

## Early reports

# In-vivo three-dimensional ultrasound reconstructions of embryos and early fetuses

Harm-Gerd Blaas, Sturla H Eik-Nes, Sevald Berg, Hans Torp

## Summary

**Background** Three-dimensional (3D) imaging of the living human embryo has become possible in the monitoring of embryological development, as described by classic human embryology. We aimed to create 3D images of organs in embryos on early pregnancy.

**Methods** We used a specially developed 7.5 MHz annular array 3D transvaginal probe to examine embryos. We included 34 women at 7–10 weeks' gestation. We measured the crown-rump length (CRL) of the embryos and fetuses and transferred the 3D ultrasound data to an external computer for further processing to calculate volume.

**Findings** The CRLs ranged from 9.3 mm to 39.0 mm. The quality of the images of the embryos and fetuses made it possible to outline in detail the outer contours and the contours of the brain cavities, and the calculated volumes corresponded well to the descriptions from classic human embryology.

**Interpretation** Our 3D imaging system allowed visualisation of structures of less than 10 mm. Therefore, development and abnormal development of fetuses can be monitored.

*Lancet* 1998; 352: 1182–86

## Introduction

Classic human embryology was established by Wilhelm His in 1880–85.<sup>1</sup> He realised the need for magnified three-dimensional (3D) imaging and the need for a model of the dissected object.<sup>1</sup> He made 3D reconstructions from free-hand drawings of histological slices.<sup>2</sup> Born,<sup>3</sup> who in 1876 was the first to describe the technique of making solid reconstructions by stacking wax plates of histological slices, made use of the camera lucida, a device that aided the accurate sketching of small objects. Wax was later substituted by more durable materials such as wood, plaster, glass, or plastic.<sup>4</sup>

Imaging by graphic reconstructions with the aid of special devices has commonly been used in modern human embryology.<sup>5–8</sup> The development of computer technology has opened new possibilities for 3D reconstructions.<sup>9</sup> The first attempt at constructing 3D images of the fetus from ultrasound recordings was made in the early 1980s.<sup>10</sup> The introduction of real-time high-frequency ultrasonography with transvaginal transducers led to improved resolution and allowed detailed imaging of the living embryo.<sup>11,12</sup> The subsequent longitudinal ultrasound studies of the embryological development<sup>13–15</sup> were in agreement with the descriptions from human embryology.<sup>16</sup> Real-time ultrasonography linked with computer technology has made 3D representation of embryonic structures feasible.<sup>17</sup>

We aimed to design a system that used a specially developed transvaginal transducer to enable the study of small embryonic organs in 3D from 7 weeks' to 10 weeks' gestation. We also wanted to describe the development of the living human embryo with emphasis on the shape and the volume of the body and of the brain compartments.

## Methods

We developed a 7.5 MHz annular array 3D transvaginal probe to enable the creation of 3D images of very small structures. The probe had an axial resolution of 0.4 mm and a lateral resolution of 0.8 mm. The scan-plane of the transducer was tilted 45° from the end-fire position, rotating inside a fixed dome. The data acquisition (181 frames/volume, range 89–297) took an average of 5.3 s (2.3–8.6). We stored the digital data in the frame buffer of the scanner (System Five, Vingmed Sound, Horten, Norway) and transferred them to an external computer for further processing with Vingmed EchoPAC-3D software (version 1.1). The rotated 2D planes were converted into a 3D dataset. The objects to be studied were segmented by manual drawing of contours in several parallel 2D slices. The parallel 2D slices could be obtained in any plane, so optimum drawing positions for each object could be determined. From these contours, polyhedrons were created to define the surface and the volume of the objects.<sup>18</sup> The objects could be rotated, scaled, and visualised with different colours and different opacity values.

