Two-Dimensional Echocardiographic Determination of Ventricular Volumes in the Fetal Heart

Validation Studies in Fetal Lambs

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The determination of ventricular volumes in the fetal heart from two-dimensional echocardiography (2DE) may give a better estimate of fetal ventricular size than simple diameter measurements, but the accuracy of this method has not been established. In fetal lambs, we tested whether ventricular volume calculations from 2DE using a biplane Simpson's rule algorithm are accurate. Calculations of left and right ventricular end-diastolic volumes from 2DE were compared with cast volumes of these ventricles. Also, at different levels of left atrial pressure, left ventricular stroke volumes calculated from 2DE were compared with stroke volumes measured simultaneously by an electromagnetic flowmeter. There was a good correlation between volumes determined from 2DE (y axis) and from casts (x axis) for both the left (r=0.92; y=0.2+1.1x; SEE=0.19 ml) and right ventricle (r=0.90; y=0.7+0.9x; SEE=0.21 ml). Left ventricular stroke volumes calculated from 2DE correlated well with those measured by the electromagnetic flowmeter (r=0.87; y=0.2+0.9x; SEE=0.27 ml). Thus, calculation of fetal ventricular volumes from 2DE images using a biplane Simpson's rule method is feasible and accurate. (*Circulation* 1990;81:325–333)

Echocardiography has become an established method for evaluating fetal cardiac anatomy.[1–5] In addition to the qualitative assessment of fetal cardiac disease, quantitative evaluation of the fetal heart from measurements of chamber size or ventricular performance by two-dimensional (2DE) or M-mode echocardiography has been reported.[6–10] Furthermore, attempts have been made to calculate ventricular volumes and output from M-mode diameter measurements in the fetal heart.[11,12] Such volume calculations in the fetus, however, may be questioned because of assumptions about ventricular geometry.

The accuracy of determining ventricular volumes in the fetus may be improved when area outlines of the ventricles on 2DE are used for the volume calculations, because this approach does not rely on "two-point" measurements but rather encompasses the entire planar outline of the ventricles. Calculation of ventricular volumes from 2DE has been validated previously in children and adults for the left ventricle[13–16] and for the right ventricle[17–21] using several planes of imaging and different geometric models. Previous studies comparing different methods used for left and right ventricular volume determination have shown that a biplane multiple slice method (Simpson's rule) gives the most accurate result.[14,22] It is not clear, however, whether such volume calculations can be extrapolated to the fetus because the fetal heart is relatively very small and its ventricles may have a different shape from that after birth. Therefore, we undertook the present study to determine the accuracy of volume calculations of fetal ventricles from 2DE using a biplane Simpson's rule method in fetal lambs. To define the accuracy of the 2DE method, we compared calculated ventricular end-diastolic volumes with cast volume measurements of these ventricles. We also compared calculated left ventricular stroke volumes with stroke

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volumes measured simultaneously by an electromagnetic flowmeter under different loading conditions.

Methods

Animals

We studied 19 pregnant sheep of mixed Western breed (term, 150 days) weighing 2.9±0.4 kg. In 10 of these fetuses, we compared echocardiographically determined left ventricle stroke volume with those determined by electromagnetic flow transducer placed on the ascending aorta. In this manner, alterations in preload could be assessed over the same time period. In 15 fetuses of the same gestational age and weight (some we used in the first experiment), we compared left and right ventricular end-diastolic volumes calculated from 2DE with the volumes of casts made from the left and right ventricle after these animals had been killed. These techniques are described below in detail. To compare the possible differences between transabdominal and transuterine measurement, we studied an additional 5 fetuses varying in age from 2.2 to 4 kg and 127 to 136 days of gestation specifically to compare transuterine measurements of left and right ventricular size compared with measurements made transabdominally simulating the situation for measurement used during human fetal echocardiographic examination.

Surgical Preparation

The ewes were fasted for 24 hours before surgery and underwent epidural anesthesia with 1% tetracaine. Polyvinyl catheters with an internal diameter of 1.27 mm were inserted into a maternal pedal artery and vein. NaCl solution (0.9%) was infused intravenously at a rate of 3–5 ml/min; ketamine (100 mg i.v. every 10–15 minutes) was used for maternal sedation; additional amounts were given as necessary. The uterus was exposed through a midline incision. Through a small hysterotomy a fetal hind limb was exposed and after local anesthesia (0.5% lidocaine), polyvinyl catheters (0.76 mm i.d.) were placed into the anterior tibial artery and vein and were advanced to the lower descending aorta and inferior vena cava.

