Ultrasound in Med. & Biol., Vol. 26, No. 3, pp. 425–432, 2000

0301-5629/00/$–see front matter

PII S0301-5629(99)00154-4

● *Original Contribution*

ACCURACY OF *IN-VITRO* VOLUME ESTIMATION OF SMALL STRUCTURES USING THREE-DIMENSIONAL ULTRASOUND

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(*Received* 28 *June* 1999; *in final form* 20 *October* 1999)

Abstract—We describe an ultrasound probe for three-dimensional transvaginal imaging. The transducer was an annular array with a center frequency of 7.5 MHz which was rotated with an internal stepper motor. The probe had no external moving parts, and the total volume covered by a full rotation defined a half sphere. The raw digital data from the scanner were transferred to an external PC for three-dimensional reconstruction. We evaluated the three-dimensional imaging system by measuring the volumes of phantoms (range 24.8–3362.5 mm^3) in a water tank, and found good correlation with true volumes (two observers' measurements gave a linear regression with a slope of 1.010 and $R^2 = 0.993$, and a slope of 0.956 and $R^2 = 0.993$, respectively). The size of the point-spread function was used in the calculations to eliminate the effect of under- or overestimation due to the limited ultrasound beam resolution. An example of data acquisition, volume estimation and imaging of an embryo less than 8 weeks old *in vivo* with the brain cavities and body is given. We conclude that the three-dimensional reconstruction and volume estimation were accurate and repeatable. © **2000 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Three-dimensional ultrasound, Transvaginal, Volume estimation, 3-D reconstruction, Point-spread function.**

INTRODUCTION

There has been a significant development of medical three-dimensional (3-D) ultrasound in the past 20 years, and obstetrics and gynecology has been one of the main areas of application. Brinkley et al. (1982) showed that fetal weight estimation was possible using a position locator system for 3-D reconstruction. Later, mechanically tilted probes were introduced and successful volume imaging of fetal structures, including malformations, has been performed (Hull and Pretorius 1998; Lee et al. 1995; Merz et al. 1995; Steen and Olstad 1994). Transvaginal 3-D imaging based on rotating the ultrasound probe with a stepper motor has been reported (Blaas et al. 1995; Kyei-Mensah et al. 1996).

Measurements in 3-D ultrasonography may be subject to errors because of the limited resolution of the ultrasound beam, or errors in the spatial position of the scan planes. The consequence of limited resolution is that small structures and object borders will be smeared out in the image and appear larger than they are. Delineation of these borders may result in overestimation of distances and volumes, or underestimation of the volume of cavities. In addition, patient movement during acquisition or errors in the 3-D rotation will result in reconstruction artifacts. Because of these problems, it is important to evaluate 3-D ultrasound systems before clinical use.

Volume estimation using 3-D ultrasound has been applied *in vivo* on such organs as the kidneys (Gilja et al. 1995) and the heart ventricles (Nosir et al. 1996), showing good correlation with other measurement methods. However, because the reference methods also are subject to measurement errors, *in vitro* validation is necessary further to evaluate the estimation accuracy. One common technique for such validation is to estimate the volumes of balloons in a water tank (Hughes et al. 1996). The volume estimated by tracing the center line of the balloon border is not much influenced by the limited ultrasound resolution. In the case of *in vivo* measurements, however, it is difficult to identify the exact object border

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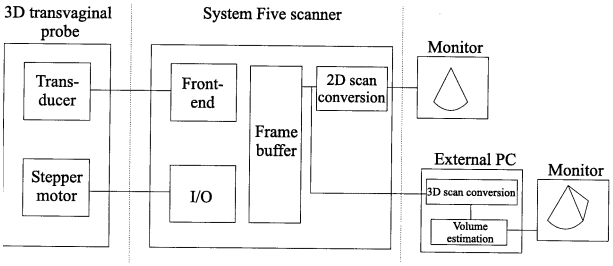


Fig. 1. Data flow diagram. The 3-D probe was coupled directly to the ultrasound scanner, and the raw digital data from the scanner were transferred to a PC and used in the 3-D reconstruction.

because we have backscattered signals from inside the object as well as from the borders.