We recruited 35 healthy pregnant women without any previous pregnancy complications from among women who were referred early in their pregnancy for a routine second-trimester

National Center for Fetal Medicine, Department of Obstetrics and Gynaecology, Trondheim University Hospital, N-7006 Trondheim, Norway (H G Blaas MD, S H Eik-Nes MD), and Department of Physiology and Biomedical Engineering, University of Trondheim (S Berg MSc, H Torp Drtech)

Correspondence to: Dr Harm-Gerd Blaas

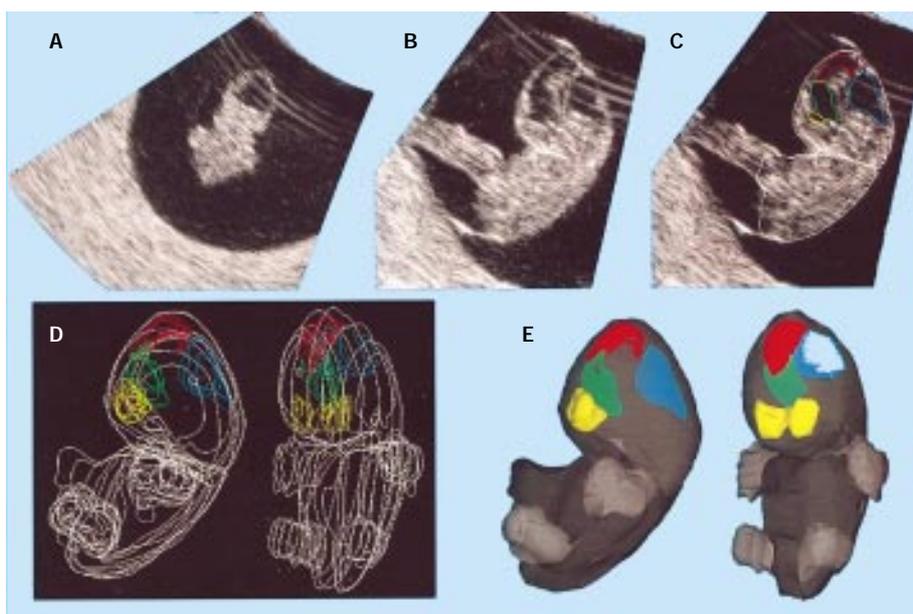


Figure 1: 3D reconstruction of embryo with CRL 17 mm (8 weeks 2 days)

A=original scan plane through gestational sac and embryo; oblique horizontal section through head with hypoechoic fourth ventricle, and upper part of thorax. B=rotated 2D sagittal section through embryo in amniotic cavity; umbilical cord leads to left. C=contours drawn manually around head and body (white), lateral ventricles (yellow), third ventricle (green), cavity of mesencephalon (red), and fourth ventricle (blue). D=3D graph of contours. E=3D graph of volumes of body and brain cavities. Opacity of volume reconstruction of body is decreased to show brain cavities.

ultrasonography at our centre. None of the women had used any hormonal treatment during the 3 months before the pregnancy. All were non-smokers. All women gave written informed consent, and the study was approved by the regional committee for medical ethics. We excluded one woman because the baby died of anomalous pulmonary venous return a few days after delivery. The 34 women included in the study gave birth to 17 boys and 17 girls at a mean of 40 weeks 3 days' gestation (range 38–43 weeks), based on the crown-rump length (CRL). The mean birthweight was 3652 g (2770–4970). All but three women delivered spontaneously. Two babies were delivered by caesarean section at 39 weeks 2 days and at 39 weeks 4 days, respectively;

defined by the point-spread function that depends on the resolution of the ultrasound beam. The surface of an embryo will appear, therefore, not as a sharp silhouette, but as a blurred line. Especially small structures will appear larger than they are on the scan, and the outlining will result in overestimation of volume proportional to the surface of the object being scanned, whereas cavities with inner surfaces may be underestimated. We corrected for the point-spread function by modelling the surface with the formula for a cube.

