weeks to 1.95 mL (95% CI, 1.02–2.88) at 30 weeks and the mean right ventricle ESV ranged from 0.03 mL (95% CI, 0.02–0.03) at 12 weeks to 1.98 mL (95% CI, 1.10–2.85) at 30 weeks. The mean predicted left ventricle EDV ranged from 0.04 mL (95% CI, 0.03–0.06) at 12 weeks to 3.27 mL (95% CI, 1.86–4.69) at 30 weeks, and the mean right ventricle EDV ranged from 0.05 mL (95% CI, 0.03–0.06) at 12 weeks to 3.43 mL (95% CI, 2.07–4.78) at 30 weeks. The right/left ventricle ratio remained constant at 1.12 for both end-systole and end-diastole. Linear regression equations provided the best fit for the relationships of right and left ESV and EDV with EFW (Figure 3).

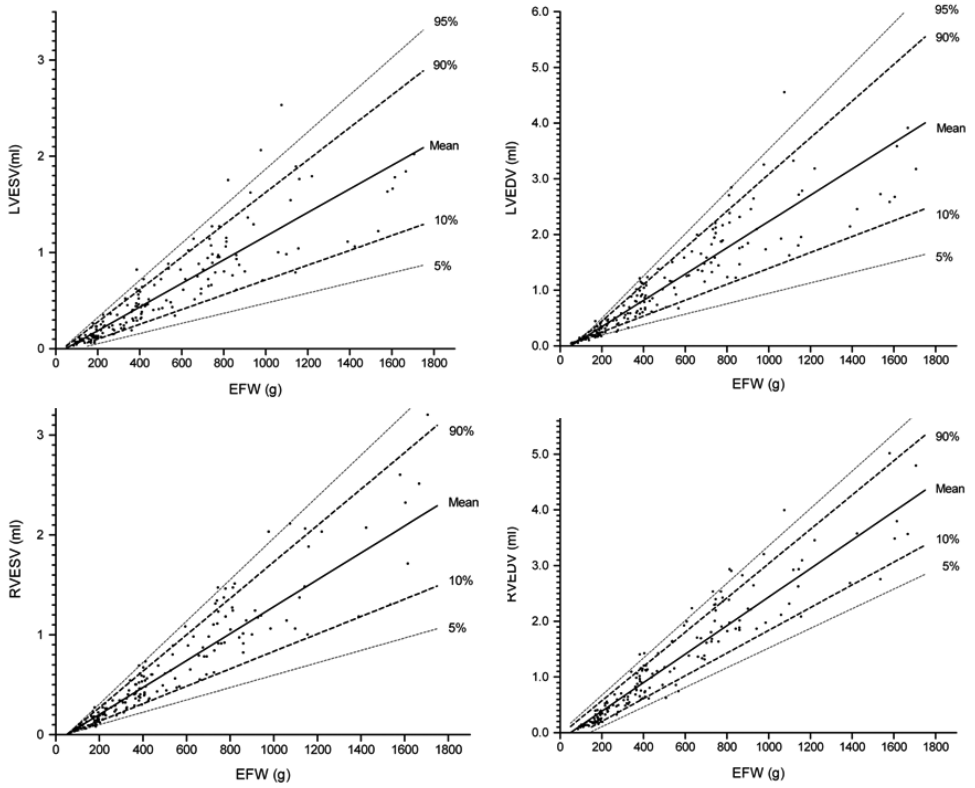

Figure 3 *Volume measurements of left and right ventricles plotted against estimated fetal weight in 174 fetuses: left ventricle end-systolic volume (ESV) (a) and end-diastolic volume (EDV) (b); right ventricle ESV (c) and EDV (d). Mean values (——), 10th and 90th centiles (- - -), and 5th and 95th centiles (····) are shown.*

Figure 4 demonstrates the increase in left and right SV with advancing gestational age. The right/left ratio for SV showed right dominance, remained constant around 1.2 throughout gestation; regression analysis showed no significant relationship between the right/left ratio and gestational age. Based on the acquired equation the predicted mean left ventricle SV increased from 0.02 mL (95% CI, 0.00-0.03) at 12 weeks to 1.41 mL (95% CI, 0.68–2.14) at 30 weeks, and mean right ventricle SV increased from 0.02 mL (95% CI, 0.00–0.04) at 12 weeks to 1.46 mL (95% CI, 0.74-2.18) at 30 weeks (Table 2). Linear regression equations provided the best fit for the relationships of left and right ventricle SV with EFW (Figure 4).

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

Table 2 *Mean, 5th (p5) and 95th (p95) centiles of left and right ventricle stroke volume, and left and right cardiac output, in relation to gestational age (GA).*

GA	Left ventricle SV (mL)			Right ventricle SV (mL)			Left CO (mL/min)			Right CO (mL/min)		
(weeks)	Mean	p5	p95	Mean	p5	p95	Mean	p5	p95	Mean	p5	p95
12	0.02	0.00	0.03	0.02	0.00	0.04	2.40	0.63	4.18	2.60	0.00	5.22
13	0.03	0.00	0.05	0.03	0.00	0.06	3.81	0.78	6.83	4.17	0.17	8.16
14	0.04	0.01	0.07	0.04	0.00	0.08	5.88	1.10	10.65	6.51	0.67	12.34
15	0.06	0.01	0.11	0.07	0.01	0.12	8.84	1.84	15.85	9.88	1.73	18.04
16	0.09	0.02	0.15	0.10	0.02	0.18	12.98	3.25	22.71	14.61	3.67	25.56
17	0.13	0.04	0.22	0.14	0.04	0.24	18.58	5.64	31.52	21.03	6.82	35.24
18	0.18	0.06	0.29	0.20	0.08	0.33	25.93	9.28	42.58	29.46	11.51	47.41
19	0.24	0.10	0.39	0.28	0.12	0.44	35.29	14.45	56.13	40.18	18.01	62.34
20	0.33	0.15	0.51	0.37	0.18	0.56	46.82	21.29	72.34	53.32	26.47	80.18
21	0.42	0.21	0.64	0.48	0.25	0.71	60.58	29.88	91.28	68.89	36.87	100.90
22	0.54	0.28	0.80	0.61	0.34	0.88	76.42	40.06	112.79	86.62	48.97	124.27
23	0.66	0.36	0.97	0.75	0.44	1.06	94.01	51.49	136.53	106.02	62.26	149.78
24	0.80	0.45	1.15	0.90	0.54	1.26	112.77	63.60	161.94	126.31	75.96	176.65
25	0.94	0.53	1.34	1.04	0.63	1.45	131.90	75.59	188.20	146.47	89.07	203.87
26	1.07	0.61	1.54	1.18	0.71	1.64	150.43	86.50	214.36	165.32	100.38	230.25
27	1.19	0.67	1.72	1.30	0.77	1.82	167.28	95.24	239.33	181.62	108.68	254.56
28	1.30	0.71	1.88	1.39	0.80	1.97	181.40	100.74	262.05	194.22	112.80	275.64
29	1.37	0.71	2.03	1.44	0.79	2.09	191.80	102.05	281.55	202.16	111.78	292.53
30	1.41	0.68	2.14	1.46	0.74	2.18	197.74	98.40	297.08	204.81	105.01	304.61

Data based on regression equations. CO, cardiac output; SV, stroke volume.

CI, 0.26-0.66) (Figure 5). To calculate left and right CO, SVs were multiplied by the fetal heart rate, as recorded within each STIC volume. Based on the acquired equation the predicted 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, and 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 2 and Figure 6). Linear regression equations provided the best fit for the relationship of left and right CO with EFW (Figure 6).

In the reliability analysis the measurement error of SV showed dependence on the mean (rank correlation test, Kendall's τ = 0.434, P=0.003) (Figure 7a). The measurement error was roughly proportional to the mean, but the relationship was removed by log transformation of the data (Kendall's τ = -0.094, P=0.512) (Figure 7b), as described by Bland and Altman[24, 25]. Therefore, the antilog of the within-subject SD for the log-transformed

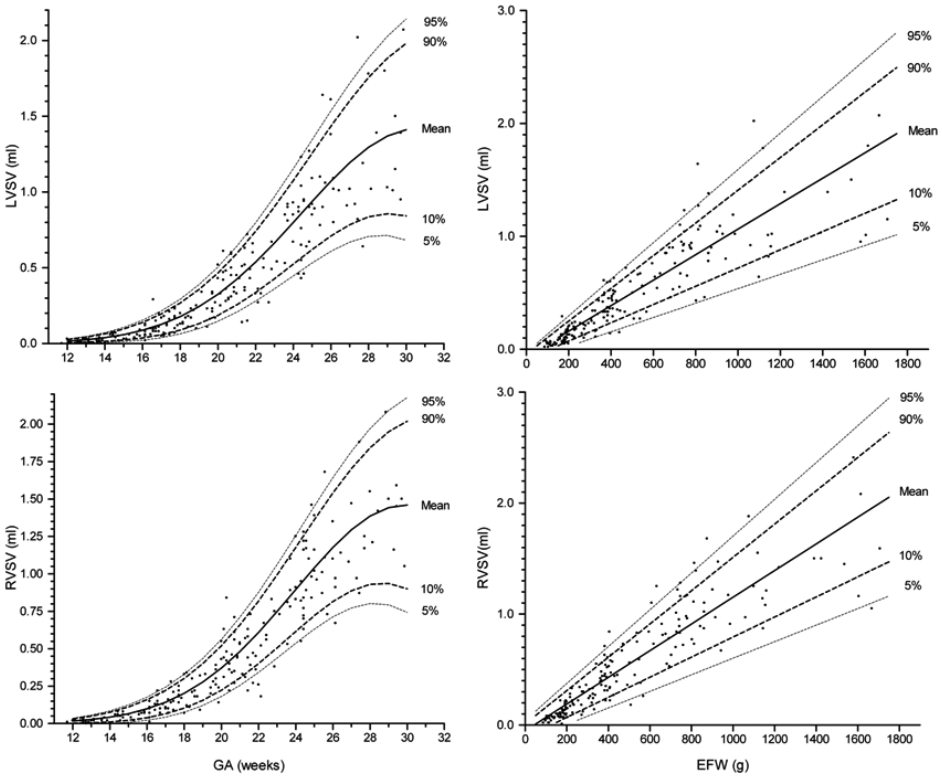

Figure 4 *Fetal stroke volumes (SVs). Left ventricle SV plotted against gestational age (GA) (ln(y)= -11,814 + 0,793 x GA - 0,013 x GA²) (a) estimated fetal weight (b); right ventricle SV plotted against GA (ln(y)= -12,039 + 0,830 x GA - 0,014 x GA²) (c) and estimated fetal weight (d). Mean values (——), 10th and 90th centiles (- - -), and 5th and 95th centiles (····) are shown. GA data are based on 202 fetuses and estimated fetal weight data are based on 174 fetuses.*

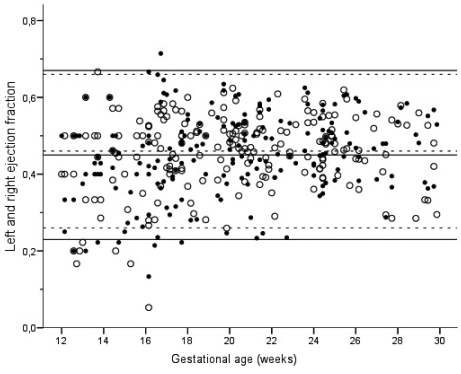

Figure 5 *Mean left (- - - -) and right (——) ventricle ejection fractions with 95% confidence intervals plotted against gestational age. Individual values are shown for left (○) and right (●) ventricle ejection fractions.*

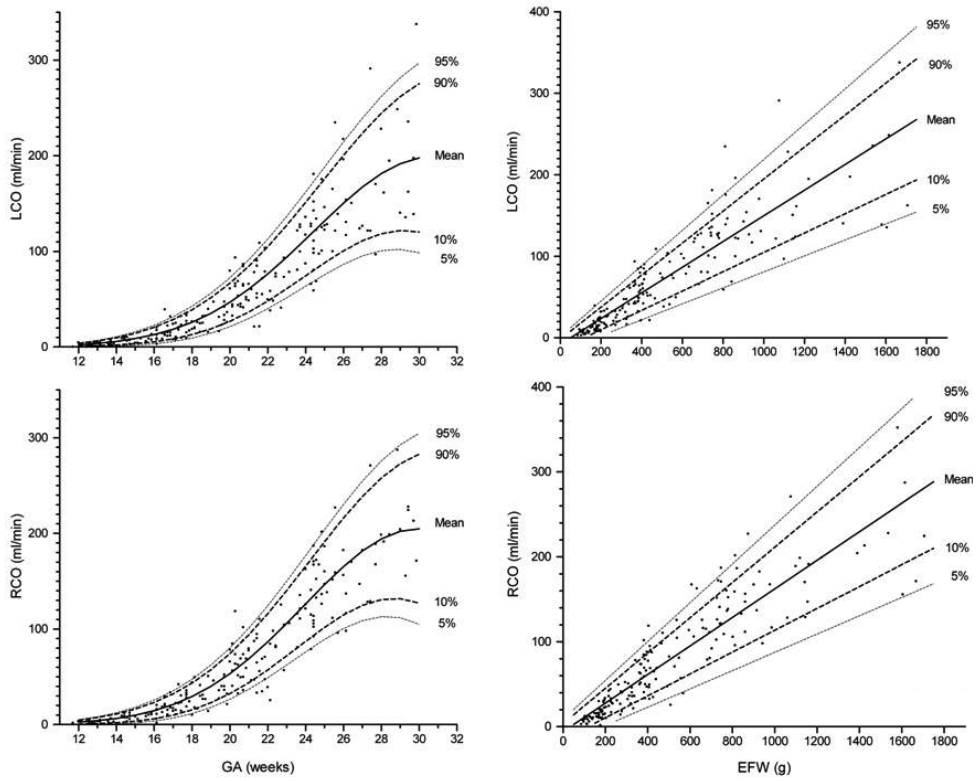

Figure 6 *Fetal cardiac output (CO). Left CO plotted against gestational age (GA) (ln(y)= -6,604 + 0,775 x GA - 0,013 x GA²) (a) and estimated fetal weight (b); right CO plotted against GA (ln(y)= -6,817 + 0,810 x GA - 0,014 x GA²) and estimated fetal weight(d). Mean values (——), 10th and 90th centiles (- - -), and 5th and 95th centiles (····) are shown. GA data are based on 202 fetuses and estimated fetal weight data are based on 174 fetuses.*