Through a second hysterotomy, the fetal thorax was exposed. Using local anesthesia (0.5% lidocaine), a left thoracotomy was performed through the fourth intercostal space; a polyvinyl catheter (0.76 mm internal diameter) was placed into the internal thoracic artery and advanced to the brachiocephalic trunk. The pericardium was then opened; a catheter (0.76 mm inner diameter) was inserted in the left atrium through the atrial appendage and fixed in place by a purse-string suture. An electromagnetic flow transducer (C&C Instruments, Culver City, California) of appropriate size was placed on the ascending aorta proximal to the brachiocephalic trunk. This flow transducer had previously been calibrated by running known volumes of 0.9% NaCl solution through tubes of adequate size for the transducer. Flow measurements by the electromagnetic flow transducer at various levels were also checked and found to be accurate in three animals by means of the radionuclide-labeled microsphere technique.[23] The pericardium was left open, the ribs were approximated, and the thoracotomy was closed in separate layers. Using the same uterine incision, the fetal neck was then exposed. Local anesthesia was administered (0.5% lidocaine), and a larger polyvinyl catheter (8F) was introduced into the right jugular vein for rapid withdrawal or infusion of blood. The incision was closed, and the fetus was returned to the uterine cavity.

A catheter with multiple side holes was placed into the amniotic cavity; lost amniotic fluid was replaced with warmed 0.9% NaCl solution and the uterine incision was closed. The catheters and the transducer cable were brought through the maternal left flank and secured in a vinyl pouch sewn to the maternal skin. The maternal abdomen was closed in separate layers. Antibiotics were given both intravenously to the ewe and into the amniotic cavity (2 million units penicillin G and 100 mg gentamycin daily) during recovery from surgery.

2DE Studies

The fetuses were studied 2–4 days after recovery from surgery. The ewes underwent epidural anesthesia and laparatomy as described above. 2DE was performed using an ATL Mark 600 instrument (Advanced Technology Laboratories, Bothell, WA) with a 4- or 3-MHz mechanical transducer directly placed on the uterine horn containing the instrumented fetus. This approach was chosen because sometimes maternal movements, intestinal gas, or the presence of a twin made adequate imaging of the fetal heart difficult. We demonstrated that imaging of the fetal heart through the intact maternal abdominal wall is possible and of similar quality in five different fetuses where volumes were measured with the ewe supine before laporatomy; then measurements were repeated with the abdomen open and the transducer placed through the uterus as observed above.

Images of the left ventricle were obtained equivalent to apical four- and two-chamber views (Figure 1). The display of both atrioventricular valves and all four chambers in the same plane served as coordinates in the four-chamber view. The mitral valve, the left ventricle forming the apex, and the ascending aorta arising from the left ventricle were seen in the two-chamber view. Images of the right ventricle were obtained equivalent to subcostal coronal and parasagittal planes (Figure 1). The display of the tricuspid and pulmonary valves as well as the apex of the right ventricle served as coordinates in the coronal plane. The mitral valve within the left ventricle as well as the pulmonary valve and the right ventricular apex were seen in the parasagittal plane. All 2DE studies were continuously recorded on half inch videotape for later analysis.