We tested the validity of the volume measurements with ten cylindrical objects of known volumes ranging from 24.8 mm<sup>3</sup> to 3362.5 mm<sup>3</sup> (table 1). The cylinders had different radii and heights, and were made from a mixture of agar gel and kaolin. Each object was measured three times from different positions in a water bath at 21°C; adjustments were made to allow for ultrasound velocity in water bath at 21°C. The deviation distance for the point spread function was calculated to be 0.2 mm, and all volume estimations were corrected by this factor. The correlation between the volume estimates and the true volumes gave a linear regression with a slope of 0.994 (SE 0.0087), and R<sup>2</sup>=0.998. The percentage error of the volume estimations was mean 15.6% (9.1) for the three smallest test volumes (<500 mm<sup>3</sup>), and mean -0.2% (5.0) for the seven largest test volumes (≥500 mm<sup>3</sup>).

We used cross-sectional regression analysis to assess the relation between the estimated volumes and CRL. Simple models to fit the data were searched for, but transformation

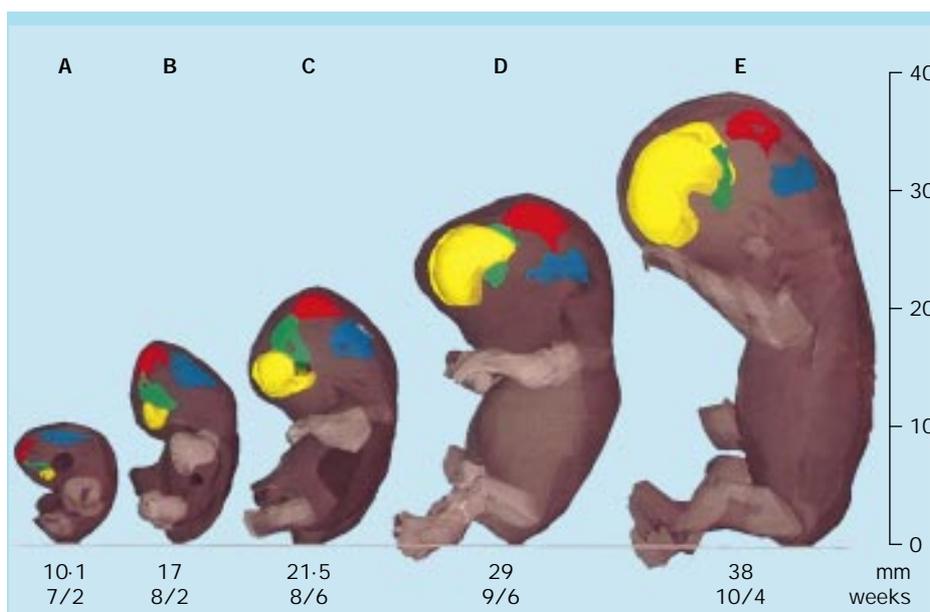


Figure 2: 3D graph of five embryos and fetuses showing development of shape and size from 7 to 10 weeks of gestation

Cavities of hemispheres (lateral ventricles)=yellow, cavity of diencephalon (third ventricle)=green, cavity of mesencephalon=red, cavity of rhombencephalon (fourth ventricle)=blue.

Measured volumes (mm <sup>3</sup> )	Estimated volumes (mm <sup>3</sup> )		
	A	B	C
24.8	27.8	30.6	28.1
132.3	175.8	159.6	149.8
449.3	509.6	492.8	454.6
950.8	892.6	1039.6	1007.6
1458.0	1406.2	1385.9	1448.4
2029.0	2112.7	2201.5	1989.7
2483.1	2480.4	2440.6	2383.0
2607.5	2531.2	2514.4	2528.2
3094.7	2991.6	3341.9	3252.5
3362.5	3166.9	3418.7	3173.0

Table 1: Comparison of measured volumes with 3D volume estimates of test objects

of the responses were necessary in all six analyses to remove the heteroscedasticity. The normality of the residuals was confirmed by visual inspection and by the Shapiro Francia W test. The assumption of independency and constant variance of the residuals was confirmed by the residual plots.