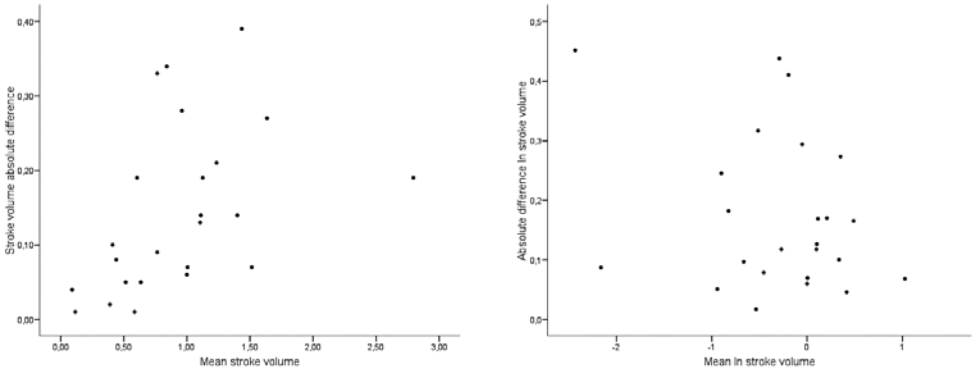

Figure 7 *Plots of absolute difference against mean for two measurements of stroke volume by the same observer (a) and absolute difference against mean after natural log (ln) transformation (b).*

data was calculated, giving 1.137; for a measured SV dividing and multiplying a measurement by the square of this number gives the approximate limits of intraobserver agreement. For example, for a SV measurement of 0.45 mL the estimated 95% CI is 0.35-0.58 mL. Hence, the coefficient of variation was estimated to be 13.7%.

DISCUSSION

This study provides reference values for both left and right ESV and EDV, left and right SV, and left and right CO from 12 to 30 weeks of gestation, measured using multiple longitudinal acquired measurements with 4D ultrasound imaging. Furthermore, this study provides reference ranges for indices of fetal cardiac function plotted against gestational age as well as EFW.

It is generally accepted that conventional 2D ultrasound examination is of limited value in the assessment of the cardiac function of the human fetus[3, 8, 26]. The origins of errors in 2D measurements are well documented in the literature[7]. Recently, Molina *et al.* published a study similar to this one concerning 4D ultrasound measurements of indices of fetal cardiac function[27]. In their study 140 cross-sectional acquired STIC volumes were measured using Virtual Organ Computer-aided AnaLysis (VOCAL) method. The present study corroborates the findings presented by Molina *et al.*, however, it differs in a number of aspects. Although cross sectional studies are generally accepted, the only valid way to express fetal changes in pregnancy is to collect the data longitudinally, as in our study. Second, this study provides reference ranges for left and right ESV and EDV and EFs. Third, we plotted our data against EFW, which is important in the evaluation of growth-restricted fetuses. Finally, this study uses the 3D slice method, which we believe is preferable in volume measurements. The few studies on 4D ultrasound imaging using STIC report the use of different methods for volume measurements; Messing *et al.*[10] use inversion method, and Rizzo *et al.*[12] and Molina *et al.* both used the VOCAL method. The advantage of 3D slice method is that, within a STIC volume, the four-chamber view often displays the clearest demarcation of the intraventricular cavity. In the 3D slice method this plane is used to trace the endocardial borders and thus avoids lower resolution in the orthogonal plane, which is used in the VOCAL method. Furthermore, there is operator dependency owing to threshold settings when using the Inversion method, which is not used in the 3D slice method. As all methods involving 4D ultrasound imaging, the 3D slice method avoids the geometric assumptions made using 2D ultrasound methods for estimating ventricular volumes, and the operator has the ability to accurately check the 3D measurement as the fetal ventricle tracings are displayed after initial drawing.

Table 3 *Combined left and right stroke volumes in previous studies using both two- and four-dimensional ultrasound imaging.*

Reference	Method of measurement	Combined stroke volume (mL)		
		20 weeks	24 weeks	30 weeks
Allan *et al.* (1987)[4]	Vessel area and TVI	1.16	2.13	4.49
Kenny *et al.* (1986)[6]	Vessel area and TVI	1.93	2.74	4.63
Rasanen *et al.* (1996)[31]	Vessel area and TVI	1.18	2.89	6.28
Mielke & Benda (2001)[30]	Vessel area and TVI	0.85*	2.06*	4.56*
Messing *et al.* (2007)[10]	STIC and Inversion method	0.41	1.26	2.95
Molina *et al.* (2008)[27]	STIC and VOCAL	0.55	1.27	2.69
Present study	STIC and 3D Slice method	0.72	1.72	2.63

**Extracted from figures. 3D, three dimensional; STIC, spatiotemporal image correlation; TVI, time velocity integral; VOCAL, Virtual Organ Computer-aided AnaLysis.*

The values for left and right ventricle volumes are in line with results presented by Schmidt *et al.*, who used 2D ultrasound imaging with summation of discs for volume determination[28]. Our results differ, however, from the results of another cross-sectional study by Messing *et al.*, especially for ESV and EDV measurements[10]. The values for ventricle volumes presented by Messing *et al.* are significantly smaller than the results we obtained. These differences might be explained by the use of the inversion method, as it is more dependent on B-mode gain, gray-scale curve and dynamic range settings. Messing's data on combined SV are, however, in line with our results. Other previous reports which estimated SV using 2D ultrasound and Doppler or 4D ultrasound show conflicting results[4, 6, 10, 29-31] (Table 3). However, all studies using 4D ultrasound imaging, including the present study, report relatively small values of SV regardless of the method used, compared with the larger values found in the 'Doppler-studies'. A study that assessed the agreement of 2D ultrasound with Doppler imaging vs. 4D ultrasonography was published recently by Rizzo *et al.*[12]. This study investigated 40 healthy fetuses and 16 fetuses with IUGR, in which close agreement between the two techniques for the measured SV, was described. This is not in line with our results or those of Messing *et al.*[10], and Molina *et al.*[27]. The difference between 2D ultrasound and 3D ultrasound findings can be explained by geometric assumptions about the ventricular shape[32], inaccuracies in Doppler calculations based on postnatal and prenatal differences in fetal myocardial contractility[27], and errors in measurement of the cross-sectional area of the vessel lumen in Doppler studies (the lumen is assumed to be round but could instead be slightly oval). Thus, the Doppler studies may be erroneous, and may have led to overestimation of fetal SV and subsequently CO. As 4D ultrasound estimations avoid geo-

metric assumption and are less susceptible to errors in measurement, our results, together with other 4D ultrasound studies, might give a more accurate reflection of fetal cardiac volumes and prenatal cardiac function.
An important limiting factor in the acquisition of STIC volumes is acoustic shadowing. As a result the acquisition became more difficult with advancing gestational age. Only a few high-quality STIC volumes could be acquired in the period beyond 30 weeks of gestation. To some extent this was also the result of the prospective longitudinal design of this study, but the high failure rate late in gestation could reveal one of the most important limiting factors for 4D ultrasound imaging using STIC. An overall success rate of 71%, however, is in line with the results of a previous study by our group[17]. In clinical practice, however, this limitation can probably be overcome without much difficulty, as clinical sonographers are less bound to examination-time limits, as in many research settings. Other limiting factors for acquisition include persistent unfavourable fetal position and abundant fetal movement. A further limitation of these 3D ultrasound methods is the time and expertise required for data analysis. In our *in-vitro* validation study in which a balloon model was used, however, the 3D slice method was proven to be less time consuming than the use of VOCAL or the inversion method.

This study presents reference ranges for indices of fetal cardiac function from 12 to 30 weeks of gestation based on longitudinal acquired measurements. It confirms the data published by Molina *et al.* but also adds valuable information for clinical use of this 4D ultrasound technique. In our opinion, more studies are needed to asses to what extent measurements in fetuses in pathological states deviate from normal and whether these measurements can aid in the prediction of fetal outcome. However, STIC volume acquisition followed by 3D volume measurement has proven to be a feasible method for assessing fetal cardiac function and seems to overcome most of the pitfalls conventional methods. STIC therefore promises to become the method of choice to asses the fetal cardiac function.

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Chapter 8

Assessment of fetal cardiac function

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ABSTRACT

Fetal cardiac function is increasingly recognized as a marker of disease severity and prognosis. Functional cardiac abnormalities may occur in cases of twin-to-twin transfusion syndrome, hydrops fetalis, intrauterine growth restriction, fetal arrhythmia, maternal diabetes, fetal anaemia or congenital heart disease. Cardiac decompensation can cause severe fetal and neonatal morbidity and mortality and can be treated in selected cases when diagnosed in time. Different non-invasive techniques have been evaluated over the years, almost exclusively by echocardiography. Sonographic evaluation of functional parameters has been performed using a wide variety of modalities. The fast development of new imaging modalities has added an enormous amount of knowledge to this field of research and provided a large number of different techniques focussing on a wide variety of functional parameters. However, no gold standard exists, for the assessment of fetal cardiac function. This paper presents an overview of the current state of the field of non-invasive assessment of fetal cardiac function.

INTRODUCTION

Congenital heart disease (CHD) is the most frequently encountered fetal congenital anomaly and is a leading cause of morbidity and mortality in infants. Consequently, a major focus of fetal echocardiography has been the timely and accurate diagnosis of congenital heart disease. More recently, non-invasive evaluation of fetal cardiac function has received an increasing amount of attention. Functional cardiac abnormalities may occur in cases of twin transfusion syndrome (TTS), hydrops fetalis, intrauterine growth restriction, fetal arrhythmia, maternal diabetes, fetal anaemia or CHD. Fetal cardiac decompensation can cause severe fetal and neonatal morbidity and mortality, and can be treated in selected cases when diagnosed in time.

Fetal cardiovascular function is the result of a complex interaction between blood volume, ventricular preload, ventricular afterload, myocardial contractile and relaxation properties, valve function, heart rate and heart rhythm. Difficulties in acquiring proper images in a moving fetus, combined with differences between fetal and neonatal circulation, make the assessment of fetal cardiac function a challenge requiring specific knowledge, some of which has been variably or inconsistently attainable.

Essential for the fetal circulation are the three shunts, the ductus venosus, the ductus arteriosus and the foramen ovale. The ductus venosus and the foramen ovale are responsible for the distribution of oxygenated blood from the placenta to the left and right atrium. Oxygenated blood ascends from the ductus venosus and flows through the inferior venous inlet where it is accompanied with blood from the inferior vena cava and left hepatic vein. It is redirected towards the left atrium via the foramen ovale[1]. The redistribution of blood between the left and right atrium is dependent on pressure changes on the left and right side of the heart.

In contrast to the postnatal situation, both left and right ventricles have a similar, relatively low, pressure during intra-uterine life[2], and oxygen saturation differences between the ventricles remain limited[3]. Both ventricles work closely together as they share the interventricular septum and are encircled by common muscle fibres. Besides the similarities, there are distinct differences between the two cardiac ventricles. The ventricles have a completely different shape and myocardial fibre orientation, as the right ventricle is virtually curled around the left. Furthermore, the left ventricle mainly feeds the coronary and cerebral circuits and the right ventricle directs most of its output to the systemic circulation via the ductus arteriosus[4]. Approaching fetal term, the right ventricle becomes more dominant and contributes 55-60% of the combined cardiac output[4, 5].