The purpose of this study was to describe and evaluate a new transvaginal 3-D ultrasound probe. We developed a method for volumetric analysis of small structures that we evaluated *in vitro* using water tank phantoms made of agar gel with kaolin particles. The size of the phantoms covered a large scale. The size of the point-spread function was used to compensate for the overestimation because of the limited ultrasound beam resolution. In this paper, we first describe the data acquisition and the transvaginal 3-D probe. The theoretical spatial sampling density requirement is then analyzed followed by a description of the volume measurement method. The results show the accuracy of the phantom volume measurements, and an application of the technique in human embryology is demonstrated.

MATERIALS AND METHODS

Data acquisition

We used a specially developed 3-D transvaginal probe, connected to a System Five digital ultrasound scanner (Vingmed Sound, Horten, Norway). Rotation of the transducer was done by an internal stepper motor. The raw digital data were transferred from the scanner frame buffer to an external PC for 3-D reconstruction (Fig. 1). The motor activity was controlled by the scanner. When the motor speed was adapted to the scanner frame rate, one scan took typically 2–5 s depending on the angle of rotation.

Probe design

The transvaginal 3-D probe had a total length of 298 mm and had a 7.5 MHz annular array transducer with an aperture of 11.5 mm (Fig. 2). The transducer midposition was tilted 45° in the 2-D scan plane (azimuth direction), with a maximum azimuth scanning angle of 90° (Fig. 3). The scan plane of the transducer was rotated inside the

Fig. 2. The transvaginal 3-D probe. Dome length: 175 mm, total length: 298 mm, dome diameter: 25.6 mm, transducer center frequency: 7.5 MHz. The probe had no external moving parts except for the cable connector.

housing (dome), so that the probe had no external moving parts except for the probe cable connector. The 2-D scan plane could be rotated 360°.

Spatial resolution

The accuracy of measurements in ultrasound images is limited by the ultrasound beam resolution. The resolution can be measured by the size of the image point-spread function that is given by the beam width and the pulse length. For an annular array transducer, the focus is symmetrical and the focal diameter D_F at the focal distance F is given by:

$$D_F = \Theta_{X\,\mathrm{dB}} \cdot F = k_X \cdot \frac{\lambda}{D} \cdot F \qquad (1)$$

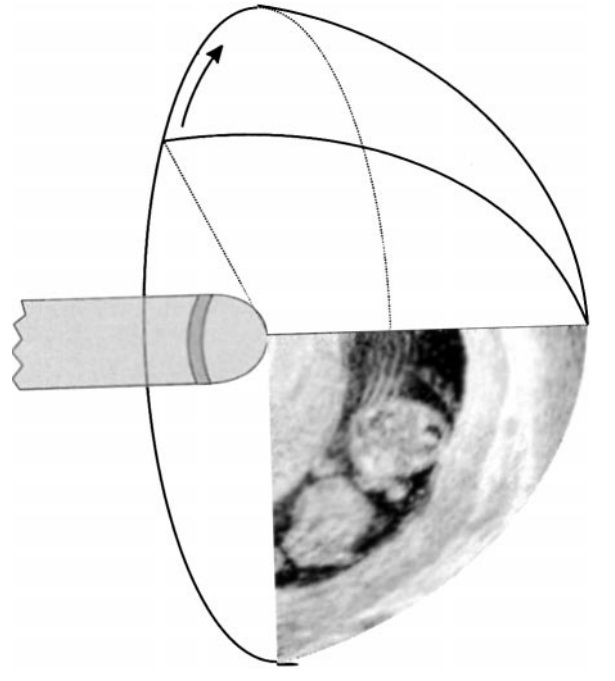


Fig. 3. The data volume covered by rotation of the scan plane with an azimuth scanning angle of 90°. The transducer midposition was tilted 45°. The scan plane could be rotated 360°.