Results

The CRL of the 34 embryos or fetuses ranged from 9.3 mm to 39 mm. It was possible to describe the development of the embryo based on the assessment of the 3D reconstructions from the 34 individuals. The shape of the embryonic bodies altered substantially from the smallest to the largest embryos and fetuses (figure 2). Initially, the embryonic body was slender in the coronal plane. In embryos with 16–24 mm CRL (figure 2), the body gradually grew thicker, becoming cuboidal and finally ellipsoid shaped with a large head in embryos of 25 mm or more CRL. The limbs were short paddle-shaped outgrowths in the smallest embryos. In an embryo of CRL 14.8 mm, the hands were distinct; the elbows became obvious in an embryo of CRL 20.6 mm (figure 2). The hand angled from the sagittal plane in embryos of 20.5 mm CRL and larger. The soles of the feet touched in the midline in 25 mm embryos. In the largest fetuses, the soles of the feet rotated from the sagittal plane. In the smallest embryos (CRL 9.3–17.0 mm), the rhombencephalic cavity was broad and shallow and lay on top, representing the largest brain cavity (figure 2). The cavity deepened gradually with the growth of the embryos, simultaneously decreasing in length. The position in the head changed as embryos grew, moving posteriorly (CRL 17 mm and larger, figure 2). The rhombencephalic cavity (future fourth ventricle) had a pyramid-like shape with the central deepening of the pontine flexure as the peak of the pyramid. In the fetuses of 25 mm CRL and more, there was a clear gap between the rhombencephalic and the mesencephalic cavity due to the growing cerebellum (figure 2). The isthmus rhombencephali was thin; in most cases it was not visible in its complete length.

In the small embryos, the curved tube-like mesencephalic cavity (future Sylvian aqueduct) lay anteriorly, its rostral part pointing caudally. The cavity of the diencephalon (future third ventricle) ran posteriorly. As the size of the embryos increased, the mesencephalic cavity

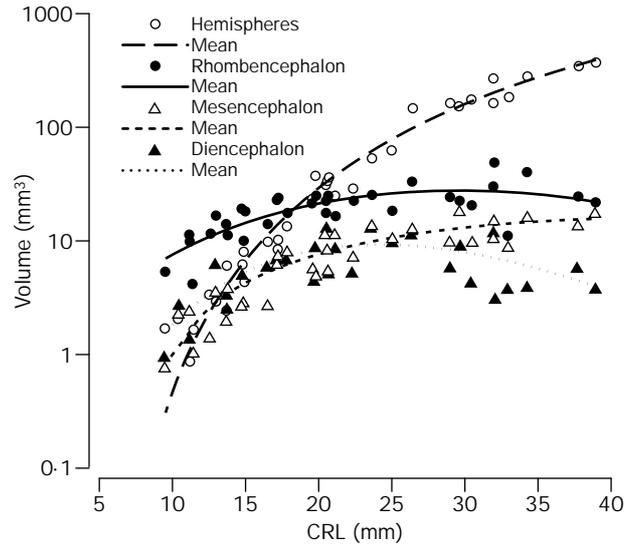


Figure 3: Measurements and mean volume of lateral ventricles, cavities of diencephalon, mesencephalon, and rhombencephalon

changed its position posteriorly. The transition from the third ventricle to the mesencephalic cavity and to the lateral ventricles was wide in the early embryos. The cavity of the mesencephalon was large in relation to total volume in all embryos or fetuses. The largest volume of the future third ventricle (11.7 mm<sup>3</sup>) was found in an embryo with 20.5 mm CRL (figure 3), but was smaller in the larger embryos and fetuses (CRL ≥ 25 mm) with a narrow upper anterior part.

In the smallest embryos, the medial telencephalon formed a continuous cavity between the lateral ventricles. The future foramina of Monro became distinct in embryos of 19.5 mm CRL. The lateral ventricles gradually changed shape from small round vesicles (CRL 9.3–13.6 mm) via thick round slices originating anterocaudally from the third ventricle (CRL 14.6–17.7 mm) into the crescent shape of the larger embryos (CRL ≥ 20.4 mm, figure 2). In the early fetuses, the thick crescent lateral ventricles filled the anterior part of the head and concealed the diencephalic cavity (figure 2), which became smaller.