Invasive assessment of fetal haemodynamics is technically challenging and ethically questionable in the human fetus. Therefore, different non-invasive techniques have been evaluated over the years, almost exclusively by echocardiography. Ultrasound combined with M-mode imaging has been thoroughly studied in the last decades and will not be discussed in this paper[6-9]. Pulsed-wave Doppler is a long standing technique that is still commonly used to study blood flow profiles of the heart and blood vessels. Other ultrasound derived modalities specifically study myocardial movement such as tissue Doppler, myocardial strain and strain rate imaging. The most commonly used non-invasive modality in adult cardiology is to record the electrical activity in the heart, the electrocardiogram (ECG). This has also been evaluated prenatally[10]. Further, new imaging techniques used in adults such as magneto resonance imaging and three and four-dimensional ultrasound have also been studied in the fetus. The fast development of new ultrasound imaging modalities such as three and four-dimensional fetal echocardiography has made it possible to measure new cardiac parameters, measure known parameters in a different way and to provide 'new access' to fetal cardiac anatomy. As such, a large amount of research has focused on these new modalities and has added knowledge to the field of fetal cardiac functional assessment. The VU University medical center and Leiden University Medical Center have combined their clinical experience and research efforts in a fetal echocardiographic collaboration. We hereby present an overview of the current state in the field of non-invasive fetal cardiac assessment. In this paper we focus on four topics in non-invasive cardiac assessment to make the information more easily accessible and act as a reference work: (1) the imaging of cardiac blood flow; (2) analysis of fetal cardiac time intervals and movement; (3) cardiac volumetry; and (4) cardiac electrical signalling.

DOPPLER IMAGING OF BLOOD FLOW

Atrioventricular (AV) flow

One of the most common modalities used in prenatal sonographic examinations is Doppler imaging. Pulsed wave Doppler was designed to detect high velocity Doppler shifts caused by movement of red blood cells. Doppler waveforms of the tricuspid and mitral valve can be acquired by placing the Doppler sample immediately distal to the valve leaflets in an apical or basal four-chamber view to ensure that the scanning angle is kept to a minimum and does not exceed 30 degrees (Figure 1). The double peaked waveform of the AV-valve flow is the result of the filling of the ventricle during the myocardial relaxation, referred to as E-wave, and during the subsequent active filling during the atrial contraction, referred to as A-wave. The ratio of these two waves (E/A ratio) is considered to be an indicator of heart compliance and diastolic cardiac function[11-13]. In adults up to

80% of ventricular filling occurs through ventricular relaxation whereas in the fetal heart this is largely dependent on atrial contraction[11]. In healthy adults a shift to a lower E/A ratio is typical for diastolic dysfunction which is known to precede systolic dysfunction, while this is reported as normal in the fetus[12, 14, 15]. The peak systolic velocity (PSV) and the mean velocity (MV) of the E wave increase as a function of gestational age while there is little or no significant change in atrial contraction mean velocity and peak velocity (A wave)[16-20]. In the fetus the E/A ratios thus normally increase with advancing gestational age[11, 13, 21-23]. This reflects increasing diastolic compliance. In growth-restricted fetuses, E/A ratio has been reported to be further increased compared to normal controls[24] while other studies found no differences in E/A ratios[25] or even reduced E/A ratios compared to normal controls[26]. In compromised fetuses, lower PSV of both atrioventricular valves might indicate diastolic dysfunction[23, 26, 27]. However, not all studies have found significant differences between growth restricted fetuses and healthy controls[27-29], and lower PSV measurements do not necessarily reflect poor ventricular function, but may instead reflect a physiological adaptation to a higher afterload or decreased vascular compliance[25]. Considering the conflicting results in the current literature, these parameters are not preferential for functional assessments prenatally.

Functional assessment

Estimation of cardiac output and stroke volume using Doppler, requires measurement of a valve or vessel orifice and the average velocity of the blood flowing through. The valve or vessel area can be estimated using two-dimensional ultrasound and the average velocity can be obtained by multiplying the velocity time integral (VTI) with the fetal heart rate. The VTI is the area under the curve of a Doppler waveform of one cardiac cycle (Figure 1). VTI values of both ventricles increase during gestation[11, 18]. Consequently, cardiac output increases with gestational age[16, 23, 30, 31], from 113 mL/min at 20 weeks to 332 mL/min at 30 weeks for the right ventricle and from 117 mL/min at 20 weeks to 305 mL/min at 30 weeks for the left ventricle[31]. However, the measurements are subject to an inherent margin for error, which can have significant impact on volume estimation. Firstly the measurement of the valve orifices is prone to measurement error, especially in early gestation with corresponding smaller cardiac dimensions. Measurement differences of 1 mm can represent a 20% error in valvular diameter and could result in 30% change in cardiac flow due to the fact that valve orifice measurement error is squared in the formula to calculate valve area[32].

Secondly, the accuracy of Doppler velocity measurement are strongly angle dependent and mathematical corrections for variations in scanning angle are subject to error as well[33]. Furthermore, aspects like fetal activity, normal diurnal rhythm and a strong individual variation in fetuses might further influence estimations of cardiac output. Because of wide variance in study results and the inherent margin of error ultrasound measure-

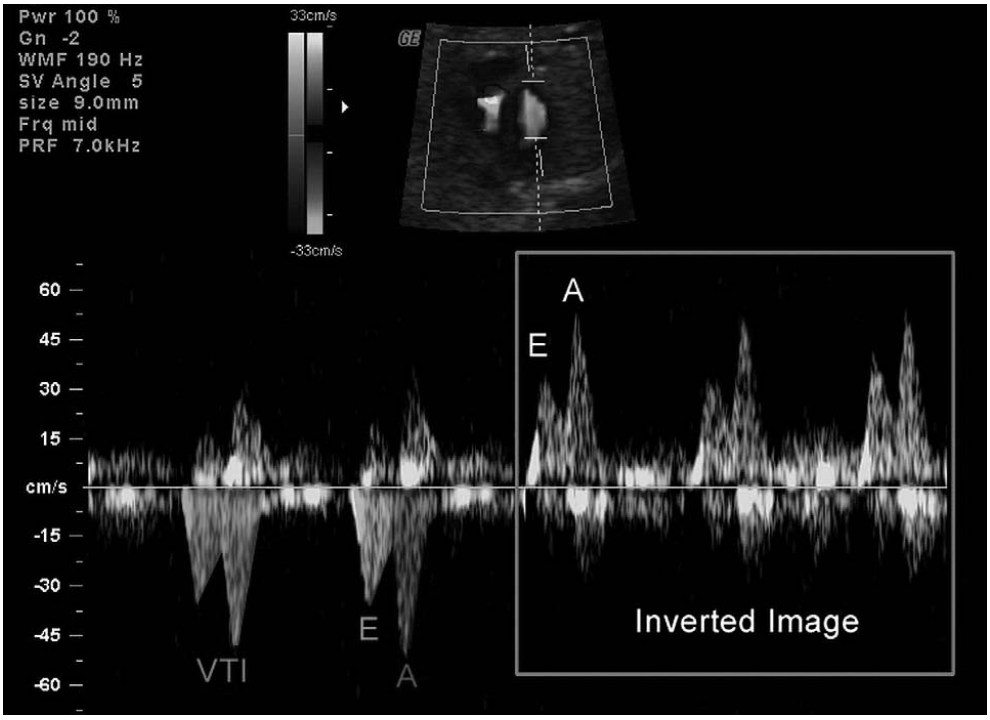

Figure 1 *A double peaked waveform of a Doppler blood flow recording over the mitral valve. The E-wave (green), A-wave (purple) and the velocity time integral (orange) are marked. For optimal Doppler recordings the angle of insonation is kept as low as possible (in this image the angle is 5 degrees as is noted in the upper left corner; SV Angle 5). Adapted from Seminars in Fetal & Neonatal Medicine (2005) 10, 515-541.*

ments, the use of these Doppler parameters as a tool for clinical function assessment remains limited and they are therefore not frequently used in daily clinical practice.

Aortic and pulmonary flow

There are different analyses of aortic and pulmonary Doppler waveforms that have been applied in the fetus, including measurement of the peak velocity, mean velocity, acceleration time, acceleration slope and ejection time. In any such assessment, the fundamental question is what clinically useful information will be obtained by the measured variable[9]. Aortic Doppler waveforms can be obtained by placing the Doppler sample just distal to the aortic valve in the long axis view of the fetal aorta (Figure 2). For pulmonary artery waveforms the Doppler sample should be placed just distal to the pulmonary valve in the short axis view or the transverse 'three vessel view'. The use of VTI of the aorta and pulmonary artery is more commonly used than the AV valve derived VTI to estimate cardiac output. With advancing gestational age, PSV in ascending aorta and pulmonary artery increase to around 1 m/sec at 40 weeks[29, 30, 34-37]. Similarly, estimated

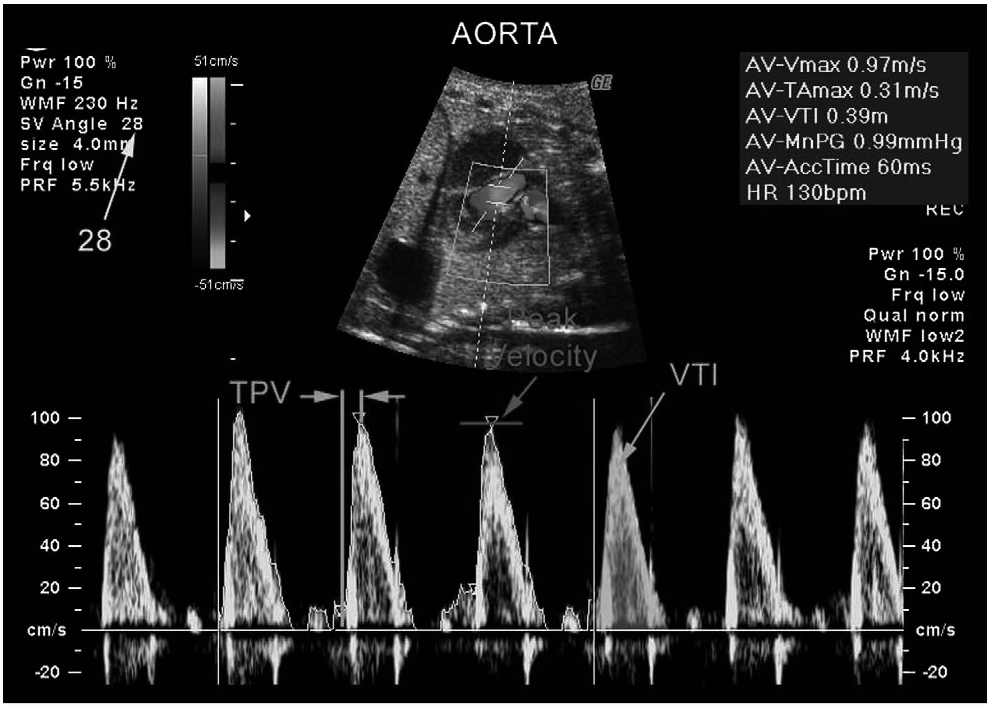

Figure 2 *Measurement of aortic blood flow with correct cursor placement for pulsed-wave Doppler interrogation. A single peaked Doppler waveform is shown below. Velocity time integral (VTI) is marked in blue, peak velocity measurement is marked in purple and measurement of the time to peak velocity (TPV) is marked in green. Adapted from Seminars in Fetal & Neonatal Medicine (2005) 10, 515-541.*

cardiac output increases as a function of gestational age[30, 35, 38-40] from 95 mL/min at 20 weeks to 389 mL/min at 30 weeks for the right ventricle and from 79 mL/min at 20 weeks to 310 mL/min at 30 weeks for the left ventricle[4, 30, 34, 35, 38, 39]. As a result, combined cardiac output shows a similar relationship, increasing with gestational age from 40 mL/min at 15 weeks to 1470 at 40 weeks, with a wide variance in study results[31, 39]. Remarkably, cardiac output values obtained with Doppler flow are generally larger than values obtained with new non-invasive methods like four-dimensional ultrasound imaging. This discrepancy will be addressed further on in this article[41]. Earlier validation studies in animals have also shown Doppler measurements to overestimate blood flow[42]. The method of estimating output from flow is hampered by the same factors mentioned above. Nevertheless, careful measurements performed by experienced sonographers can have coefficients of variation of less than 10%[16, 43]. The technical limitations combined with the variability in measurement outcome limit the use in clinical practice. Despite the variability involved in output estimations, however, this method is still regularly used in research settings.

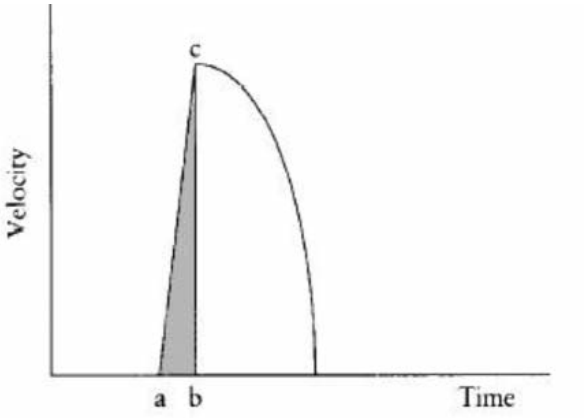

Figure 3 *Ejection force can be calculated using the formula: F = (1.055 x valve area x velocity time integral of acceleration(VTIa)) x peak systolic velocity/acceleration time). VTIa refers to the area under the curve of the doppler waveform in the acceleration phase only (grey), peak systolic velocity is marked with (c) and acceleration time is the time between (a) and (b). Adapted from Ultrasound Obstet Gynecol 1997; 10: 325-332.*

Ventricular ejection force

Newton's second law of motion defines force as the product of mass and acceleration. The ejection force is an approximation of the energy transferred from the ventricular myocardial shortening to blood accelerating into pulmonary and systemic circulation. This value can be calculated from valve area estimates and Doppler outflow recordings with the same limitations as mentioned earlier (Figure 3). Ejection force (F) can be calculated using the formula: *F = (1.055 x A x VTIa) x PSV/aT)*, where VTIa refers to VTI of acceleration that is the area under the curve of the doppler waveform in the acceleration phase only. Ejection force has been shown to be more sensitive than Doppler indices such as PSV for the assessment ventricular (dys)function in adults, and appears to be less influenced by changes in loading conditions than other Doppler indices[44-46]. Validation and reliability studies, however, are eagerly awaited. From 20 weeks to term, force increases as a function of increased myocardial mass, with a wide variance in values ranging from 3.3–4.1 to 28.8–39.5 mN for the left and 0.06–5.6 to 19.5–59.8 mN for the right ventricle from 22 to 40 weeks[40, 47, 48]. Growth restricted fetuses have decreased ejection forces secondary to placental insufficiency[48]. A direct relationship between this force and umbilical vein pH values has been found. Ejection force below the 5th percentile has been reported to predict a poorer perinatal outcome[48]. As with many ultrasound parameters for fetal cardiac function, there are several limitations concerning the validity of the measurement itself. This, combined with the lack of validation and reliability data, has limited its role in clinical settings.