where $\Theta_{X\,\mathrm{dB}}$ is the opening angle defined by the X dB beam amplitude reduction. We used 12 dB two-way reduction to define the lower amplitude value limit. Furthermore, λ is the ultrasound wavelength, and D is the aperture diameter. The parameter k_X is the amplitude reduction factor, and for 12 dB two-way reduction we get $k_{12\,\mathrm{dB}} = 1.4$ (Angelsen, 1995). The depth of focus, L_F, is defined as the region where the beam diameter is limited by diffraction, and can be expressed as where the on-axis beam amplitude has dropped 1 dB:

$$L_F\,(1\ \mathrm{dB}) = 2 \cdot D_F\,(12\ \mathrm{dB}) \cdot \frac{F}{D} \tag{2}$$

The resolution in the radial direction was given by the length of the transmitted pulse T_p:

$$\Delta r_p = c \cdot \frac{T_p}{2} = \frac{\lambda}{2} \cdot f \cdot T_p \tag{3}$$

where c is the sound velocity and f is the transmit frequency. Here, $c = 1540$ m/s was used as the mean value for tissue. The product $f \cdot T_p$ is the number of periods in the transmitted pulse.

The spatial sampling density must be sufficient in all three dimensions to prevent undersampling in the reconstructed 3-D data. The lateral sampling density was determined by the angle increment in the original scan plane, and in our set-up we used 60% overlap. We used the same overlap in the elevation direction, so the corresponding 3-D rotation angle increment was $\theta = 0.4 \cdot \Theta_{12\,\mathrm{dB}}$. The radial sampling density was determined by the radial resolution. The rotational speed w (degrees/s) depended on the scanner frame rate, fr (frames/s): $w = fr \cdot \theta$.

Volume reconstruction

The data were scan-converted and interpolated into a regular 3-D volume using the EchoPAC-3D software (Vingmed Sound, Horten, Norway), and visualized by extracting 2-D slices at different positions (Martens et al. 1997). The accuracy of the rotation was evaluated by measuring the actual probe movement, and by imaging a grid phantom placed in a water bath. The probe was kept in a fixed position during rotation, and the grid was placed at a 45° angle to the probe rotation axis. Since the probe needed a few milliseconds to accelerate, a few frames were left out from the reconstruction at the start and at the end of the rotation to avoid artifacts.

Volume measurement

In this study, we used manual delineation of the object borders; this was performed by manually tracing contours in different 2-D slices of the data. The contours did not have to be parallel, as long as they did not intersect. This resulted in a set of planar contours which were converted into a polyhedron created by a triangulation of consecutive contours. The volume of this polyhedron was calculated, giving an estimate of the object volume. This volume estimation algorithm has been described in detail (Thune et al. 1996).

The value D_p defined the size of the point-spread function (i.e., the lateral image resolution), and Δr_p defined the radial image resolution. All structures in the ultrasound image were smeared out or blurred with an amount corresponding to these values. An object of true length l had length $l + D_p$ when positioned horizontally, and length $l + \Delta r_p$ when positioned vertically in the image. In the focal zone L_F, $D_p = D_F$.

If the lengths of the object for all directions are known, the measurement error caused by the limited resolution of the ultrasound beam can be corrected by appropriate scaling of the object. If the lengths are almost equal, we can use the volume of a cube to approximate the measured volume, V_m. Here, such a cube was oriented in the radial/lateral direction. The length of each side was then $l_m = V_m^{1/3}$. The true length l in the lateral direction and r in the radial direction was corrected to be $l = l_m \pm D_p$ and $r = l_m \pm \Delta r_p$. The minus sign was used for overestimated organ volumes, and the plus sign was used for underestimated cavity volumes. The following correction factor, k_{psf}, was introduced to estimate the true measured volume, $\hat{V}_m$:

$$\hat{V}_m = k_{psf} \cdot V_m \tag{4}$$

Using the scaled dimensions of the cube, the correction factor for the point-spread function was:

$$k_{psf} = l^2 \cdot r / V_m \tag{5}$$

Phantom volumes

Ten cylindrical phantoms were made out of agar gel mixed with kaolin (range 24.8–3362.5 mm^3) and placed in a water bath on top of a block of clear agar gel. Each object was scanned three times from different, random angles ($n = 30$), with the 3-D rotation angle in the range 27–111°. The water had a temperature of 21°C. At this temperature, the sound velocity was 1485.4 m/s (Lubbers and Graaff 1998). To compensate for the difference in sound velocity for the water and the scanner setting of 1540 m/s, we scaled each measured volume by a factor $k_{vel} = (1485.4/1540)^3$. The outer boundary of the object was traced by two observers and the corresponding volume was estimated from the following equation:

Table 1. Ultrasound resolution and sampling density values for the 3-D transvaginal probe.

Transmit frequency (f)	7.5 MHz
Wavelength (λ)	0.21 mm
Aperture diameter (D)	11.5 mm
Focal distance (F)	25 mm
Radial resolution (Δr_p)	0.32 mm
Elevation opening angle ($\Theta_{12\ dB}$)	2.1°
Focal diameter (D_F (12 dB))	0.64 mm
Focal depth (L_F (1 dB))	2.6 mm
Rotation angle increment (θ)	0.84°

$$\hat{V}_m = V_m \cdot k_{psf} \cdot k_{vel} \quad (6)$$

The true volume, V_t, was calculated by measuring the diameter and the height of each object with a vernier caliper. We used the same phantoms as described in an earlier study (Blaas et al. 1998); but in this study, the contours were retraced by different observers, thus, we obtained new volume estimates.

Statistical analysis

All volumes were traced once by two observers blinded for each other's results. The estimates were compared with the true volumes by linear regression with the line fit curve passing through zero. The mean percentage error ± standard deviation (SD) was calculated for two ranges of the volumes, larger and smaller than 500 mm^3. The mean percentage error was defined as the mean of the percentage difference between true and estimated volumes. Interobserver variability was expressed as the SD of the difference of the two observers' estimates divided by the true values. The volumes were also analyzed according to Bland and Altman (1986). We compared the percentage difference between the estimated and true measured volumes with the true measured volumes. Similarly, we found the impact of the mathematical corrections by comparing the estimated values without any corrections with the true volumes.

Measurement of embryonic volumes in vivo

Transvaginal 3-D data were acquired of an embryo from a healthy pregnant woman with no pregnancy complications. The gestational age based on the last menstrual period was 7 1/2 weeks. The image was first adjusted for optimal quality and range. Then the probe was manually rotated so that the scan plane was placed to the side of the embryo. Finally, the motor rotated the scan plane to cover just the embryo. The outer border of the embryo body, the embryonic brain cavities, the yolk sac and the amnion sac were all manually outlined. The estimated volumes were then given by: $\hat{V}_m = V_m \cdot k_{psf}$.

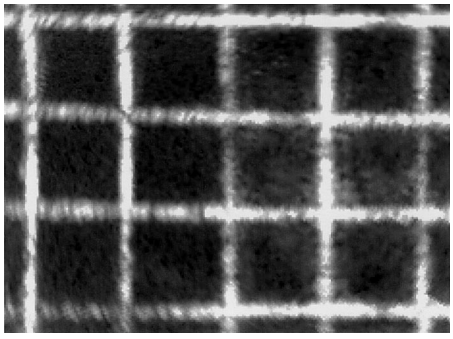


Fig. 4. Slice through the 3-D reconstruction of a grid phantom. The grid size is 1 cm; the grid is placed 45° to the probe rotation axis.