The range of the estimated volumes was: bodies 122.0–4987.6 mm<sup>3</sup>; all cavities of the brain 6.6–354.1 mm<sup>3</sup>; cavities of the hemispheres 0.8–323.8 mm<sup>3</sup>; cavities of the diencephalon 0.9–11.7 mm<sup>3</sup>; cavities of the mesencephalon 0.7–15.9 mm<sup>3</sup>, and cavities of the rhombencephalon 3.8–42.4 mm<sup>3</sup>. The estimates of the regression coefficients are shown in table 2.

We combined the measurement of the CRL with the external form of the embryos and the casts of the brain cavities;<sup>8,16</sup> this approach gave an estimate of the development stages (table 3). The correlation of the volumes of the embryo/fetuses and their brain cavities to the CRL are presented in figures 3 and 4.

Response	Intercept	CRL	CRL <sup>2</sup>	Residual SE	R <sup>2</sup>
Body*	-10.7 (1.1)	2.05 (0.048)	..	2.33	0.983
Cavities of brain*	-3.25 (0.47)	0.554 (0.020)	..	0.986	0.958
Cavity of hemispheres†	-1.34 (0.14)	0.215 (0.0061)	..	0.293	0.975
Cavity of diencephalon*	-1.22 (0.59)	0.316 (0.056)	-0.0063 (0.0012)	0.446	0.526
Cavity of mesencephalon*	-1.17 (0.51)	0.257 (0.048)	-0.0035 (0.0010)	0.385	0.826
Cavity of rhombencephalon*	..	0.325 (0.017)	-0.0054 (0.0006)	0.641	0.978

\*Square root transformed, cubic root transformed. †Residual and multiple R<sup>2</sup> are shown.

Table 2: Estimates (SE) of regression coefficients by linear regression analysis

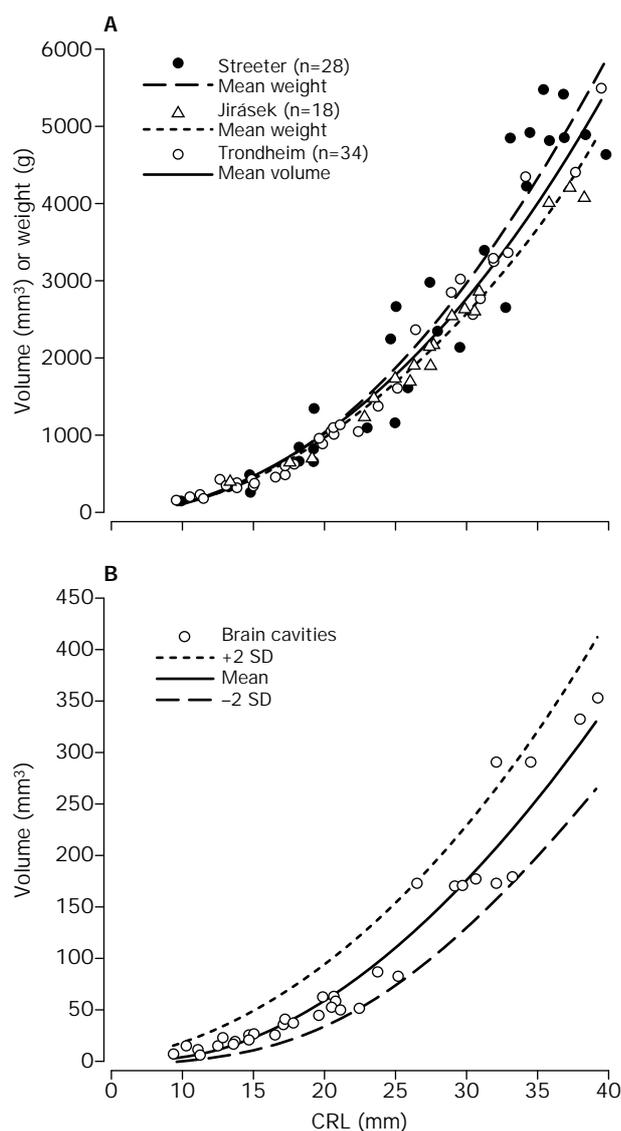


Figure 4: **Volumes of embryos and fetuses and brain cavities**  
 A=mean volume of embryos and fetuses compared with mean weights from Streeter<sup>23</sup> and Jirásek<sup>24</sup> in embryos CLR  $\leq 40$  mm.  
 B=measurements of total volume of brain cavities, regression line, and  $\pm 2$  SD.