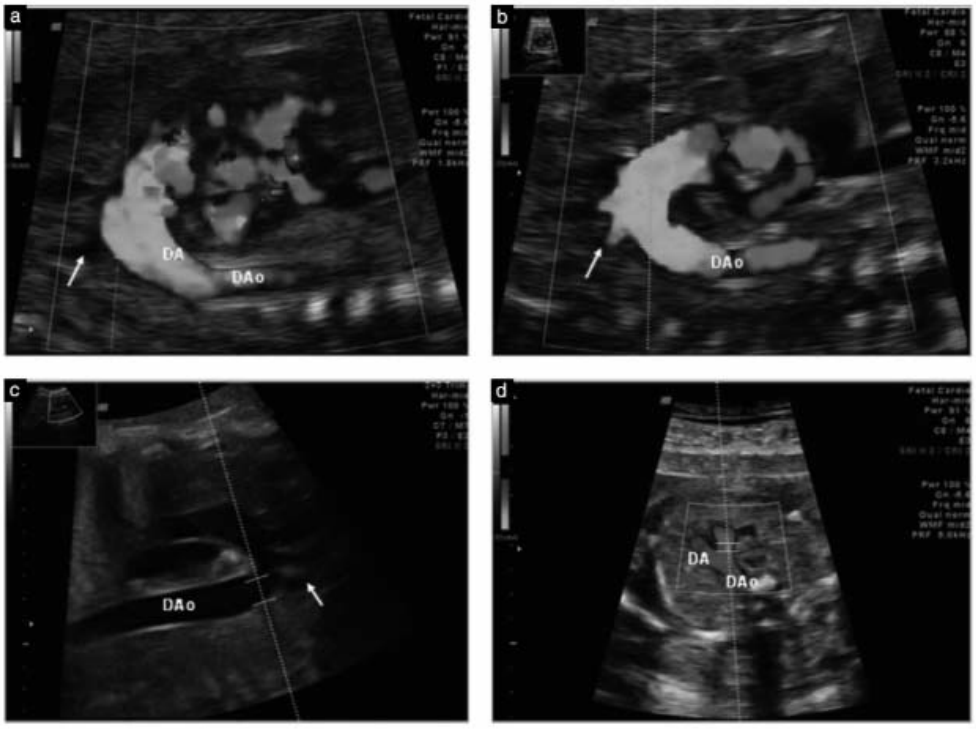

Figure 4 *Longitudinal (a–c) and cross-sectional (d) imaging planes demonstrating the aortic isthmus with correct cursor placement for pulsed-wave Doppler interrogation. The arrow indicates the left subclavian artery. DA, ductus arteriosus; DAo, descending aorta. Adapted from Ultrasound Obstet Gynecol 2009; 33: 628–633.*

Aortic isthmus flow

The fetal aortic isthmus is the vascular segment located between the origin of the left subclavian artery and the segment of the ductus arteriosus that ends in the aorta. Aortic isthmus Doppler waveform can be obtained in a longitudinal plane of the fetal thorax or the transversal three vessel trachea view. It has been demonstrated that the retrieval of Doppler flow velocity waveforms of this part of the aorta is relatively easy in trained hands, but the placement of the Doppler sample volume may be challenging depending on fetal position (Figure 4)[49-51]. Its unique location makes it the only arterial connection between the right and left fetal vascular systems. In systole, the amount and direction of flow are influenced by the relative performance of the two ventricles and by the balance between the vascular impedances of the upper body and cerebral circulation for the left ventricle and the placental and lower body circulation for the right ventricle. In diastole the direction of blood flow is solely dependent on the placental and cerebral vascular impedances. As a result of this unique 'balancing' position, blood flow patterns

reflect the balance between the ventricular output and the impedance of any of the cerebral (left ventricle) and placental (right ventricle) vascular networks[50, 52-54]. Normal and abnormal waveforms can be identified by simple qualitative assessment as antegrade or retrograde diastolic flow. Aortic isthmus retrograde diastolic blood flow signifies redistribution of fetal circulation, indicating lower cerebral resistance compared to the placental resistance. The reversal of net blood flow during diastole indicates that the fetus has problems maintaining cerebral oxygenation[52, 55-57]. Changes in aortic isthmus waveforms become evident before abnormalities in the umbilical artery, descending aorta or ductus venosus in placental insufficiency[52,58]. It remains unclear, however, whether aortic isthmus flow measurement adds to the prediction of perinatal death[48]. Aortic isthmus Doppler waveforms provide, due to the aorta's unique position in the fetal circulation, valuable information on fetal cardiac function and changes in the vascular resistance relevant to the left and right ventricle. Due the relatively short length of this segment of the fetal aorta, and to the close anatomical location with relation to other vessels, this measurement can be technically challenging. These complicating factors have hampered its implementation in routine fetal monitoring practice.

Aortic pulse velocity

Pulse velocity reflects vascular stiffness and elastic properties. Increased placental impedance will reduce fetal aortic end-diastolic flow, and may even cause flow reversal, thus increasing the ventricular afterload and compromising fetal cardiovascular function. In the fetus pulse, velocities can be measured using phase-locked echo-tracking[59]. Lower values of aortic pulse velocities in growth restricted fetuses are thought to reflect the chronic fetal ventricular and vascular responses to increased placental impedance[60]. Pulse pressure waveform of the aorta has been shown to correlate with cardiovascular preload, total peripheral resistance and stroke volume, myocardial contractility and cardiac output in animal studies[61]. Pulse pressure, the amplitude of the pressure wave, has been determined in normal and growth restricted fetuses[62, 63]. Based on these findings authors have suggested that in growth restricted fetuses there is an increase in diastolic pressure and a reduction in stroke volume, while in large for gestational age fetuses there is an increase in the pulse pressure and stroke volume. Recently, a non-invasive method has been presented to determine fetal arterial blood pressure using a technique of simultaneously obtained blood flow and diameter waveform recordings[64]. The technique currently involves cumbersome calculations and is insufficient to allow measurements in individual fetuses. However, technological advances and further research may bring it to a point where blood pressure characterization of fetuses becomes possible to discriminate between hypertensive and hypotensive blood pressures.

Ductus venosus flow

Although flow in the ductus venosus reflects cardiac function only indirectly, flow measurements in the ductus have received a high level of attention in literature recently as a potent marker of fetal hemodynamics. The ductus venosus (DV) is a small venous shunt connecting the umbilical vein to the fetal heart. The portion of well-oxygenated blood from the placenta that is diverted to the fetal heart flows through the foramen ovale to the left side of the circulation. Normally at mid gestation around 30% of blood from the umbilical vein is shunted through the DV decreasing to 20% from 30 weeks of gestation onward, but with wide variations1. Although flow patterns in DV and vena cava inferior have been studied in detail, the exact mechanism of blood diversion in DV is not yet fully understood[65-67]. The redistributional mechanisms of increased shunting during hypoxaemia found in animal experiments seem to occur in the human fetus as well[68]. Alterations in venous flow waveforms can be explained by alterations in cardiac afterload, cardiac contractility and compliance, intravascular volume status and heart rate (Figure 5)[69]. Because the DV has high velocity blood flow, it is easily identifiable by the aliasing seen with color Doppler. The blood flow velocity waveform of the ductus venosus reflects the normal cyclic cardiac events with a peak during ventricular systole, a peak during passive diastolic filling and a nadir during atrial contraction. A general increase in velocities reflects an increased portocaval pressure gradient (e.g. liver disease, anaemia). An additional augmented atrial contraction wave reflects increased end-diastolic pressure (e.g. increased preload, adrenergic drive) commonly seen in placental compromise. A further deterioration would be a reversed A-wave[1]. The liver receives most of the umbilical venous return and thus seems to have a high developmental priority[66]. An increased shunting through the ductus venosus, possibly resulting in hypoxia in parts of the liver, plays an important compensatory role during acute fetal hypoxaemia and hypovolaemia and, probably, a prolonged adaptational role during chronic placental compromise[1, 70, 71]. The utility of DV Doppler seems greatest in fetal conditions that have impact on afterload, cardiac compliance and contractility. It is important to bear in mind that all venous Doppler measurements are easily influenced by sampler placement and examination technique[69]. Although the DV pulsatility index has been found an important indicator for the optimal timing of delivery before 32 weeks of gestation[72], there is still no consensus on which method of fetal monitoring is most useful for the timing of delivery. Currently an international randomized trial is performed in early preterm fetal growth restriction to compare timing of delivery based on early and late fetal Doppler venous changes versus cardiotocography. The results are eagerly awaited.

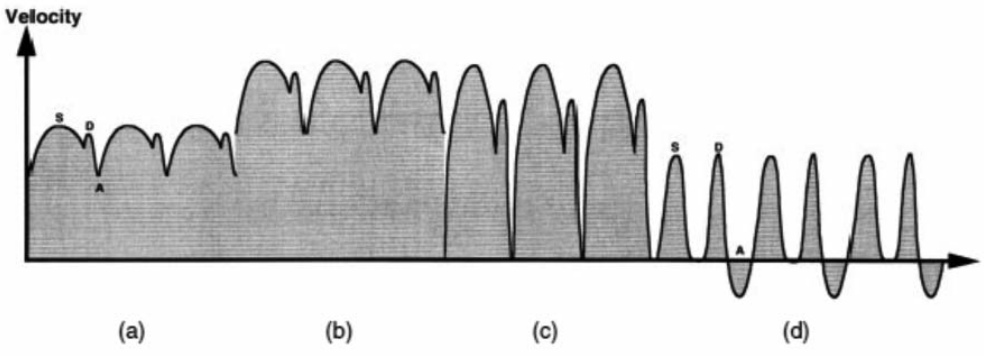

Figure 5 *The blood velocity in the ductus venosus reflects the normal cyclic cardiac events (a) with a peak during ventricular systole (S), a peak during passive diastolic filling (D) and a deflection during atrial contraction (A). A general increase in velocities (b) reflects an increased portocaval pressure gradient (e.g. liver disease, anaemia). An additionally augmented atrial contraction wave (c) reflects increased end-diastolic pressure (e.g. increased preload, adrenergic drive) commonly seen in placental compromise. A further deterioration (d) would be a reversed A-wave. With increasing myocardial hypoxia and acidosis, the muscle is less compliant, causing a dichotomy of the S- and D-wave (e.g. preterminal placental compromise). Adapted from Prenat Diagn 2004; 24: 1049–1059.*

DOPPLER MEASUREMENT OF CARDIAC TIME INTERVALS

Myocardial Performance Index

The index consists of the time required for intraventricular pressure to exceed the systemic pressure (the isovolumetric contraction time, ICT), plus the time of ventricular relaxation in which intraventricular pressure drops before ventricular filling starts (the isovolumetric relaxation time, IRT) and divided by the ejection time (ET) *(MPI=(ICT+IRT)/ET)*. The MPI was introduced in 1995 for cardiac assessment in adults and has subsequently been adapted in pediatric and fetal cardiology[73-77]. The left ventricle myocardial performance index (MPI) is obtained from a single Doppler wave form, by placing the sample on the medial wall of the ascending aorta close to the mitral valve in the five-chamber view (Figure 6). For the right ventricle two separate measurements have to be obtained, at the TV and just distal to pulmonary valve[75]. The measured parameters are not only dependent on myocardial function but are also influenced by pre- and afterload conditions. The MPI therefore indirectly reflects global cardiac function[78]. To improve LV MPI reproducibility the use of valve clicks of the AV valves and aortic valve as landmarks was introduced[76, 79, 80]. Nevertheless, the very short time intervals, as well as the use of different methods of acquisition, result in a wide variance in reported values of mean MPI[75-77, 80-84]. The MPI has been shown to increase in growth restriction, indicating cardiac dysfunction. As an reflection of decreasing cardiac function the MPI is an independent predictor for perinatal mortality[24, 81, 85]. Also, the fetuses of diabetic mothers have been