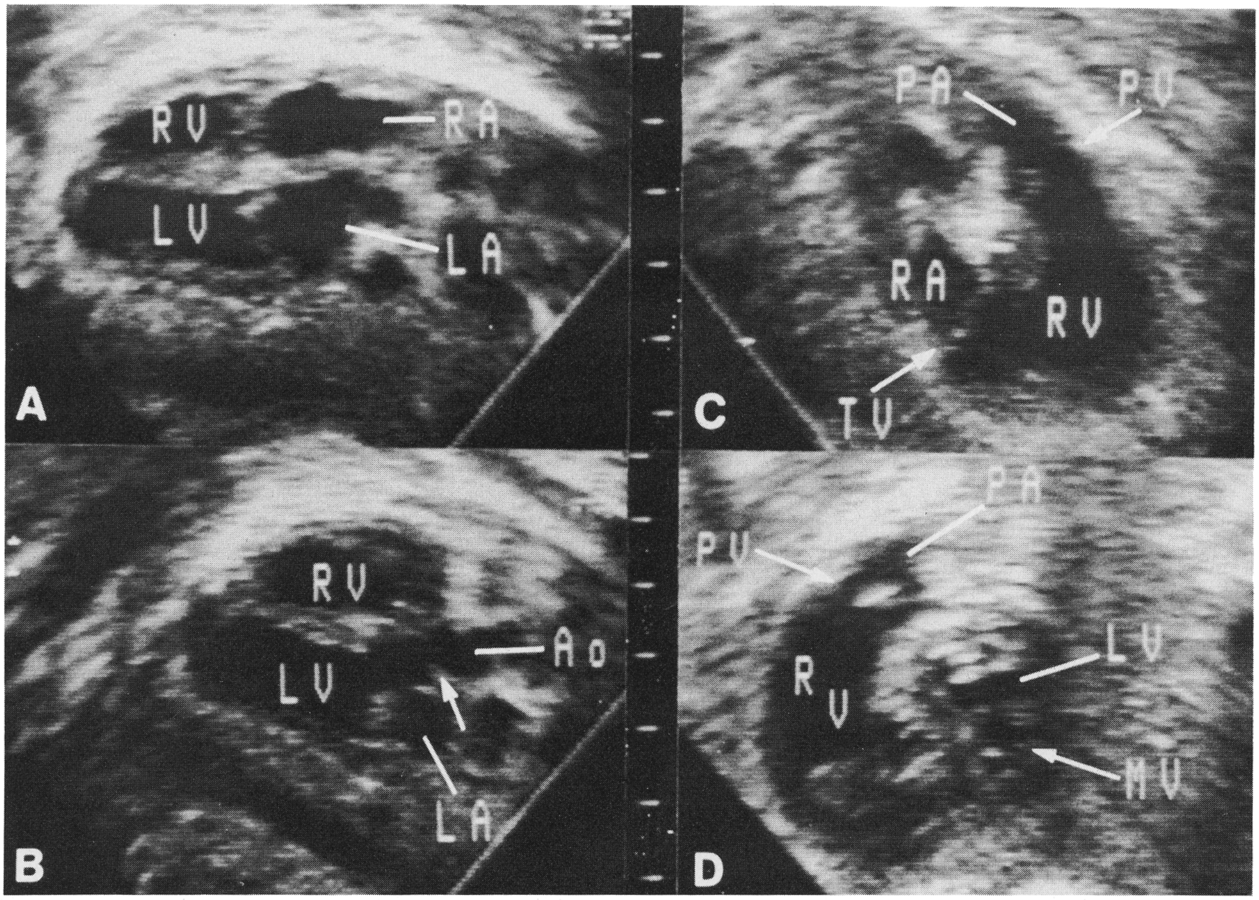

FIGURE 1. *Two-dimensional echocardiographic images of the left (A, B) and right (C, D) ventricle in fetal lambs.* A: *The left ventricle (LV) is shown in an equivalent of an apical four-chamber view; the right ventricle (RV), the right atrium (RA), and the left atrium (LA) are also seen.* B: *In an equivalent of an apical two-chamber view, the left ventricular outflow tract and the aortic valve (arrow) as well as the proximal ascending aorta (Ao) are seen.* C: *This frame depicts the right ventricle in an equivalent of a subcostal coronal view, where the tricuspid valve (TV) bordering to the right atrium as well as the pulmonary valve (PV) and the proximal pulmonary artery (PA) are seen.* D: *In the equivalent of a subcostal sagittal view of the right ventricle, the left ventricle and the mitral valve (MV) within it are imaged in cross-section.*

In nine fetal lambs not subjected to surgery 2DE, determinations of end-diastolic volumes from both the left and right ventricles were performed at baseline conditions only. In the 10 instrumented fetal lambs, end-diastolic ventricular volumes under baseline condition were determined first; subsequently, left ventricular stroke volumes were determined at different preload levels. Preload changes were achieved by hemorrhage of 20 ml/kg estimated fetal reinfusion of this blood, and additional transfusion of 20 ml/kg blood obtained from a twin fetus or from the ewe. Simultaneously, 2DE imaging as well as left atrial, carotid artery, and amniotic pressures, ascending aortic flow, and heart rate were recorded with a Beckman eight channel direct-writing recorder (Beckman Instruments, San Jose, California). The echocardiographic volume determinations, therefore, were made from beats recorded during the period where the cardiac output was measured by flow transducer. Two hundred forty such comparisons were made.

Additionally, five ewes underwent epidural anesthesia as described above. In these animals, a four-chamber view of the fetal heart was obtained by placing the transducer on the intact maternal abdominal wall; this procedure was repeated after one minute. Subsequently, a laparatomy was performed, and without any notable change in fetal heart rate the 2DE study was repeated twice by placing the transducer directly on the uterine wall. Therefore, a comparison between 20 transabdominal and transuterine area and volume measurements were made.