## Discussion

3D reconstructions and measurements of embryos and their brain cavities showed similar results to those from classic embryology, and estimations of the stage of embryos could be made. We confirmed the classic descriptions of the external appearance of the embryonic body and limbs, and of the shape, size, and position of the brain compartments.<sup>6,8</sup>

In previous studies, volume reconstruction of large structures was tested *in vitro* and *in vivo*.<sup>18-21</sup> The objects we measured were small, some of them near the limits of the ultrasonographic resolution. Therefore, even small errors in the surface-setting would lead to incorrect volumes. Our test study showed that 3D reconstructions were acceptable, with only a small correction to account for the point-spread function. We believe that the relatively high percentage error in the volume estimation of the small test object of less than 500 mm<sup>3</sup> was due to the surface setting in the segmentation procedure, and we may assume that the volumes of embryos up to 17 mm CRL (<500 mm<sup>3</sup>) became overestimated, whereas volumes of all brain

cavities may have been underestimated. We assume that the volume estimations of the embryos of 17 mm CRL and more ( $\geq 500$  mm<sup>3</sup>) are good, since the percentage error in the test study only was mean  $-0.2\%$ , with an acceptable SE of 5.0.

Because of the difference between the embryonic tissue and the amniotic fluid and between the brain tissue and the fluid-filled brain cavities we could analyse the embryonic structures very early, despite their small size. Limitations were set mainly by the resolution of the ultrasonographic equipment. The short duration of the recordings (2.3–8.6 s) was enough to obtain pictures of the fetuses in a quiet phase. Movements of the pregnant woman, such as breathing or pulsation of the abdominal aorta, did not affect the quality of the images.

The difference between the fetal weight (g) and the volume (mm<sup>3</sup>) is less than 2%.<sup>22</sup> We compared the volumes of the body with measurements of embryos and fetuses that had a corresponding size (CRL  $\leq 40$  mm) from two other studies.<sup>23,24</sup> The regression curves were best fitted by a square-root transformation in all three studies (figure 4). The comparison of these different studies involved several possible biases. Streeter<sup>23</sup> studied fresh and formalin-fixed samples; the latter were substantially heavier and longer.<sup>23</sup> In addition, the largest fetuses in Streeter's group were reported to be flexed, and the CRL might have been larger. Drumm and O'Rahilly<sup>25</sup> analysed the relation between measurements of the CRL *in vivo* by ultrasonography and measurement of the embryo or fetus after abortion. They found that the lengths of embryos in embryological studies was about 1–5 mm less than equivalent *in vivo* CRL measurements.<sup>25</sup> Therefore, the condition of the embryos and fetuses in the different studies was not uniform, which may have affected the shape of the curves.

If the mesencephalic cavity had a cylindrical shape, the volume could be calculated by the formula  $\pi r^2 \times \text{length}$ . The embryos in O'Rahilly and Müller's study<sup>6</sup> (Carnegie stage 23 is about 10 weeks 0 days gestational age and CRL 30 mm) would then have a mean volume of 15 mm<sup>3</sup> based in the measurements indicated from graphic reconstructions, or 23 mm<sup>3</sup> based on the values corrected for shrinkage, whereas the mean volume of the mesencephalic cavity at 10 weeks 0 days in a 2D ultrasound study was only 7.4 mm<sup>3</sup>.<sup>13</sup> In our study the mean volume in embryos and fetuses of 30 mm CRL was calculated to be 11 mm<sup>3</sup> (figure 3). As the rhombencephalic cavity moved posteriorly, it shortened in the sagittal section. In 1890, His showed the changes of the pontine flexure with a decreasing angle in older fetuses.<sup>26</sup> The growth of the cerebellar hemispheres further restricted the volume of the fourth ventricle. In this way, the rhombencephalic cavity did not increase in volume despite the rapid growth of the brain (figure 3). In a previous study, the volumes of the lateral ventricles were estimated