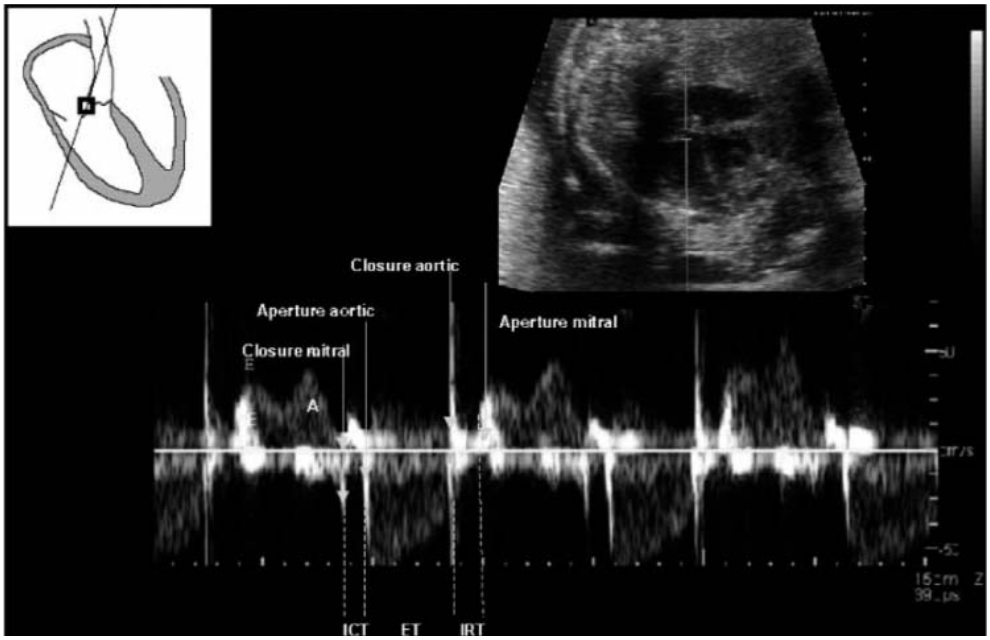

Figure 6 *Left ventricle modified myocardial performance index (Mod-MPI). The sample volume is located over the lateral wall of the aorta, close to the mitral valve. The E/A waveform is always displayed as positive flow. ET, ejection time; ICT, isovolumetric contraction time; IRT, isovolumetric relaxation time. Adapted from Ultrasound Obstet Gynecol 2005; 26: 227-232.*

observed to display higher MPI values in the third trimester, possibly due to hypertrophic cardiomyopathy[81, 83]. To assess global cardiac function in uncomplicated monochorionic twins, Van Mieghem et al constructed reference ranges for left and right MPI[80]. Within this sample set, he compared in cases of TTS. He found preoperative left and right MPI of the recipient twin to be above the 95th percentile[86]. Whether this index can be used as a management tool to predict the prognosis in TTS or IUGR remains an interesting subject for further research.

DOPPLER IMAGING OF CARDIC MOVEMENT

Tissue Doppler Imaging

As opposed to conventional Doppler imaging, tissue Doppler imaging (TDI) focuses on the low velocity movement of ventricle walls. Tissue velocities are much less influenced by cardiac loading conditions compared to transvalvular blood flow velocities[87, 88]. In adult echocardiography, TDI has been available as a clinical tool for more than a decade. Peak systolic myocardial velocities (Sa) of the mitral valve annulus have been reported

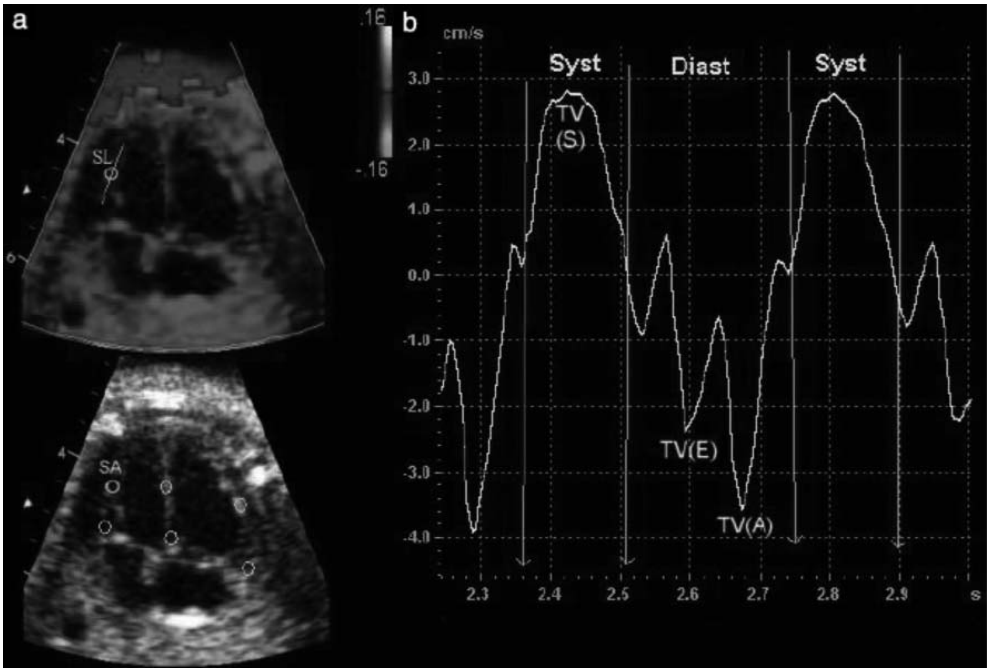

Figure 7 *Four-chamber view in B-mode with color Doppler myocardial imaging superimposed. Red indicates movement toward the transducer and blue movement away from the transducer. (b) Example displaying tissue velocities of the middle portion of the left ventricular wall. SA, sample area; SL, strain length; TV(A), peak velocity during atrial contraction; TV(E), peak velocity during early diastole; TV(S), peak velocity during systole. Adapted from Ultrasound Obstet Gynecol 2006; 27: 210–213.*

to have excellent correlation with LV ejection fraction and pressure changes[89-91]. TDI has also been reported as a sensitive technique for the detection of right ventricular systolic dysfunction[92]. The E/Ea ratio, or ratio between the peak blood flow velocity (E) and the atrioventricular annular velocity (Ea) during early diastolic filling, has been shown to correlate with ventricular filling pressures[93, 94].

Velocity profiles can be obtained with two different techniques: *pulsed-wave* (PW)-TDI and *color-coded* (CC)-TDI. PW-TDI is a real time measurement, while CC-TDI requires post-processing of data. PW-TDI has a very high temporal resolution. The velocity curves are imaged real time and peak myocardial velocities can be measured instantaneously. CC-TDI velocities are superimposed on two-dimensional images (Figure 7). A major advantage of CC-TDI is that it allows repositioning of the sample volume and simultaneous comparison of various segments, which is impossible with PW-TDI. The temporal resolution is, however, much lower compared to PW-TDI. Furthermore, CC-TDI velocities are usually measured 10-20% lower than PW-TDI derived measurements[95].

Several studies have presented normal values for fetal PW-TDI velocities[96-99]. Left ventricle ratios of early (Ea) and late (Aa) myocardial wall movement found in IUGR fetus might reflect reduced myocardial compliance[100]. Signs of impaired fetal diastolic function have been reported in fetuses of diabetic mothers, regardless of the existence of myocardial hypertrophy[101] and in fetal hydrops[102]. On the other hand, PW-TDI has also been stated to be not sufficiently sensitive to distinguish fetuses with heart failure (TTS and cardiomyopathy) from healthy controls[103]. However, two indices have been reported to be useful and sensitive indicators of global right ventricular dysfunction: (1) the E/Ea ratio (reflecting ventricular filling pressures); and (2) the TDI-measured MPI. Notably, for calculation of the MPI, TDI and conventional PW Doppler cannot be used interchangeably, as measurements of PW Doppler are based on blood flow events and TDI measurements on myocardial motion[104]. Further studies in complicated pregnancies are needed to establish the clinical relevance of these techniques.

Strain rate imaging

Recently a TDI derived technique has been introduced: strain rate imaging. Myocardial strain is defined as the change in length of an object relative to its baseline length caused by an applied stress, with strain rate being derived from the velocity of the deformation over time. Positive strain-rate thus indicates lengthening of the tissue whereas a negative strain rate indicates shortening. Strain-rate reflects the movement of one tissue site relative to another within the sample volume, in contrast to tissue velocity data, which merely reflect movement of one site relative to the transducer (Figure 8)[105]. Larsen *et al.* showed that with good-quality imaging, measurements of MPI indices, strain, strain rate and tissue tracking have also become feasible in the human fetus[106]. Others have found fetal strain and myocardial deformation to be almost identical to pediatric normal values[107]. Although there is still a significant variability in TDI-derived parameters because of differences in machine characteristics and lack of guidelines for standardization of sample positioning and machine settings, strain rate imaging promises to become another technique available for the fetal sonographer to use in the evaluation of fetal cardiac function, although its place in routine clinical practice has still to be determined.

Velocity vector imaging (VVI)

Velocity vector imaging is another novel technique to study myocardial movement[108]. It is a software package that allows offline evaluation of myocardial tissue motion and velocity, using two-dimensional images. A combination of speckle tracking and geometric analysis enables the software to follow myocardial movement throughout the cardiac cycle, resulting in analysis of the myocardial mechanics (Figure 9). From these datasets, myocardial velocity, strain, and strain rate can be calculated. Advantages of this technique are that it is independent of the angle of acquisition and it is able to measure motion in

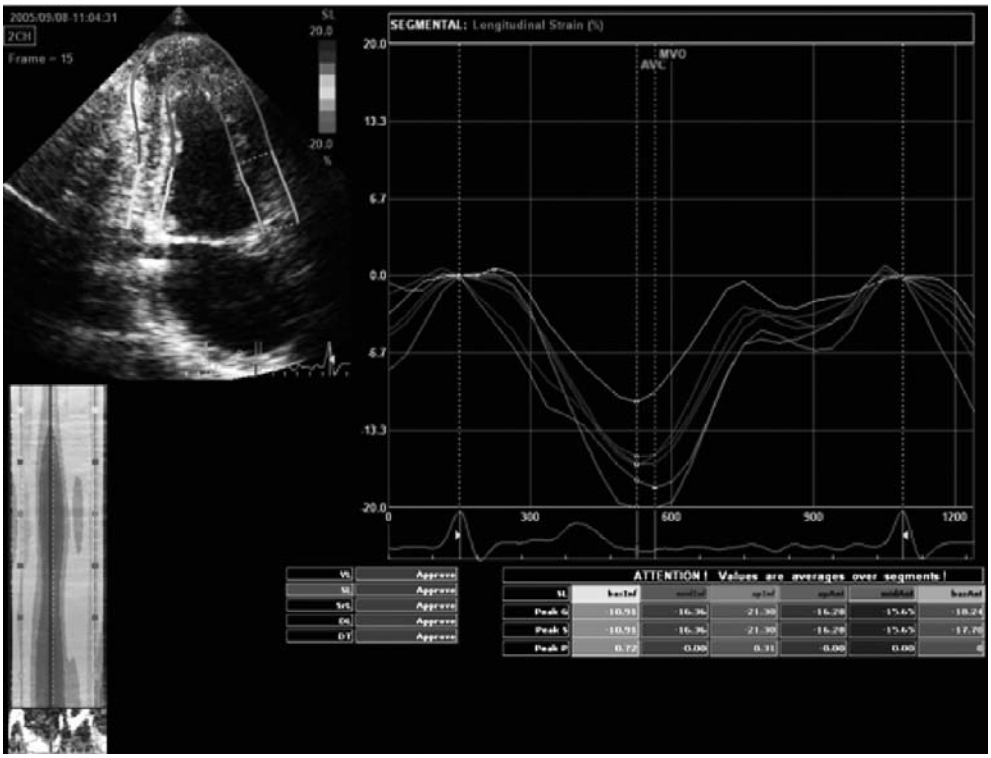

Figure 8 *Strain rate curves and values in the apical two-chamber view, the average longitudinal strain over time is displayed graphically, with each segment represented by the corresponding color. Numerical values are displayed below, with identical corresponding color scheme. Adapted from J. Am. Soc. Echocardiogr. 2007;3: 234-243.*

several directions (longitudinal and radial) and in several segments resulting in global myocardial values. Furthermore, unlike Doppler-based measurements, VVI is not influenced by overall heart motion or the tethering of motion due to contraction of adjacent segments. Disadvantages are that the technique strongly relies on image quality, especially in late gestation when acoustic shadowing increasingly hampers sonographic assessments.

