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

L. B. UITTENBOGAARD*, M. C. HAAK*, R. J. H. PETERS†, G. M. VAN COUWELAAR* and J. M. G. VAN VUGT*

*Departments of *Obstetrics and Gynecology and †Physics and Medical Technology, VU University Medical Center, Amsterdam, The Netherlands*

KEYWORDS: 3D ultrasound; 4D ultrasound; fetal echocardiography; inversion mode; spatiotemporal image correlation; STIC; VOCAL; volume measurement

ABSTRACT

Objectives *To assess 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 coefficients 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 the 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 had the smallest LOA. This method may prove useful when applied to* in-vivo *investigations. Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.*

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 the literature[4]. Ejection fraction and stroke volume are both indices of systolic cardiac function[2,5–7]. Both indices can be calculated from systolic and end-diastolic ventricular volumes. Prenatal quantitative measurements of ventricular volumes using 2D ultrasound imaging 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 three-dimensional (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,

Correspondence to: Dr L. B. Uittenbogaard, VU University Medical Center, Postbus 7057, 1007 MB, Amsterdam, The Netherlands (e-mail: lb.uittenbogaard@vumc.nl)

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

		Combined stroke volume (mL)		
Reference	*Method of measurement*	*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 and 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.

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 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 STIC in volumes smaller than 5 mL. This is remarkable because all recently published studies show fetal ventricular 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 the 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 assess 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.

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.

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 cardiac ventricular 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 a 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 center of the wheel, the operators were able to adjust accurately the 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 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 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 ventricular 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 per 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 weighed repeatedly 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, Zipf, Austria) with a motorized curved-array 6–12-MHz transvaginal 3D/4D transducer. To avoid bias owing to image enhancement software, Speckle Reduction Imaging and Cross XBeam were not used. The impact of postprocess gain or contrast changes on the 3D volume measurements was evaluated by measuring 30 volumes using different settings in the gray-scale curve.

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.

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

Three-dimensional slice method

The 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 an 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 for obtaining 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,

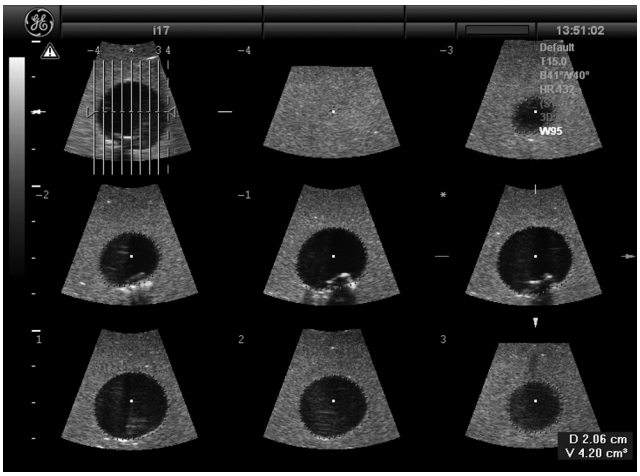

Figure 2 Three-dimensional slice method; nine parallel slices (only eight shown here) at equal distances were traced manually to measure the balloon volume.

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 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 that 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 artifacts 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 measure only the true echolucent volume of the fluid-filled balloon.

To avoid measuring the end of the catheter inside the balloon as 'liquid' volume, the catheter tip volume was calculated and subtracted from the measurements done with the 3D slice and VOCAL methods. 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 percentage 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 error of < 5%. Stroke volumes and injection fractions were calculated from the observed volumes. Stroke volume was defined as end-diastolic volume minus end-systolic volume, and the injection fraction was defined as stroke volume divided by end-systolic volume. Intraobserver reliability was assessed by calculating the intraclass correlation coefficient (ICC)[25,26] and coefficient of variation (CV), which was defined as the SD of 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 $P < 0.05$.

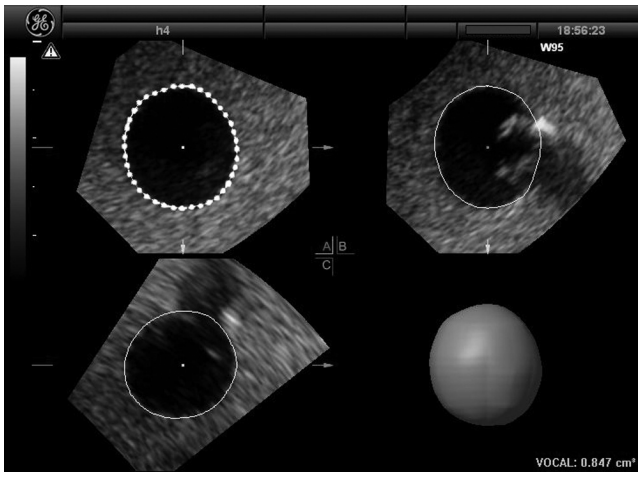

Figure 3 The Virtual Organ Computer-aided AnaLysis (VOCAL) method rotates the selected image around a fixed vertical axis. Semiautomated tracing was used to measure the volume.