Carnegie stage	Number of embryos and fetuses	CRL range (mm)
16	6	9.3–12.8
17	5	13.5–14.8
18	4	16.4–17.7
19	2	19.5–19.7
20	4	20.4–21.0
21	2	22.3–23.6
22	2	25.0–26.4
$\geq 23$	9	$\geq 29.0$

Table 3: **Distribution of embryos and fetuses according to Carnegie staging system**

by the formula of two ellipsoids and the rhombencephalic cavity by the formula of a pyramid.<sup>14</sup> Our sonographic 3D reconstructions of the lateral ventricles were in the range of the estimated values from the 2D study; our 3D reconstructions of the rhombencephalic cavity were larger than the range of the estimated values from the 2D study, in which the measurements of the width involved only the main part of the fourth ventricle, not the lateral recesses. The size of the diencephalic cavity has not been measured before. Through the growth of the dorsal and ventral thalami, the lateral walls of this cavity narrow, giving it a slit-like shape. This change in shape explains the decreasing volume of the cavity in the older embryos and fetuses in our study, in which only part of the third ventricle between the dorsal thalamus and the mesencephalon, which is the region near the pineal gland, was discernible. According to embryologists, the recesses of the pineal region and near the infundibulum are distinct in older fetuses.<sup>27</sup>

In 1921, Jenkins<sup>28</sup> carried out a volumetric study of the developing brain. Only two of the embryos he studied were smaller than 40 mm (CRL 16 mm and 25 mm), and the volumes of the brains were calculated to be 41 mm<sup>3</sup> and 126 mm<sup>3</sup>. In our study, the mean volume of the brain cavities (figure 4) was 37 mm<sup>3</sup> at 16 mm CRL, and 112 mm<sup>3</sup> at 25 mm CRL; these measurements must be taken to be in agreement with Jenkins' measurements. Jenkins also looked at the proportional growth of the brain compartments, finding a larger share of the diencephalon and the mesencephalon than our measurements showed. At the same time he found less of a shift of the share of the telencephalon and the rhombencephalon between the 16 mm and the 25 mm embryo than we showed (figure 4).

Classic human embryology is based on aborted human embryos and fetuses, as previously summarised.<sup>16</sup> In the Carnegie staging system, embryos are classified by external form and development of the organ system analysed by light microscopy. Such exact staging by ultrasonography is not possible, but we could estimate the stages in the embryos by comparing the external form of the body, the limbs, and the casts of the brain cavities with embryological descriptions.

We showed that it was possible to develop a dedicated transducer system and additional software for 3D imaging of objects of less than 10 mm. The quality of the images allowed description of the outer contours and the development of organ systems, as well as the staging of the embryos between 7 weeks' and 10 weeks' gestation. Our system will also make possible study of abnormal development of the embryo or early fetus and elucidate its progression. The 3D imaging system will help in the avoidance of artefacts and other difficulties in in-vitro assessment of the aborted embryos and may revitalise human embryology.

#### Contributors

Harm-Gerd Blaas planned the study, did the ultrasound examinations and the 3D-reconstructions, and wrote the paper. Sturla H Eik-Nes supervised the planning and revision of the study and its results. Sevald Berg assisted with the technical performance of the 3D reconstructions of the test series and of the study objects. Hans Torp assisted with the mathematical assessment of the 3D analysis and the validity of the method.

#### Acknowledgments

We thank Nancy Lea Eik-Nes for revision of the paper.