Perk *et al.* were the first to use this new technique for fetal cardiac assessment[108]. Systolic strain and strain rate of several wall segments were not found to correlate significantly with gestational age[109, 110]. Segmental measurements were also not significantly different from global measurements and these may be a useful tool to quantitate fetal cardiac function[111]. Di Salvo *et al.* assessed 100 normal Italian fetuses[112] and normal values for a Chinese population of 132 fetus were formed by Peng *et al.*[109]. VVI is a new, promising and non-invasive tool for objective quantification of myocardial function, with the ad-

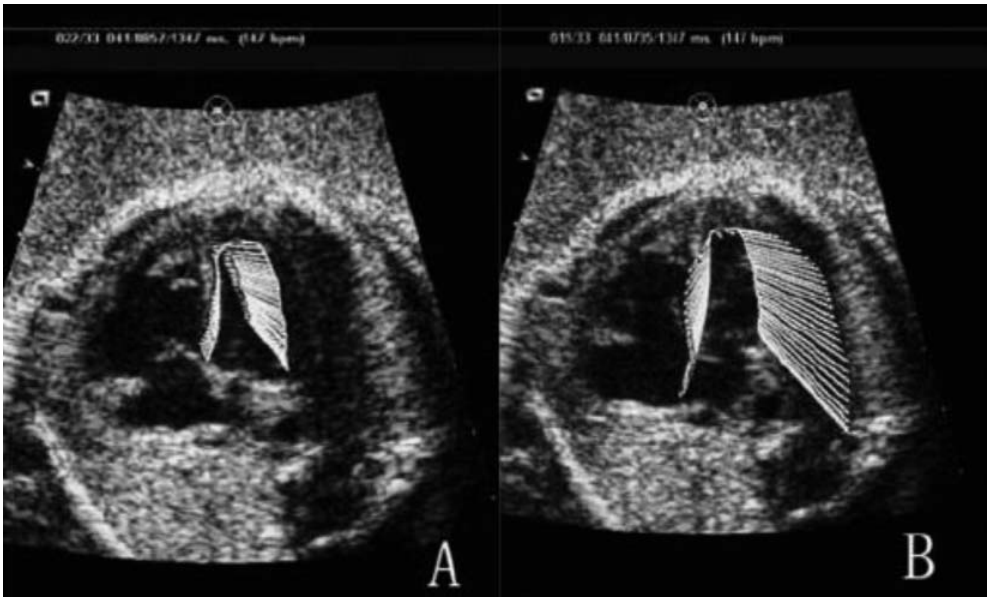

Figure 9 *Vlocity vector profile of the left ventricle in two still frames. The length of the arrow represents the amplitude of the velocity and the direction represents the myocardial motion during (a) systole and (b) diastole. Adapted from Prenat Diagn 2009; 29: 1149–1155.*

vantage of being angle independent and measuring global myocardial values compared to segmental values.

Notably, tissue Doppler-derived velocities are always measured in two dimensions and might therefore not properly reflect the full three-dimensional complexity of the cardiac movement. With the ongoing advances in prenatal three-dimensional ultrasound techniques, a next step in prenatal TDI might be the development of 3D TDI or triplane TDI, which is currently available on some high-end machines in adult echocardiology[113].

CARDIAC VOLUMETRY

Two-dimensional imaging

A relatively simple and reproducible measurement method to assess cardiac volume and size, as index of cardiac overload, is the measurement of the cardiothoracic (CT) ratio by two-dimensional ultrasound. CT-ratio is obtained from a frozen image of a transverse scan of the fetal thorax with the four-chamber view of the heart in diastole. And although the ratio as such is not an extremely sensitive index, it has been used in the evaluation and follow-up of heart disease[114], and in the evaluation of fetoscopic laser

treatment of twin-twin transfusion syndrome[115]. In the evaluation of fetal anaemia, however, the CT-ratio combined with contractility assessment and fetal heart rate, showed insufficient to detect severe anaemia[116]. Although others report a combination of a Doppler measurement of peak flow in the mean cerebral artery and the CT-ratio to predict homozygous α-thalassemia[117]. Quantitative volumetric measurements of fetal ventricles have also been performed using two-dimensional ultrasound[118] and have been validated using animal studies[119]. In these studies ventricle volumes have been obtained by using a multiple disk method (Simpson's rule) and functional measurements showed good agreement with Doppler derived values.

Three-dimensional ultrasound imaging

Advances in ultrasound technology have provided the possibility of assessment of the fetal heart using static three-dimensional ultrasound (3DUS). This technique avoids erroneous assumptions about spherical or elliptical shape of the heart used in two-dimensional measurements. The earliest studies evaluated volumetric parameters with 3DUS on remote computers and electromagnetic position sensors to acquire 3D data volumes[120-122]. Three-dimensional measurements of cardiac volume were reported to have better reproducibility compared to two-dimensional measurements[122]. Other studies assessed ventricular volumes in healthy fetus and in fetus with CHD and reported estimates of ventricular volume changes[121]. However, the initial studies employing 3DUS were hampered by the static nature of 3DUS and included cumbersome manual and time consuming postprocessing to acquire estimates of functional parameters[120].

Four-dimensional ultrasound imaging

As a result of further advances in scanning technologies static 3DUS has developed into dynamic four-dimensional ultrasound (4DUS). Spatiotemporal image correlation (STIC) is a fully integrated automatic volume acquisition technology that realigns two-dimensional images according to their spatial and temporal domain[123]. By analyzing the rhythmic movements within the volume, fetal heart rate is detected and used to create a cineloop of three-dimensional cardiac motion. Since the first paper on STIC was published in 2003 by DeVore *et al.*, numerous researchers have investigated the possibilities of this technique in the assessment of fetal cardiac anatomy[5, 124-129]. Recent studies have shown that this new technique is also feasible in early pregnancy[130-132]. Researchers have been attracted to this new modality for the evaluation of fetal cardiac function because 3D volume measurements might overcome some of the difficulties related to the geometric assumptions of ventricular shape necessary in conventional 2DUS. Because of the temporal nature of the technique, cardiac phase specific events can be tracked and 3D volume measurements in end-systolic and end-diastolic phase can provide data for the calculation of fetal stroke volume *(stroke volume = diastolic volume – systolic volume)*,

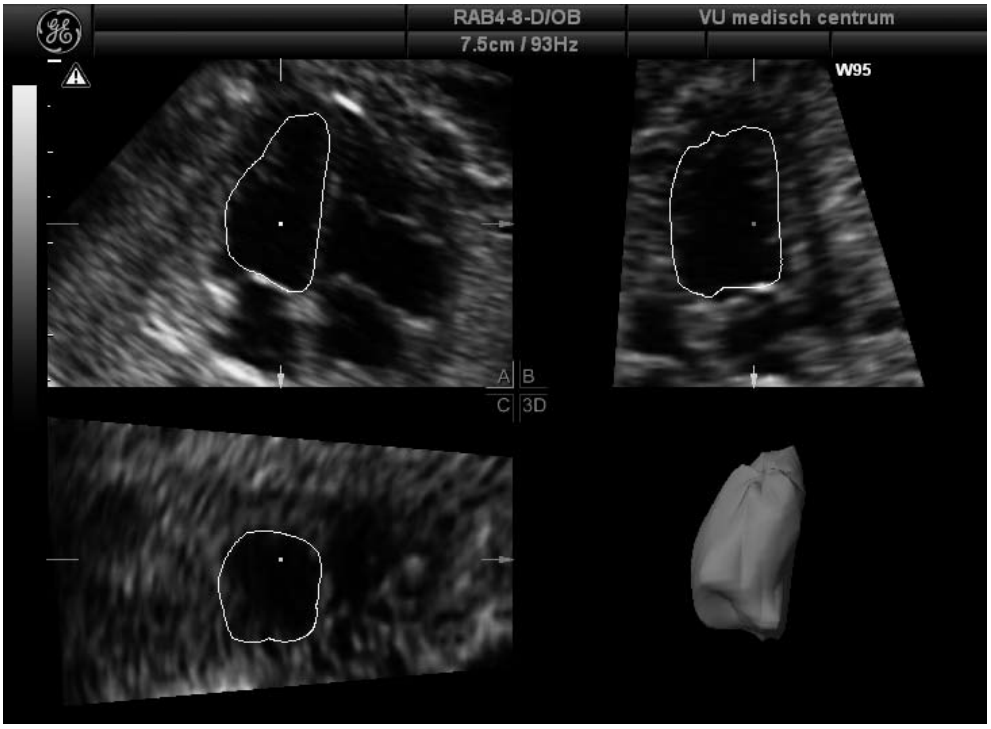

Figure 10 *Fetal left ventricular volume has been measured using Virtual Organ Computer-aided AnaLysis in a spatiotemporal image correlation volume. The irregular volume has been rotated in steps of 15 degrees around the vertical axis and endocardial borders have been manually traced to create a three-dimensional volume (bottom right).*

ejection fraction *(ejection fraction = stroke volume / diastolic volume)* and cardiac output *(cardiac output = stroke volume x heart rate)*.

In adult echocardiography, 3D volume measurements have been proven to be more accurate than conventional 2DUS, compared to magnetic resonance imaging as gold standard[133]. Also in prenatal ultrasound has 3DUS proven to have a better validity and reliability than 2DUS[134-136]. Additionally, validation studies in the small volume ranges comparable with mid- and late gestation fetal hearts have shown that STIC is a feasible method for fetal cardiac volumetry[137, 138]. Over the last few years several methods have been described to obtain volume measurements from 3D volumes[137, 139-144]. The most commonly used method is VOCAL (Virtual Organ Computer-aided AnaLysis), which is an automated rotational technique (Figure 10)[139, 140, 143-145]. Other methods are inversion mode[142], 3D slice method[41] and manual segmentation[141].

Over the last years these methods have been used to establish normal values of fetal cardiac ventricle volumes and estimations of fetal cardiac stroke volume and cardiac output[41, 142, 143]. These studies reported combined left and right fetal cardiac output to increase from around 5 mL/min at 12 weeks to around 600 mL/min at 34 weeks of gestation[41, 143]. The values of cardiac output measured with 4DUS, are however smaller than those reported in earlier studies using VTI and valve or vessel areas as described above[146]. Whether this is the cause of volume underestimation by 4DUS based methods or an overestimation by PW-Doppler calculated volumes is unclear. There is, however, also a study that reports good agreement between the different modalities[147]. Nonetheless, the extent to which measurements made using 4DUS and STIC, in fetuses in pathological states deviating from normal, and whether these measurements are sensitive enough to aid in the prediction of fetal outcome, has yet to be determined.

Fetal magnetic resonance (MR) imaging

The high resolution and excellent soft tissue contrast are attractive characteristics of MR imaging. Currently fast MR imaging protocols have been used in the evaluation of cardiac malformations[148]. However, the use of MR imaging in the evaluation of the fetal cardiac function is hampered by limitations well known in 3D fetal echocardiography: the fast movement of the fetal heart and fetal body movements. As in 3D echocardiography, electric triggering of image acquisition would be ideal. In catheterized fetal sheep, pulse wave triggered cardiac MR imaging of the fetal heart indeed allowed evaluation of anatomical structures and allowed assessment of functional information[149]. Additionally, blood oxygen level–dependent (BOLD) MR imaging can be used to depict changes in cardiac and placental oxygenation in fetal sheep induced by maternal hypoxia[150, 151]. Recently, a new non-invasive technique has been reported that reorganizes and reconstructs MR data retrospectively by recognition of fetal cardiac and breathing cycles[152]. Using this technique cardiac MR images of fetal chicken hearts were produced free of motion artefacts. Thus, with the ongoing development of scanning protocols, MRI gating technology and post process software, non-invasive evaluation of the human fetal cardiac function will presumably be feasible within the next decade.

MYOCARDIAL ELECTRICITY

Fetal electrocardiogram (fECG)

For nearly a century, it has been known that the fetal electrocardiogram (fECG) can be detected using electrodes placed on the maternal abdomen[153]. The use of fECG, however, has always been limited owing to problems of poor signal-to-noise ratio. As an alternative, one of the most commonly used modalities for fetal surveillance is cardiotoco-

graphy (CTG). CTG uses Doppler ultrasound based on movement of the intracardiac structures to demonstrate a fetal heart rate. CTG recordings do not show a true beat-to-beat heart rate but an average over three neighbouring beats, analysed to give baseline rate, baseline variability and periodic changes. Thus, the ultrasound derived CTG signals are therefore only an approximation of those derived from a fetal electrocardiogram. Non-reassuring features on a CTG trace are unusually rapid or slow rates, a flat pattern (reduced variability), and certain types of heart rate decelerations (especially 'late' or 'severe variable' decelerations).

Heart rate variability as recorded by fECG and CTG also is fairly nonspecific and its value is heavily dependent on subjective interpretation by clinicians. Therefore, the process of decision making during labour based on the sometimes subtle differences between normal and abnormal CTG patterns to identify fetal hypoxia remains a challenge[154]. In addition to the widely used CTG recordings an invasive technique has been developed, aiming to increase the specificity in identifying hypoxia and to reduce unnecessary interventions by analysis of the ST-segment in a fECG recording. A recent large multi centre randomized trial performed in the Netherlands shows that the addition of this ST-analysis, however, did not significantly reduce the number of newborns with metabolic acidosis[155].

Over the last decade several studies have demonstrated renewed interest the feasibility of non-invasive fECG recordings[10, 156-158]. Complete fECG waveform recordings combined with uterine contraction patterns can be obtained antepartum and intrapartum[158]. Examinations are, however, still limited by the use of extensive electrodes applied to the maternal abdominal skin[158]. These electrodes are associated with adverse effects, consisting mainly of transient skin irritation[10], in 20% of cases. Furthermore, the success rate of obtaining good fetal heart rate detection is around 80%, which is still suboptimal[10, 158]. Long-term transabdominal fECG monitoring, however, has been reported to be feasible from 20 weeks of gestation onwards[10]. Technical advances must ideally lead to the development of a system that measures to an accuracy of 1 millisecond, which would allow true beat-to-beat measurement and a more accurate assessment of fetal cardiovascular physiology[10]. Results of future studies could possibly provide insight in true fetal myocardial electricity and heart rate patterns.