Analysis of Imaging

Analysis of echocardiographic recordings was performed using a computer-assisted analysis system and commercially available software (CAD 886, Microsonics, Indianapolis, Indiana). End-disastolic and end-systolic frames from the left and right ventricles in both orthogonal views were selected and digitized. To select appropriate frames for analysis,

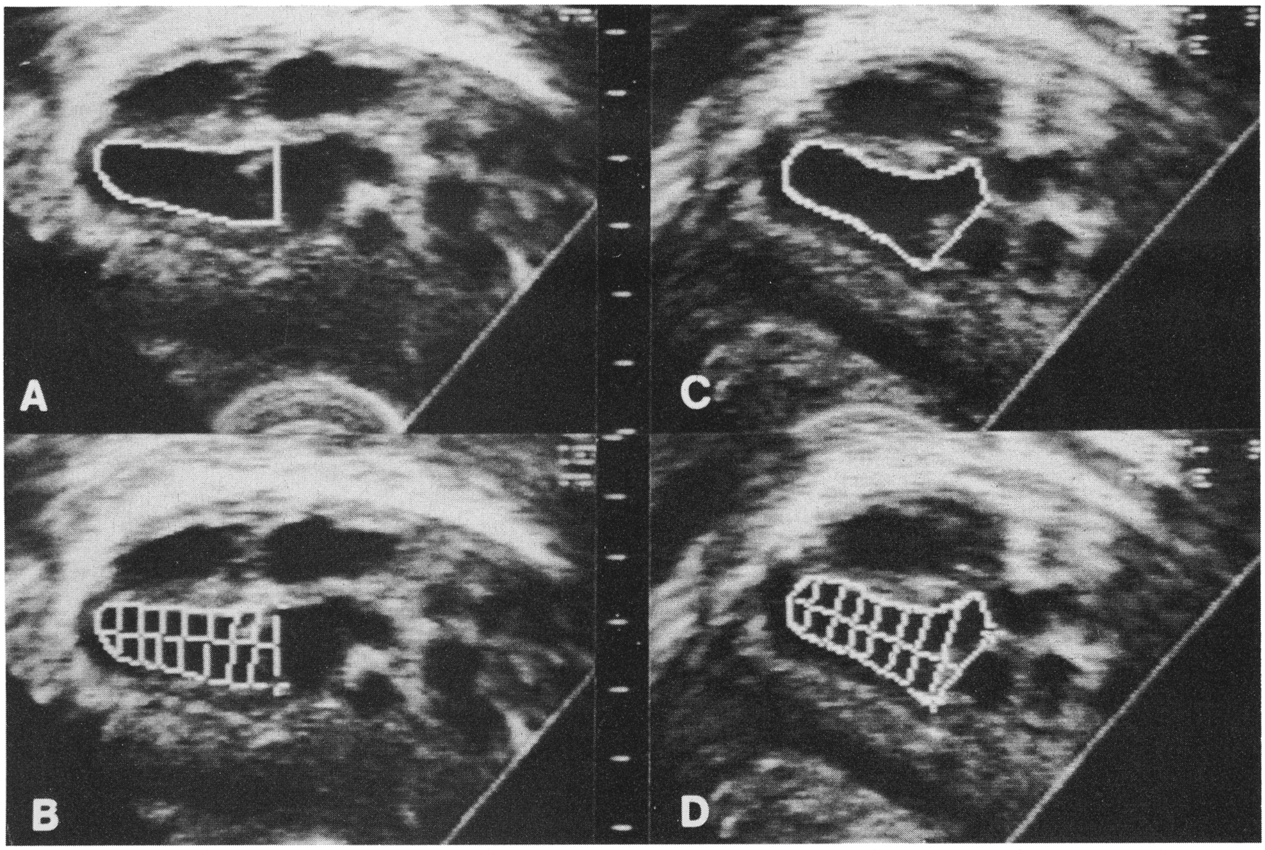

FIGURE 2. *Calculation of end-diastolic left ventricular volume in a fetal lamb from area outlines in two perpendicular planes using Simpson's rule. In the four-chamber view, the left ventricular endocardium is traced* (A) *and the long axis is placed between the apex and the center of the mitral valve* (B). *In the two-chamber view* (C, D), *the same procedure is performed; the ventricular volume is equal to the sum of volumes of the eight slices.*