RESULTS

Spatial resolution

In Table 1, the ultrasound resolution values for the 3-D transvaginal probe are shown. The length of the transmitted pulse, T_p, is a function of the transducer response. We assumed that the propagating pulse had three periods. The radial resolution then was $\Delta r_p = 0.32$ mm. In the original scan plane, the radial sampling density was less than 1 mm, and the lateral angle increment was less than 0.5°. The rotation step angle increment was: $\theta = 0.4 \cdot \Theta_{12\ dB} = 0.84°$. This means that the 3-D data were recorded with no loss of resolution. With a typical frame rate of 23.7 frames/s, the rotational speed was: $w = 23.7 \cdot 0.84$ degrees/s $= 19.9°$/s.

Volume reconstruction accuracy

After the raw data were transferred to the computer, the 3-D reconstruction was performed within 3 s. Figure

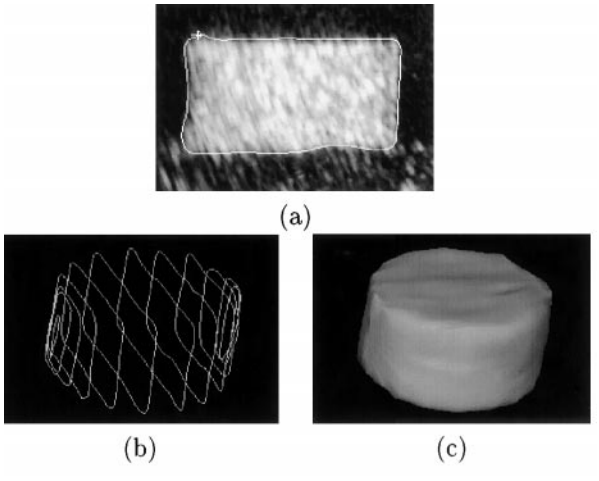
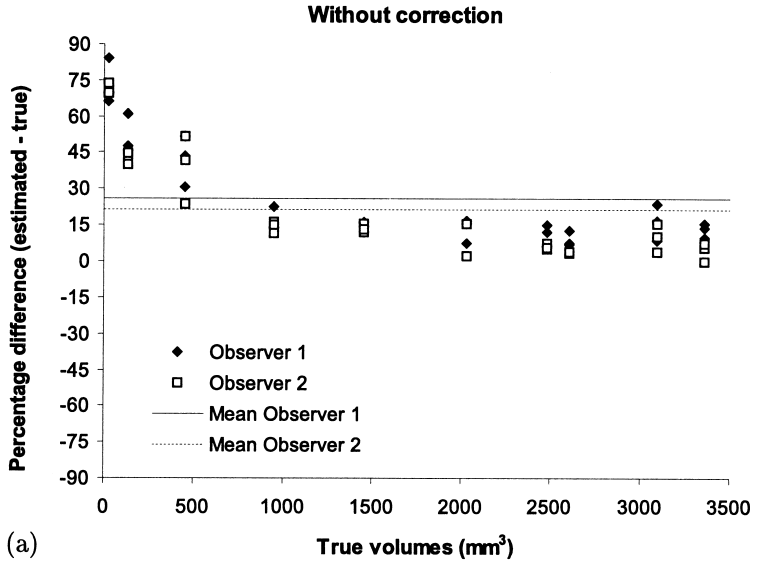


Fig. 5. Volume estimation of phantom object with true volume 3094.7 mm^3. (a) The contour of a manually delineated border; (b) all planar contours; (c) reconstruction of phantom based on contours.