COMMENT

A wide variety of non-invasive methods are currently available for the evaluation of different aspects of the fetal cardiac function. However, finding a completely load-inde-

pendent non-invasive tool or index, reflecting the true cardiac function, remains a challenge. It is especially complicated as this 'gold standard' has to be able to differentiate between normal fetuses and those at risk for intrauterine compromise or even demise. In the case of growth restriction, for example, some parameters of the cardiac function have been reported to remain within relatively normal ranges (i.e. cardiac output) throughout different stages of growth restriction, while other parameters (i.e. MPI and peripheral Doppler indices) show signs of cardiac dysfunction dependent of the severity in the pathophysiologic process[24]. Also, the ejection phase parameters stroke volume, ejection fraction and cardiac output are heavily load dependent. Therefore, these measurements might be of limited value in characterizing the contractile state of the heart in both normal and growth restricted fetus[159]. Furthermore, these parameters might not be able to distinguish between abnormalities in the contractile state from compensatory or dangerous alterations in loading conditions in the compromised fetus[24, 38]. While some of the methods discussed have proven their use over the years (i.e. venous and arterial Doppler indices and cardiotocography), others are still hampered by lack of standardization and considerable interobserver variability (i.e. MPI and tissue doppler imaging). Several new modalities, however, provide high potential subjects for further research projects. Three-dimensional vector velocity imaging and 3D tissue doppler might provide new insights in fetal myocardial movement. The development of 3D matrix ultrasound transducers able to acquire four-dimensional cardiac volumes from multiple angles might overcome some of the limitations encountered in sonographic cardiac volumetry. The development of fast MR imaging protocols and image correlation software would provide researchers with a new modality to study fetal hemodynamics with the high resolution of MR imaging and the possibilities of imaging tissue oxygenation.

Combining of a number of measurements in a cardiovascular profile score (CVPS) has been proposed as a way to overcome the lack of a 'gold standard'[160]. This CVPS has been found to correlate with fetal outcome in cases of congenital heart disease[161], fetal hydrops[162], twin to twin transfusion syndrome[163], in cases fetal therapy for congestive heart failure[164] and also in cases of growth restriction[165]. The CVPS now consists of echocardiographic markers of cardiac dysfunction: cardiomegaly, valve insufficiency, hydrops, and abnormal venous and arterial Doppler flow profiles. Once some of the new modalities have been thoroughly validated, they might be added to the CVPS for the prediction of fetal outcome. But first they have to find their way into clinical practice. Most of the discussed methods require specific ultrasound machines or software for acquisition of data or for postprocessing. And what is perhaps most important, operators need dedication, extensive training and perseverance to master the different techniques. Even the simplest techniques, e.g. recording a peak velocity in the aorta using Doppler, can most likely be improved by training and standardization, not to mention the more complicated

measurements that include complicated postprocessing or calculations. Nevertheless, now that researchers have all these available modalities at their disposal and newer technologies still being developed, the aspiration of clinicians to gain full insight into the functioning of the fetal heart may be not so far away.

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Chapter 9

General discussion

CONCLUSIONS

This thesis provides an exploration of a new technique in fetal echocardiography, three-dimensional (3D) ultrasound imaging. Several conclusions can be drawn from the results. Automated 3D echocardiography as a tool for prenatal screening purposes is still insufficient. However, despite differences in individual learning curves, sonographers do not need extensive training in 3D ultrasound in order to incorporate the acquisition of 3D volumes satisfactorily into their routine practices. Four-dimensional (4D) ultrasound combined with spatiotemporal image correlation (STIC) can also be easily introduced into routine third-level ultrasound practice. In the assessment of fetal cardiac function, STIC combined with a method using summation of manually traced parallel slices (3D slice method) can be used to calculate fetal cardiac chamber volumes. The validity of these volumetric measurements is good in-vitro, in the volume range comparable to fetal cardiac dimensions. The volumetric data can be used to provide parameters like stroke volume in the assessment of the fetal cardiac function. Both interobserver and intraobserver reliability of these measurements are good. Further, this thesis provides reference ranges for both fetal ventricular volumes and functional parameters for the clinical assessment of fetal cardiac function. The findings indicate an overestimation of conventional Doppler methods in the assessment of fetal cardiac output. Finally, reviewing many of the existing non-invasive methods used prenatally, it can be concluded that, currently, no gold standard exists for the prenatal assessment of the cardiac function. Based on the studies presented, this thesis could be the start of a new field of research to further explore the possibilities of 3D echocardiography in the evaluation of the cardiac function in the human fetus.

GENERAL DISCUSSION

Three-dimensional imaging as a screening tool

Several developments of 3D imaging techniques facilitate the application in the clinical setting. Examples of this are automated retrieval of anatomically relevant imaging planes and the ability of different motion display modalities. We evaluated the clinical feasibility of automated cardiac screening from static 3D volumes. Static volumes can be acquired very quickly, which is a great advantage in fetal sonography, but obviously do not offer the extensive post-processing possibilities of dynamic spatiotemporal image correlation (STIC) volumes. Initial studies on the automated retrieval of relevant cardiac planes reported excellent performance of this newly developed software tool but were strongly influenced by selection bias[1, 2]. In this thesis we showed that, when applied in a routine clinical setting to avoid selection bias, current software still lacks the consistency to be implemented into every-day practice. On the other hand, our results showed that sonographers, albeit experienced in fetal echocardiography, did not have to be extensively trained in the use of 3D imaging techniques to acquire volumes of high quality, which makes the technique relatively easy to implement into routine clinical programs. The quality of the acquired volumes proved of crucial importance and a prerequisite for adequate post-processing. Further training and familiarization with the techniques is therefore expected to further improve the quality of the acquired volumes and subsequently the results of postprocessing software. Regardless, the initiative towards improvement of quality of fetal assessment and the standardizing of sonographic examination by the automated retrieval of planes has potential and offers interesting possibilities for future studies.

Spatiotemporal image correlation in clinical practice

The proposed potential benefits of 4D ultrasound imaging techniques such as STIC are numerous. Benefits include that STIC allows review of an unlimited number of images and allows correlation of images that are perpendicular to the acquisition plane. The digital STIC volumes have great storage capability, and the three-dimensional nature of the data provides new ways to acquire volumetric measurements. Further, it also allows cardiac assessment at a remote site. However, the important issue of feasibility in clinical practice of this sophisticated and complex technique remains. Several investigators previously evaluated the clinical feasibility of STIC in specialized clinical settings and reported high success rates[3, 4]. The studies presented in this thesis corroborate these findings and confirm that the application of routine STIC volume acquisition is relatively easy and feasible. Although differences in individual learning curves exist, sonographers do not necessary have to be experienced in the use of 3D ultrasound imaging to acquire STIC volumes of high image quality. Comparable to findings in static 3D ultrasound, the

results demonstrated that image quality is of crucial importance for the postprocessing of STIC volumes.

This thesis did not focus on the diagnostic properties of 4D ultrasound imaging. But with regard to diagnostic accuracy, other studies have shown promising results[5, 6]. We learned from our study that training increases success rate of acquisition and reduces the amount of time needed for review. To exclude congenital heart disease, however, it is our experience that in a routine setting for screening purposes an experienced sonographer needs lesser time to perform an extended basic cardiac screening than to acquire and review a STIC volume. In case of an cardiac anomaly the difference between the modalities might be even larger. Despite the promising results with regards to cardiac screening using STIC in several reports[7-9], STIC still can not, in our opinion, replace conventional 2D cardiac examinations. Another disadvantage of STIC concerns the postprocess review of STIC volumes which, even with extensive experience, remains a time-consuming undertaking. Another important issue is the angle of insonation. Because a STIC volume is acquired from one optimal angle selected by the sonographer, ultrasound beams within the data volume will inevitably be parallel to some anatomical structures in the heart. Sonographic reflections of structures parallel to the ultrasound beam are less clearly displayed than structures perpendicular to the ultrasound beam. The possibility to review an unlimited number of planes within the STIC volume does not change the initial acquisition quality. Future advances in scanning equipment, as 4D matrix ultrasound probes, might simplify volume acquisition techniques and might provide possibilities to acquire data from multiple angles at the same time. Regardless of the issues mentioned above and awaiting further technological advances, the diagnostic accuracy achieved by the review of STIC volumes alone, without a 2D cardiac examination, is still a promising field for further research. When there are no experienced hands available, however, digital STIC volumes might already be used perfectly for telemedicine and for consulting a remote expert10. The use of telemedicine with 4D ultrasound imaging with STIC is therefore another field that awaits further exploration.

Estimating fetal cardiac function parameters

Non-invasive measurement of fetal cardiac function can help clinicians to fully understand fetal hemodynamics and fetal adaptations to changes in utero. It can also help in the follow up of cases with congestive heart disease or other functional cardiac diseases. To date no gold standard exists for non-invasive measurement of fetal cardiac function. This thesis provides an overview of several sonographic and other non-invasive methods to assess fetal cardiac function parameters, most of which are ultrasound based. A number of studies in this thesis focused on cardiac volumetric measurements. It has long been known that end-systolic volumes and end-diastolic volumes can be used to esti-

mate stroke volume and output. Before the availability of 3D ultrasound these volumetric measurements had to be obtained by measurements of successive 2D planes[11]. The necessary assumptions about the cylindrical shape of fetal ventricles limit this method. Other methods used to estimate cardiac output involve Doppler measurements of blood flow and estimations of valve area which are also known to have important limitations[12-14].

We established reverence ranges for cardiac volumes during pregnancy. These volumes provided estimates of fetal cardiac function and were plotted against both gestational age and estimated fetal weight. Values found in other studies employing 3D ultrasound are in line with our results despite the use of different measurement techniques[15, 16]. Interestingly, values of fetal cardiac stroke volume obtained using four-dimensional ultrasound and STIC were remarkably smaller compared to studies employing the conventional methods applying Doppler and vessel orifice measurement[17, 18]. Earlier validation studies in animals have also shown Doppler measurements to overestimate blood flow[19]. Validation studies employing 3D ultrasound for volumetric measurements of other fetal organs have been found to be accurate and reliable[20-22]. We confirmed the validity and reliability for volumetric measurements using 3D ultrasound and STIC in the small volume range comparable to fetal cardiac sizes in our validation studies. It might therefore be argued that 3D ultrasound based techniques give a more realistic estimate of fetal cardiac function parameters. An accurate technique *in vitro* is, however, not necessarily the best method *in vivo*. Therefore, ideally, additional animal studies should be performed to further validate the use of 3D ultrasound based methods *in vivo*. If the method would proof to have a certain amount of measurement variation this could make the method less suited for individual comparison. Nevertheless the method proves to be useful for the detection of changes in functional parameters over time in the follow-up process of compromised fetus. These are all interesting subjects for future research projects.

Both studies, the study by Molina *et al.*[23] and the study presented in chapter 7 in this thesis[24], show considerable variation in cardiac output. The variation in these estimates of cardiac output could certainly be caused by measurement error. The data in chapter 7 and a recent study by Hamill *et al.*, however, show good agreement in measurements of ventricular volumes[25, 26]. A large portion of the variation found could also be due to physiological variation in the studied population. It can also have its origin in a whole variety of causes including diurnal rhythm, active/passive state and pure individual variation. The relative size of the variation in stroke volumes or the subsequent estimate of cardiac output will remain unclear until it is possible to measure the values without significant measurement error. But what might be even more important, it needs to be

determined which parameter reflects fetal cardiac function best. Moreover, one has to know to what extent this parameter is able to differentiate between normal and compromised fetuses. This thesis focuses on a new method of estimating fetal stroke volume and cardiac output. These are well known parameters of cardiac function in both adults and children. Recent studies however, indicate that in case of intra uterine growth restriction, compensatory mechanisms probably keep cardiac functional parameters within normal ranges until fetal compromise has progressed to a very severe state[27]. The effect is known as 'heart-sparing effect'[28] and has been described for the fetal brain as 'brain sparing effect'[29]. If this indeed proves to be an adaptational mechanism in the compromised fetus, output estimates might not be of any significant clinical value in the follow up of growth restricted fetuses. Thus in order to aid obstetricians in the sometimes difficult decisions regarding the timing of delivery in cases of fetal compromise, any method should ideally be relatively easy to perform, valid and reliable, and the measured parameter should be able to identify the pre-pathologic state in which delivery can be initiated before fetal hypoxia occurs. In that light another limitation of STIC has to be mentioned. The results of the studies presented in this thesis show that the acquisition of STIC volumes is feasible in the window of 20 to 22 weeks of gestation. The results of chapter 7 also show that acquisition becomes increasingly more difficult as pregnancy progresses. This presents another important limitation of the technique as the decisions about the timing of a delivery are often made in the window between 24 to 32 weeks. One of the major causes of the failure to acquire a good STIC volume is the increasing acoustic shadowing of the fetal ribs. Until these issues have been overcome the quest for the ideal non-invasive technique for the assessment of the fetal cardiac function continues.