cine-loop acquired images were carefully observed over several cardiac cycles. The frame preceding that showing opening of the semilunar valve or the frame at atrioventricular valve closure was defined as the end-diastolic frame; the frame preceding that showing atrioventricular valve opening or the frame at semilunar valve closure was defined as the end-systolic frame. Alternatively, when the precise points of valve closure could not be clearly identified, frames that reflected the largest and smallest of the ventricles were selected. The endocardium was traced, and a biplane Simpson's rule algorithm using seven cords and a common long axis was used to calculate left and right ventricular volumes (Figure 2). The common long axis in left ventricular calculations was from the apex to the center of the mitral valve, and the common long axis in right ventricular calculations was from the midportion of the right ventricular diaphragmatic surface to the center of the pulmonary valve (Figures 1 and 2). Calculations in both views from at least three consecutive cardiac cycles were averaged for volume determinations. Stroke volume was calculated as the difference between end-diastolic and end-systolic volume.

For the comparison of measurements in transabdominal and transuterine images, left and right ventricular area outlines and volumes calculated by an area-length method were determined in the four-chamber view.

Volume Validation

2DE determination of end-diastolic ventricular volumes at baseline were compared with cast volumes of these ventricles. After completion of the study, the lambs were killed and weighed. The fetal chest was opened, and the inferior vena cava ligated. The superior vena cava, the distal pulmonary artery, and the brachiocephalic trunk were cannulated, and intracardiac blood was removed by repeated rinsing with saline. We observed—in accordance with reports by others[24]—that in the fresh heart it is possible to induce considerable distension by injecting into the ventricles at high pressure. To avoid overt ventricular distension, we allowed the heart to stop beating and then perfused the heart through the great veins for 24 hours until the heart became fixed with 10% formalin solution at a filling pressure of 3–4 mm Hg. This procedure keeps the heart in its diastole and allows the subsequent injection of casting material into the ventricles without monitoring pressures.[24] Casts of both of the ventricular chambers were made using a silicone rubber material (RTV 11; General Electrics,

FIGURE 3. *Left ventricular* (top) *and right ventricular casts* (bottom) *from a fetal lamb are seen in similar planes as depicted echocardiographically. The left ventricular cast is seen as in a four-chamber view* (top left) *and as in a two-chamber view* (top right). *The right ventricle is seen as in a coronal view* (bottom left) *and as in a sagittal view* (bottom right).

Waterford, New York[24] injected simultaneously into both ventricles through the semilunar valves. All sites of cannulation were closed after silicone rubber material began to pour out.[24] After the casts had hardened, the ventricular walls were removed using concentrated sodium hydroxide solution, and atria or any great arteries were cut off at the level of the atrioventricular or semilunar valves, respectively (Figure 3). True ventricular volumes were measured from the weight of ventricular casts divided by the specific gravity of the silicone rubber material (1.18 g/cm^3).

Additionally, in instrumented animals, left ventricular stroke volumes calculated by 2DE were compared with stroke volumes determined from the simultaneous flow transducer measurement of mean ascending aortic flow divided by heart rate.

Interobserver and Intraobserver Variability

To test the variability in volume calculations from 2DE, 15 randomly selected echocardiographic calculations were repeated by a second observer (N.H.S.) and by the first observer (K.G.S.), each unaware of previous results. Variability was expressed as difference from the mean of the two results in percent of the mean.

Statistical Analysis

Values are given as mean±1 SD. Linear regression analysis was used to compare end-diastolic volumes derived from 2DE with cast volumes, stroke volumes derived from 2DE with simultaneous flow transducer measurements, and ventricular areas and volumes measured by the transabdominal and transuterine approach.