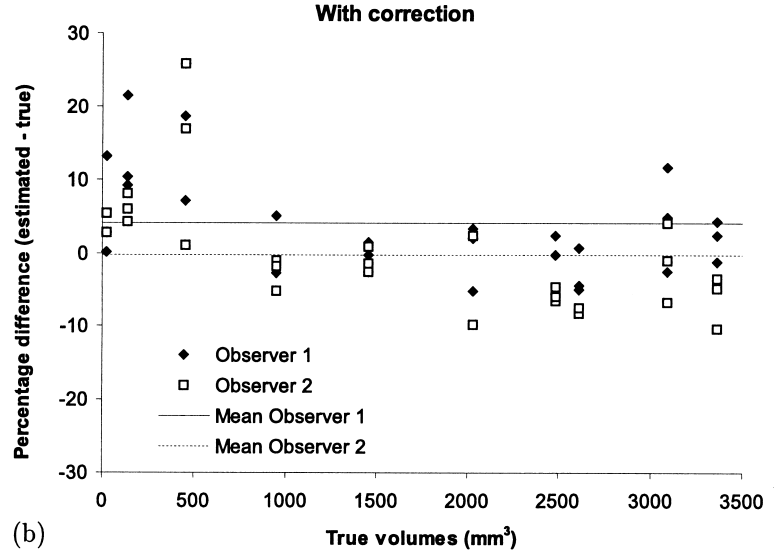


Fig. 6. The percentage difference between the estimated and true volumes compared to the true volumes. (a) The mathematical corrections for the point-spread function has been removed from the estimated volumes; (b) the estimated volumes with the mathematical corrections.

4 shows a slice of the reconstructed grid phantom data. Within the area of the phantom, the reconstruction error was less than the ultrasound beam resolution. This was also confirmed by measuring the amount of rotation on the probe itself.

Volumes of phantoms in a water tank

The delineation of a phantom object is shown in Fig. 5. The resolution correction parameters were set to the values of the point-spread function: $D_p = 0.64$ mm and $\Delta r_p = 0.32$ mm. The correlation between the estimated and the true volumes for the two observers gave linear regression with a slope of 1.010 and $R^2 = 0.993$, and a slope of 0.956 and $R^2 = 0.993$, respectively. In Fig. 6, the percentage difference between the estimated and true measured volumes are plotted against the true measured volumes. The impact of the mathematical corrections can be seen from Fig. 6a. The mean percentage error of the volume estimates was higher for the volumes <500 mm^3 (12.1% $\pm$ 8.6%, and 8.1% $\pm$ 8.1%) com-