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 owing 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 methods, 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 to −0.01; $n = 10$) and manual injection of 1.5 mL showed a mean difference of −0.02 mL (95% LOA, −0.03 to −0.02; $n = 10$). Further, measurement outcome was not significantly influenced by postprocess gray-scale curve variation as assessed by the 95% LOA (mean difference (darker subtracted from original), 0.03 mL (95% LOA, −0.02 to 0.08; $P = 0.12$); mean difference (lighter subtracted from original), 0.04 mL (95% LOA, −0.01 to 0.08; $P = 0.08$)).

Three-dimensional slice method

The average time taken to perform a measurement using the 3D slice method was 118 (range, 95–134) s ($n = 10$).

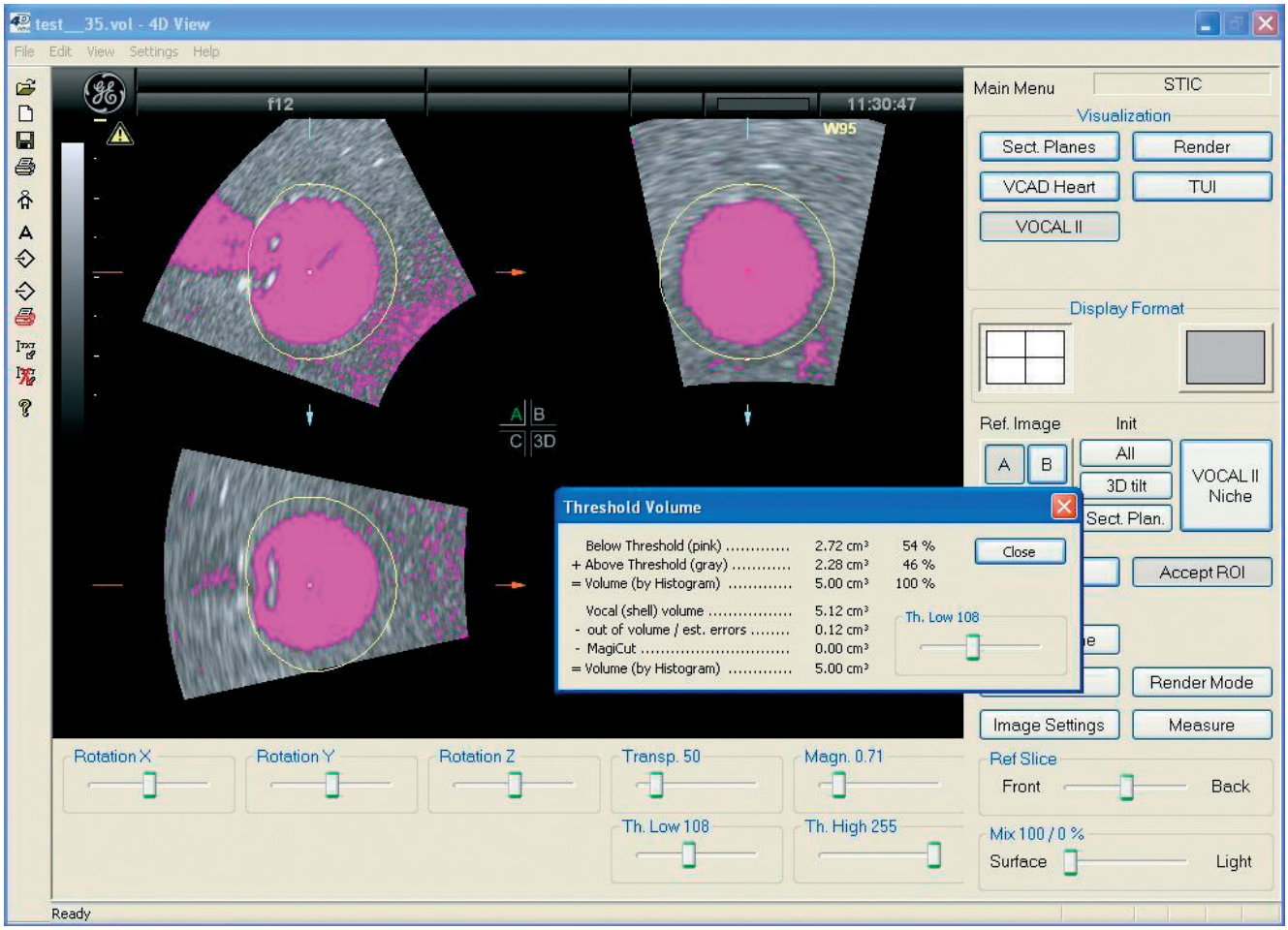

Figure 4 The inversion method measures the volume of the echolucent voxels only, adjustable through threshold setting, within a selected area defined by an automated contour using Virtual Organ Computer-aided AnaLysis (VOCAL) software.

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%).

Virtual Organ Computer-aided AnaLysis

The average time taken 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 percentage 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

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.