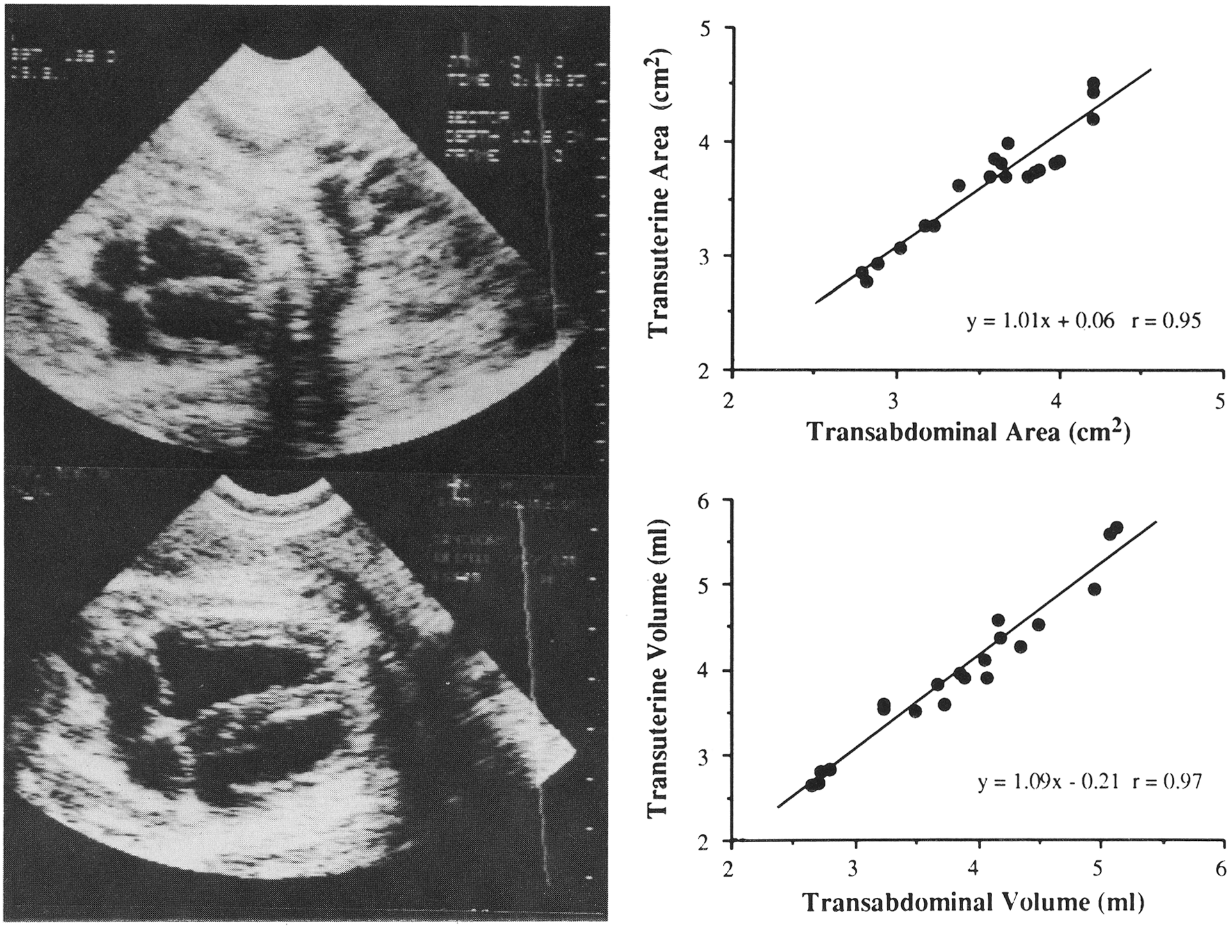

FIGURE 4. *Comparison of fetal four chamber views obtained by placing the transducer on the intact abdomen of the ewe* (top left) *and directly on the uterine wall* (bottom left). *There was close agreement between calculations in transabdominal* (x *axis) and transuterine* (y *axis) four-chamber views for both the ventricular area outline* (top right) *and the volume calculations* (bottom right).

Results

Ventricular area and volume estimates in a four-chamber view obtained by placing the transducer on the intact maternal abdominal wall correlated excellent with these measurements in images obtained by placing the transducer on the uterine wall (Figure 4).

Appropriate 2DE images for volume calculations of the left ventricle were obtained in all animals; in three animals, it was technically impossible to obtain appropriate biplane images for end-diastolic volume calculations of the right ventricle. Cast measurements averaged 1.8±0.3 ml (range, 1.1–2.4 ml) for the left ventricular end-diastolic volume and 2.3±0.4 ml (range, 1.4–2.8 ml) for the right ventricular end-diastolic volume. Left ventricular end-diastolic volumes estimated from 2DE averaged 2.2±0.4 ml (range, 1.4–3.1 ml) and correlated well with left ventricular end-diastolic cast volumes (r=0.92; y=0.2+1.1x; SEE, 0.19 ml) (Figure 5.) Right ventricular end-diastolic volumes estimated by 2DE averaged 2.6±0.4 ml (range, 1.8–3.4 ml) and correlated well with end-diastolic right ventricular cast volumes (r=0.90; y=0.7+0.9x; SEE, 0.21 ml) (Figure 6).