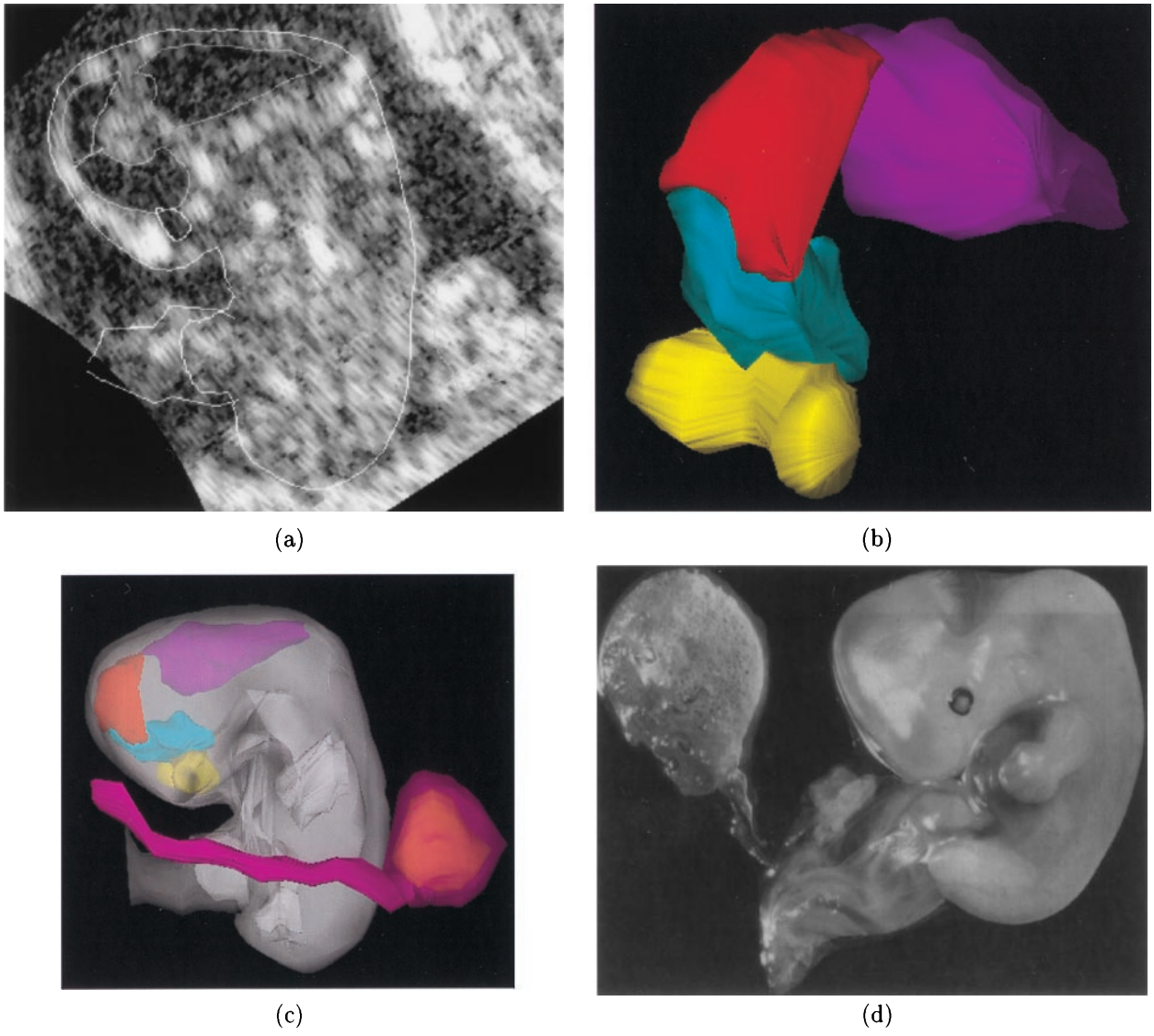


Fig. 7. (a) The delineation of brain cavities and body of an embryo with CRL 13 mm, age 7 1/2 weeks based on the last menstrual period; (b) reconstruction of the brain cavities: hemispheres (yellow) 5.5 mm^3, diencephalon (green) 6.0 mm^3, mesencephalon (red) 4.0 mm^3, and rhombencephalon (violet) 17.8 mm^3; (c) reconstruction of the embryo (329.4 mm^3) with part of the umbilical cord, the yolk sac and the vitelline duct lie in front (rose); (d) for comparison: photography of an aborted embryo. The umbilical cord and the vitelline duct leading to the yolk sac on the left side (Barnea et al. 1992; reprinted with permission).

pared to the volumes >500 mm^3 (0.6% $\pm$ 4.3%, and $-3.8\% \pm 4.1\%$). The interobserver variability for the volumes <500 mm^3 was 6.8%, and for the volumes >500 mm^3 it was 3.4%.

Embryonic brain cavities and body volumes

Figure 7 shows the delineation and 3-D reconstruction of an embryo with brain cavities and the yolk sac. The embryo had a crown-rump length (CRL) of 13 mm. The CRL is the greatest length of the embryo from the top of the head to the caudal end of the body. The 3-D reconstruction is compared with a photograph of an aborted embryo (Fig. 7d).