Future prospects

If something can be said about technological advances and new technologies it is that they cannot be undone. This definitely holds true for 3D ultrasound imaging. It is a valuable new development in ultrasonography and offers a variety of new opportunities to sonographers. An important objection that may be targeted against fetal 3D ultrasound imaging concerns the fact that the principal acquisition technique for 3D imaging is still based on 2D ultrasound technology. This inevitably includes that the new technology adopts all the limitations inextricably bound to fetal 2D ultrasound discussed above. This, however, may change in the nearby future with the developments in transducers and signal processing. Ultra-high resolution matrix ultrasound transducers might acquire fetal data simultaneously from multiple angles to optimise image quality. This might overcome some of the limitations caused by acoustic shadowing of fetal ribs. Future sonographers might also be able to use machines with sophisticated automated software programs that will integrate all available data and thus overcome other limi-

tations of 2D ultrasound. One of the main issues that hampered the widespread use of 3D ultrasound is the necessity of expensive ultrasound scanners and software packages which restrict availability of the most recent techniques to only highly specialized centers. Also the cumbersome postprocessing may discourage new users of the technology to further explore its possibilities. The centers that have the ability to use these new techniques should continue to do so because training, standardization and familiarization have proven to be a prerequisite for a widespread integration into clinical practice.

This thesis provides a first exploration of the possibilities of this new technique in echocardiography. It outlines a number of interesting areas ready to be further explored and could therefore be the start of a whole new research approach on the diagnosis and screening of fetal cardiac disease, which may offer researchers a new modality for the exploration of functional parameters.

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Chapter 10

Summary

Chapter 1 During the last decade prenatal sonographic assessment has become an integral part of obstetric care. Fast technological advances over the last years have made it possible to assess the human fetus in three dimensions. The clinical use of cardiac three-dimensional (3D) ultrasound, however, is still unclear.

The detection rates of congenital heart disease in conventional ultrasound screening programs are suboptimal. The examination of the fetal heart is a technically difficult part of a fetal ultrasound scan. 3D ultrasound might offer a solution to this problem. The acquisition of digital 3D volumes might make the screening less dependent on the skills of the sonographer. Moreover, with a digital data volume available, automated cardiac screening becomes a possibility.

Additionally, 3D ultrasound offers a new way to obtain measurements of the fetal heart. There are different conventional methods to evaluate the fetal cardiac function. These methods, however, are known to be limited in accuracy and reliability. An accurate and reliable method to assess the fetal cardiac function could be very useful in the management of a number of fetal conditions. Using 3D ultrasound and spatiotemporal image correlation (STIC) it is possible to measure the volumes of fetal heart chambers on different moments during the cardiac cycle. These measurements could provide estimates of parameters of the fetal cardiac function.

In **Chapter 2** we evaluate the feasibility of an automated 3D software tool for the extended basic cardiac screening in routine ultrasound practice (SonoVCAD). In this study we have shown that 3D ultrasound can be introduced into routine ultrasound practice without much difficulties. All four sonographers acquired images of sufficient to good quality in more than two-thirds of the cases. Automated retrieval of all cardiac planes necessary for an extended basic cardiac examination was successful in less than half of the cases. Currently, SonoVCAD still lacks the consistency to be clinically feasible for cardiac screening purposes.

In **Chapter 3** the feasibility of four-dimensional (4D) ultrasound using STIC in routine fetal echocardiography was systematically analyzed. STIC creates a virtual 3D cardiac cycle displayed in a cineloop. In three-fourths of the examined women successful STIC volumes could be acquired. Around two-thirds of the acquired volumes was of sufficient to high quality. Our results showed that sonographers do not have to be specifically experienced in 3D/4D ultrasound imaging to acquire high-quality STIC volumes. 4D ultrasound using STIC is feasible in clinical practice. Our result further stress the importance of training and experience of the sonographers in the successful application of 4D ultrasound.

The results in **Chapter 4** show that it is possible to perform valid volumetric measurements using 4D ultrasound and STIC. Using a customized miniature balloon model, volumes comparable to fetal cardiac chambers were measured. Three different methods to obtain volumetric measurements were compared. 3D volumetric measurements were found to be accurate from 0.5 mL onward although all methods underestimated the actual volumes to a certain extent. A method of manual tracing and summation of multiple slices (3D slice method) proved most useful when applied to in-vivo investigations.

The reliability of volumetric measurements obtained from 4D volumes acquired using STIC was evaluated in **Chapter 5**. We collected volume datasets from two sources: fetuses over a range of gestational ages and also from a miniature balloon model. Volume calculations were obtained by the 3D slice method. The datasets were analysed by three observers repeatedly. Measurement errors of balloon volumes were small and reliability was good. In the fetus, measurement errors shown to decrease with operator experience and reliability were better for stroke volume than for ejection fraction.

In **Chapter 6** image reconstruction artefacts during the use of 4D ultrasound and STIC are presented. These artefacts were encountered during the validation study presented in chapter 4. The distorted shape, the thickened wall and the blurred speckles observed in the spatiotemporal rearranged volumes, in our opinion, were the result of gating artefacts. These observations can be important for the understanding of the methodology of STIC and thus for the analysis of STIC volumes in daily practice.

Reference values for left and right ventricle volumes, and for indices of fetal cardiac function are presented in **Chapter 7**. In this prospective longitudinal study, STIC volumes were acquired periodically from 12 weeks of gestation onwards. This study establishes reference values from 12 to 30 weeks of gestation. STIC seems to overcome many of the pitfalls of conventional ultrasound methods and has the potential to become the method of choice for cardiac volumetric assessments.

Different non-invasive techniques have been evaluated over the years, almost exclusively by echocardiography. The fast development of new imaging modalities has added an enormous amount of knowledge to this field of research. New non-invasive techniques, some adopted from adult cardiology have been studied in the fetus. **Chapter 8** presents an overview of the current state of the field of non-invasive assessment of fetal cardiac function.

Chapter 9 provides a general discussion of the results of this thesis and suggests future research strategies to further explore the many possibilities of 3D ultrasound of the fetal heart.

Appendices

1 Summary in Dutch
Samenvatting

Hoofdstuk 1 vormt een algemene introductie en zet het doel en de opzet van deze dissertatie uiteen.

In het afgelopen decennium is prenataal echoscopisch onderzoek een standaard onderdeel geworden van de verloskundige zorg. De snelle technologische ontwikkelingen van de laatste jaren hebben het mogelijk gemaakt de foetus in drie dimensies te bestuderen. Onduidelijk is echter wat de waarde van deze driedimensionale (3D) echoscopie is voor de obstetrische praktijk.

Bij conventionele echoscopische screening naar cardiale afwijkingen is het detectiepercentage nog suboptimaal. Een van de oorzaken is de technische moeilijkheidsgraad van het onderzoek van het foetale hart voor echoscopisten. Hiervoor biedt 3D echoscopie mogelijk een uitkomst. De digitale 3D volumes van het hart kunnen na afloop van het onderzoek op alle mogelijke manieren worden weergegeven. Op deze manier wordt het echoscopisch onderzoek mogelijk minder afhankelijk van de vaardigheden van echoscopisten. Bovendien gaat hierdoor digitale geautomatiseerde echoscopische screening tot de mogelijkheden behoren.

Daarnaast biedt 3D echoscopie mogelijkheden om op een nieuwe manier metingen te verrichten aan het foetale hart. Er bestaan verschillende conventionele methodes om het functioneren van foetale hart te meten. Van deze methodes is echter bekend dat zij onnauwkeurig zijn en gevoelig zijn voor meetfouten. Een nauwkeurige en precieze 3D methode zou kunnen bijdragen aan het nemen van klinische beslissingen bij een aantal foetale aandoeningen. Met 3D echoscopie kan de inhoud van foetale hartkamers gemeten worden in verschillende fasen van de hartcyclus. Zo kunnen parameters berekend worden van de foetale hartfunctie.

In de eerste hoofdstukken onderzoeken we de klinische toepasbaarheid en de haalbaarheid van 3D echoscopie in de praktijk. In Hoofdstuk 2 evalueren we de haalbaarheid van geautomatiseerde foetale cardiale screening met behulp van een nieuwe toepassing van 3D echoscopie, SonoVCAD. We ontdekten dat 3D echoscopie zonder veel moeite geïntroduceerd kan worden in de huidige echopraktijk. Alle echoscopisten verzamelde kwalitatief voldoende tot goede 3D beelden in meer dan tweederde van de gevallen. Het geautomatiseerd afbeelden van alle structuren nodig voor basale cardiale screening was echter maar mogelijk in minder dan de helft van de gevallen. Vooralsnog is de klinische toepassing van geautomatiseerde cardiale screening met SonoVCAD daarom niet haalbaar.

In Hoofdstuk 3 wordt de klinische toepasbaarheid van vierdimensionale (4D) echoscopie met spatiotemporal image correlation (STIC) geëvalueerd. STIC creëert een dynamische 3D weergave van een foetale cardiale cyclus. In drievierde van de populatie waren de opnames succesvol. Ongeveer tweederde van deze opnames waren van voldoende tot goede kwaliteit om relevante basale anatomische structuren te beoordelen. Wij toonden aan dat echoscopisten niet noodzakelijkerwijs veel ervaring nodig hebben om kwalitatief goede 3D beelden te verzamelen. Toepassing van STIC in de klinische praktijk is dus goed haalbaar. Wel wordt duidelijk aangetoond dat training en ervaring van echoscopisten de resultaten verbeterd.

In de volgende hoofdstukken focussen we ons op het gebruik van STIC om de foetale hartfunctie te beoordelen. De bevindingen in Hoofdstuk 4 laten zien dat het mogelijk is om nauwkeurige volume metingen te gebruik makend van 4D echoscopie en STIC. Met behulp van een in vitro model werden miniatuur ballonnen gemeten met een grootte vergelijkbaar met foetale hartkamers. Ook werden drie verschillende methoden vergeleken. Alle methoden onderschatte de inhoud van de verrichten volumes in zekere mate. De beste resultaten werden verkregen met een methode waarbij 3D volumes in parallelle doorsneden (3D Slice) worden geanalyseerd. Deze methode heeft de meeste potentie om klinisch te worden toegepast voor het verrichten van metingen in het foetale hart.

Precisie en nauwkeurigheid zijn beiden van groot belang voor een methode. Daarom wordt de variatie in metingen van verschillende onderzoekers en de variatie binnen metingen van dezelfde onderzoeker geëvalueerd in Hoofdstuk 5. Met 4D echoscopie en STIC werden foetale hartvolumes gemeten evenals volumes uit een in vitro model. Wij toonden aan dat, gebruikmakend van 3D Slice methode precieze volume metingen kunnen worden verricht. Tevens vonden wij dat de vorm en de grootte van het volume en de ervaring van de onderzoeker van invloed zijn op de precisie van volumemetingen.

In Hoofdstuk 6 wordt een verschijnsel beschreven waarbij faseringsfouten in het algoritme van STIC kunnen leiden tot vertekening van de gecreëerde echobeelden.

De resultaten van een prospectief longitudinaal onderzoek worden gepresenteerd in Hoofdstuk 7. We hebben referentiewaardes gecreëerd voor foetale linker en rechter ventrikel volumes en indices voor de foetale hartfunctie van 12 tot 30 weken zwangerschap. 4D echoscopie met STIC is een goed alternatief voor conventionele echoscopische methoden om de foetale hartfunctie te bepalen.

In Hoofdstuk 8 wordt een uiteenzetting gegeven van verschillende non-invasieve methodes die tegenwoordig beschikbaar zijn om de foetale hartfunctie te evalueren. Van relatief bekende en langer bestaande methodes, maar ook van nieuwere modaliteiten worden de technieken, toepassingen en de tekortkomingen besproken.

Hoofdstuk 9 vormt een algemene beschouwing van de bevindingen in dit proefschrift. Ook wordt er in dit hoofdstuk gefilosofeerd over de toekomst van de foetale echocardiografie en de implementatie van de nieuwe technieken. Afsluitend worden suggesties gedaan voor verder onderzoek binnen dit nieuwe onderzoeksgebied, foetale 3D echocardiografie.

2 Words of thanks in Dutch
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Lotte, lief klein schatje van me, veel meer dan van jou kan ik niet van iemand houden denk ik. We gaan nu nog meer van jouw genieten!

3 Curriculum Vitae

Lukas Bastiaan Uittenbogaard werd op 4 september 1977 te Hilversum geboren. Hij studeerde geneeskunde aan de Vrije Universiteit te Amsterdam. Tijdens zijn studie was hij betrokken bij de onderzoekergroep 'de Vasculisten' verbonden aan de afdeling vasculaire geneeskunde van het Academisch Medisch Centrum. Ook was hij betrokken bij een onderzoeksproject naar angiogenese aan de University of Otago, Wellington, Nieuw Zeeland.

Na het behalen van zijn doctoraal startte hij in 2004 als arts-assistent bij de afdeling Gynaecologie & Verloskunde in het Spaarne Ziekenhuis, Hoofddorp.

Van april 2005 tot maart 2009 was hij als arts-prenatale diagnostiek en promovendus werkzaam op de afdeling Prenatale diagnostiek en Screening van het VU medisch centrum te Amsterdam onder leiding van prof. dr. J.M.G. van Vugt. In deze periode heeft hij de onderzoeken verricht die geresulteerd hebben in deze dissertatie.

In april 2009 is hij gestart met zijn opleiding tot gynaecoloog in het Kennemer Gasthuis te Haarlem (opleider dr. J.P. Lips). Sinds oktober 2010 vervolgt hij zijn opleiding aan het VU medisch centrum (opleider prof. dr. H.A.M. Brölmann).

Lukas woont samen met Margrita Slagter en heeft met haar sinds april 2010 een dochter Lotte.