As a consequence of the preload alterations in instrumented fetuses, mean left atrial pressure ranged from 0 to 15 mm Hg. Over this range of left ventricular filling pressures, left ventricular stroke volumes as determined by 2DE correlated well with stroke volumes measured by the electromagnetic flowmeter (r=0.87; y=0.2+0.9x; SEE, 0.27 ml) (Figure 7). Left ventricular function curves derived from 2DE stroke

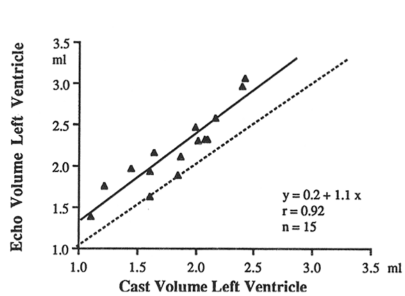

FIGURE 5. *Plot of left ventricular end-diastolic volumes determined by cast measurements (x axis) compared with those calculated from echocardiographic images (y axis). Solid line is the regression line: the broken line is the line of identity.*

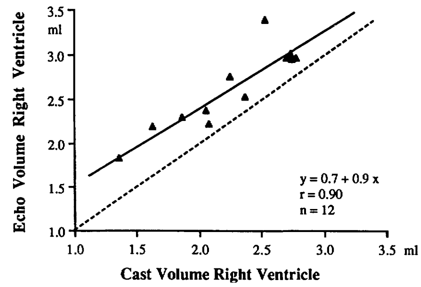

FIGURE 6. *Plot of right ventricular end-diastolic volumes determined by cast measurements* (x *axis*) *compared with those calculated from echocardiographic images* (y *axis*). *Solid line is the regression line; broken line is the line of identity.*

volume estimates and flowmeter measurements had a similar shape (Figure 8).

Intraobserver variability for the echocardiographic volume calculations was 4%, and interobserver variability was 9%.

Discussion

This study demonstrates that 2DE can be used to accurately estimate ventricular volumes in the fetal sheep heart. We found a good correlation between cast volumes and end-diastolic volumes calculated from 2DE both for the left and right ventricle (r=0.92 and 0.90, respectively; Figures 5 and 6). Although placing the transducer on the uterine wall is not practice in human fetal echocardiography, we found no difference in the quantitative analysis of images that were obtained by the transabdominal or the transuterine approach (Figure 4). This suggests that the accuracy of the measurements made in this study may be extrapolated to the clinical situation.

Earlier studies on the echocardiographic assessment of ventricular volumes in humans after birth tended to underestimate the true ventricular volume but showed good agreement with angiographic measurements when ratios of end-diastolic and end-systolic volumes such as the ejection fraction were compared.[13,15–17] This was the case particularly in right ventricular volume calculations by 2DE, where the irregular shape and limited accessibility of the

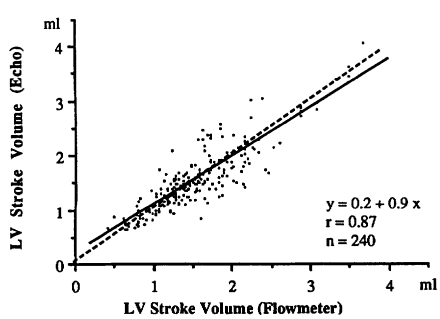

FIGURE 7. *Plot of left ventricular (LV) stroke volumes at different levels of preload measured by an electromagnetic flow transducer* (x *axis*) *compared with those calculated from echocardiographic images* (y *axis*). *Solid line is the regression line; broken line is the line of identity.*

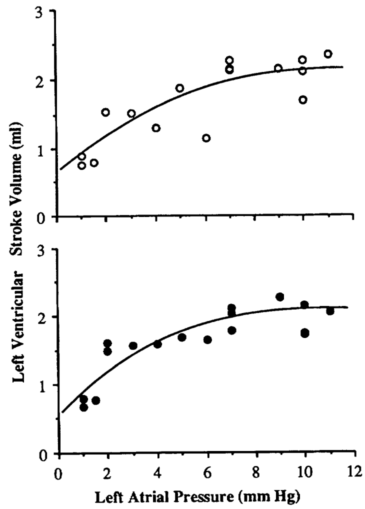

FIGURE 8. *Plots of left ventricular stroke volumes* (y *axis*) *against left atrial pressures* (x *axis*) *as observed in one animal.* Upper panel: *Measurements by the electromagnetic flow transducer* (○), *whereas the* (lower panel) *shows the simultaneous echocardiographic calculations* (●). *Note that the shapes of the left ventricular function curves are similar.*

right ventricle led to the use of complex models for volume assessment.[16–20] More recently, close agreement between angiographic and echocardiographic volume determinations has been reported when a biplane Simpson's rule algorithm was used both for the left ventricle imaged in apical four- and two-chamber views[14] and for the right ventricle imaged in subcostal coronal and sagittal views.[21] Direct comparisons of different methods for left and right ventricular volume calculation also underlined the superiority of the Simpson's rule.[14,22]