DISCUSSION

In this study, we have shown that it was possible to estimate volumes of phantoms >500 mm^3 *in vitro* with excellent accuracy using a transvaginal 3-D probe. The estimates were less accurate for the phantoms <500 mm^3, illustrating the problem of measuring very small structures with ultrasound. The percentage error was a

function of the volume size, because the volume correction was based on the ultrasound beam resolution. The size of the smallest volumes was comparable to the beam resolution giving higher uncertainty, whereas the impact of the beam resolution was smaller for the larger volumes. The interobserver variability for the volumes >500 mm^3 was small, indicating that the volume estimation method was repeatable. The exact placement of the contours was subject to individual variation as is shown by the small bias between the two observers. Such differences are inevitable using manual segmentation of ultrasound images and are in this case regarded as small.

The volume estimation algorithm used in this study has previously been tested on the stomach and kidney *in vitro* (Gilja et al. 1994). Using a 3-D tilting probe, the percentage error $\pm$ SD between estimated and true volume was at most mean 9.8% $\pm$ 3.1%. The method has also been applied on human kidneys *in vivo*, and compared with 3-D magnetic resonance imaging (MRI) (Gilja et al. 1995). Compared to MRI, the 3-D ultrasound method underestimated the volumes ($-9.4\% \pm 8.4\%$). Other methods for estimating volumes from 3-D ultrasound have been reported. King et al. (1991) estimated volumes with a mean error of 1.6%, and Basset et al. (1991) reported a maximum error of 10%.

The results from the current study indicate that our 3-D ultrasound system is adequate for volume estimations. However, we must be aware of the following measurement pitfalls: (1) volume measurement with ultrasound can give large estimation errors because of the limited resolution of the beams; in this study, we have reduced this error by including a measure for the beam resolution in the estimation algorithms; (2) the speed of sound in water tank experiments must be considered with caution. A small deviation of this value compared to the listed value results in a different volume. It is affected by several factors such as the contents of gas (air), salt and temperature. The measured volume must be compensated for the differences in sound velocity in the tank and in the scanner setting. The consequence of different sound velocities inside the phantoms was not regarded as significant in the experiments.

In an *in vivo* study including 34 embryos and fetuses, with CRLs ranging from 9.3 to 39.0 mm, we measured brain cavities and body volumes and found that the estimates corresponded well with the earlier findings from classic human embryology (Blaas et al. 1998). In the present study, slightly different resolution correction parameters were used because the contours were traced at the outermost boundary of the object with the gain set at a high value. In a previous study (Blaas et al. 1998), a different observer traced the contours closer to the object. This compensated, to a certain extent, the blurring of the object borders caused by the limited beam resolution.

There are several advantages of having the transducer midposition tilted 45° instead of being aligned with the rotation axis. In the latter case, it is often necessary to do a full 180° rotation to cover a sufficiently large volume. Objects that are in the rotation center of a 180° rotation are likely to be distorted, because the first and the last 2-D images must be at the exact same position; this is difficult to achieve because of movement of the probe and the patient. The tilted midposition transducer may be rotated a smaller angle to cover the same volume, and the object does not have to be placed in the rotation center. The total volume that is covered in a full 360° rotation is $2\sqrt{2}$ times larger than for the probes with the transducer aligned with the rotation axis, when the transducer's scan planes are 90°.

CONCLUSIONS

With a specially developed 7.5-MHz 3-D probe for transvaginal imaging, ultrasound data could be acquired and volume reconstructed with high accuracy. The 3-D data were reconstructed with no loss of spatial resolution using the raw digital data from the scanner. Accurate and repeatable *in vitro* volume estimations were achieved for small phantoms (500–3400 mm^3) by including a measure for the ultrasound beam resolution in the volume estimation algorithm. For even smaller phantoms (25–500 mm^3), the estimations were less accurate, but satisfactory considering the small sizes and the limited resolution of the ultrasound beams. Successful reconstruction and visualization of a 7 1/2-week embryo *in vivo* shows the potential application of studying organ development of embryos and fetuses at an early stage of gestation.

Acknowledgements—We thank Ditlef Martens, Christian Michelsen Research, Bergen, Norway, for software support, and Nancy Eik-Nes for revision of the paper.

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