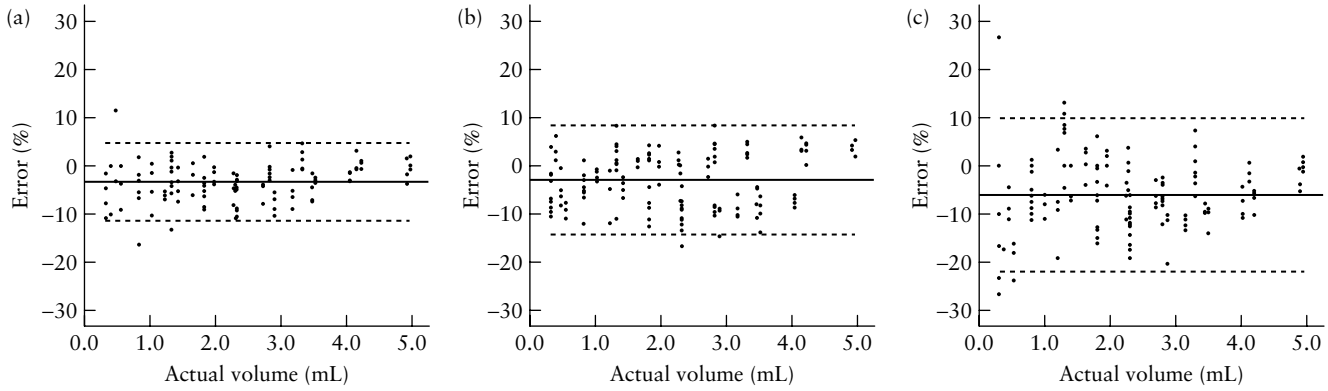

Figure 5 Percentage errors (difference/actual volume × 100; ———) of systolic and diastolic balloon volumes with limits of agreement (- - - -) plotted against actual volume for three-dimensional slice method (a), Virtual Organ Computer-aided AnaLysis (VOCAL) method (b) and inversion method (c).

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%).

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 the 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 the 3D slice, VOCAL and inversion methods 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 imaging has made volume measurements more accurate than those made by two-dimensional ultrasonography[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 methods were most accurate, with a mean underestimation of approximately 3% in the range 0.3–5.0 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 accuracy (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 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

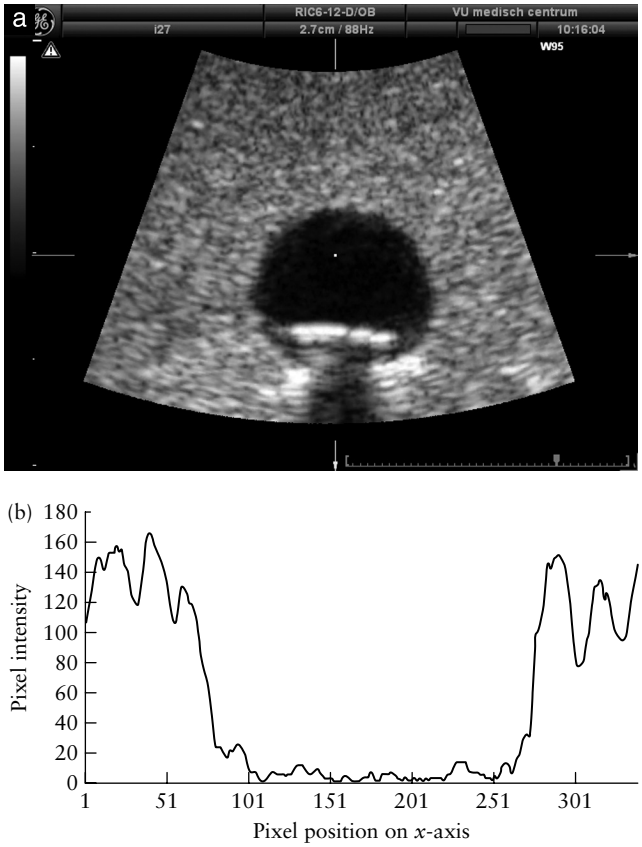

Figure 6 (a) Ultrasound image of a balloon model clearly illustrating that the hard-edged contrast balloon margins are displayed as a gradual transition in pixel intensity. (b) Histogram of an image showing pixel intensity against position on the *x*-axis through the reference point in Figure 6a.

software. We therefore hypothesize 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 reliable.

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 3D slice method will prove more useful than the 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 owing to threshold settings, and shadow artifacts, which are a frequent limiting factor in postprocess 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 gray-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 comparable to systolic fetal ventricular volumes before approximately 20 gestational weeks[5,6].

Three-dimensional ultrasonography is not real-time ultrasound imaging. Therefore, the measurements were not obtained from real-time images. STIC volumes display a virtual cycle, computed from 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 beats/min) which last less than 0.5 s. We therefore feel that 4D ultrasound imaging 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 gray-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, gray-scale curve and dynamic range setting of the ultrasound machine as postprocess gray-scale variation did not significantly change the results. Accordingly, very low-volume measurements in the fetus 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 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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