Further support for the use of Simpson's rule to calculate fetal ventricular volumes by 2DE is that the shape of fetal ventricles may be different from that after birth.[25] It is unlikely that such a different shape of the fetal ventricles has influenced our results because the biplane Simpson's rule algorithm we used makes few assumptions about the geometric shape of the ventricle under consideration. The method requires only imaging of the ventricles in two planes that are perpendicular and share a long axis. This was possible for the left ventricle in all and for the right ventricle in 12 of 15 fetuses in our study. It also has been shown recently that 2DE can be used to estimate small ventricular volumes such as that of the right ventricle in infants and young children with pulmonary atresia and intact ventricular septum.[26,27] The slight overestimation of the end-diastolic volumes calculated from 2DE that we have observed may be due to the inclusion of papillary muscle and trabeculation volume into the ventricular volume by the echocardiographic method. Although this inclusion is of little importance in larger hearts, it may be more critical in hearts of this small size.

In a recent report,[12] fetal ventricular volumes have been calculated from simple diameter measurements by M-mode echocardiography using the Teichholz formula. While the Teichholz formula is valid for the normal left ventricle in adults where the ratio of long to short axis is about two,[28] its validity for fetal ventricular volume calculation must be questioned. Teichholz himself reported poor results when M-mode measurements were used to calculate the volumes of left ventricles that had a different shape than normal[28]; certainly, such caution is even more relevant for the fetal right ventricle that has a fundamentally different geometry than the left ventricle. Additionally, even little errors in measuring the diameter of small fetal ventricles will result in substantial errors in volume calculation because the Teichholz formula uses the diameter raised to its third and fourth power.[28] Furthermore, it is often impossible in the fetal heart to steer the M-mode line through a standardized plane as is practice after birth because fetal lie varies and the heart may be imaged only with its long axis parallel to the ultrasound beam. Therefore, fetal ventricular volumes should be calculated from 2DE using two planes of imaging.

Fetal volume calculation by 2DE may also be useful in the assessment of changes in ventricular chamber size occurring with different loading conditions of the heart. When we altered the preload of the left ventricle in the fetal lambs, we were able to demonstrate the resulting changes in stroke volume accurately by 2DE; we found a good correlation between left ventricular stroke volumes measured by an electromagnetic flow transducer and those calculated from echocardiographic imaging (Figure 7). These estimates of stroke volume over a wide range of filling pressures are particularly gratifying because they represent a difference of two volume estimates—one at end diastole and the other at end systole, which accounts for some variability. The comparison of left ventricular function curves derived from flow transducer measurements and from echocardiographic calculations showed good agreement (Figure 8). This finding indicates that 2DE may well be used for such physiologic observations when instrumentation of the fetus must be limited.[29]

There are possible limitations in the 2DE assessment of fetal ventricular volumes. With an echocardiographic frame rate of 30/sec, it may be difficult to identify the precise points of end-diastolic and end-systolic frames in the rapidly beating fetal heart. In lambs, this is a greater problem than in human fetuses because they have faster heart rates. This potential error was reduced in our study by averaging the volume estimates from several consecutive cardiac cycles. Another possible limitation may arise from inaccessibility of the fetal heart to imaging in planes required for these calculations. Although we found little difficulties in imaging these fetal lambs when placing the transducer on the uterine wall, imaging of the human fetal heart may sometimes be difficult due to fetal lie or fetal movements. However, in our preliminary experience with 2DE estimation of ventricular volumes in human fetuses, we have not encountered these potential difficulties, suggesting that accurate calculation of ventricular volumes in the human fetus is feasible.

The determination of fetal right and left ventricular end-diastolic and end-systolic volumes by 2DE using a biplane Simpson's rule algorithm is possible and accurate. This method may provide a more reliable measurement of cardiac chamber size and function in human fetuses than the measurement of ventricular diameters from 2DE or M-mode echocardiography. Furthermore, at various filling pressures stroke volumes can be calculated, allowing ventricular output to be estimated by a different approach than by Doppler echocardiography.[30,31] This additional approach for ventricular output calculation may be particularly useful when Doppler interrogation cannot be performed adequately.

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