#### References

- 1 His W. Anatomie menschlicher Embryonen. Leipzig: Vogel: 1880–85.
- 2 His W. Über Methoden plastischer Rekonstruktion und über deren Bedeutung für Anatomie und Entwicklungsgeschichte. *Anat Anz* 1887; **2**: 382–94.
- 3 Born G. Die Plattenmodelliermethode. *Arch Mikr Anat* 1883; **22**: 584–99.
- 4 Gaunt PN, Gaunt WA. Solid reconstruction. In: Gaunt PN, Gaunt WA, eds. Three dimensional reconstruction in biology. Kent: Pitman Medical, 1978: 63–73.
- 5 Müller F, O'Rahilly R. The human brain at stages 21–23, with particular reference to the cerebral cortical plate and the development of the cerebellum. *Anat Embryol* 1990; **182**: 375–400.
- 6 O'Rahilly R, Müller F. Ventricular system and choroid plexuses of the human brain during the embryonic period proper. *Am J Anat* 1990; **189**: 285–302.
- 7 O'Rahilly R, Müller F. General embryology and teratology: introduction and general concepts. In: O'Rahilly R, Müller F, eds. Human embryology and teratology. New York: Wiley-Liss, 1992.
- 8 O'Rahilly R, Müller F. The embryonic human brain: an atlas of developmental stages. New York: Wiley-Liss, 1994.
- 9 el-Gammal S. ANAT3D: a computer program for stereo pictures of three dimensional reconstructions from histological serial slices. In: Elsner N, Creutzfeld O, eds. New frontiers in brain research. Stuttgart, New York: Thieme, 1987: 46.
- 10 Brinkley JF, McCallum WD, Muramatsu SK, Liu DY. Fetal weight estimation from ultrasonic three-dimensional head and trunk reconstructions: evaluation in vitro. *Am J Obstet Gynecol* 1982; **144**: 715–21.
- 11 Timor-Tritsch IE, Farine D, Rosen MG. A close look at the embryonic development with the high frequency transvaginal transducer. *Am J Obstet Gynecol* 1988; **159**: 678–81.
- 12 Timor-Tritsch IE, Peisner DB, Raju S. Sonoembryology: and organ-oriented approach using a high-frequency vaginal probe. *J Clin Ultrasound* 1990; **18**: 286–98.
- 13 Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR. Early development of the forebrain and midbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 183–92.
- 14 Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR. Early development of the hindbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. *Ultrasound Obstet Gynecol* 1995; **5**: 151–60.
- 15 Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR. Early development of the abdominal wall, stomach and heart from 7 to 12 weeks of gestation: a longitudinal ultrasound study. *Ultrasound Obstet Gynecol* 1995; **6**: 240–49.
- 16 O'Rahilly R, Müller F. Developmental stages in human embryos. Washington: Carnegie Institution Publishing, 1987.
- 17 Blaas HG, Eik-Nes SH, Kiserud T, Berg S, Angelsen B, Olstad B. Three-dimensional imaging of the brain cavities in human embryos. *Ultrasound Obstet Gynecol* 1995; **5**: 228–32.
- 18 Thune N, Gilja OH, Hausken T, Matre K. A practical method for estimating enclosed volumes using 3D ultrasound. *Eur J Ultrasound* 1996; **3**: 83–92.
- 19 Gilja OH, Thune N, Matre K, Hausken T, Ødegaard S, Berstad A. In vitro evaluation of three dimensional ultrasonography in volume estimation of organs. *Ultrasound Med Biol* 1994; **20**: 157–65.
- 20 Gilja OH. In vivo comparison of 3D ultrasonography and magnetic resonance imaging in volume estimation of human kidneys. *Ultrasound Med Biol* 1995; **21**: 25–32.
- 21 Riccabona M, Nelson TR, Pretorius DH. Dreidimensionale Sonographie: zur Genauigkeit sonographischer Volumbestimmungen. *Ultraschall Klin Prax* 1995; **10**: 35–39.
- 22 Meban C. The surface area and volume of the human fetus. *J Anat* 1983; **137**: 217–78.
- 23 Streeter GL. Weight, sitting height, head size, foot length, and menstrual age of the human embryo. *Contr Embryol Carneg Instn* 1920; **11**: 143–70.
- 24 Jirásek JE, Uher J, Uhrová M. Water and nitrogen content of the body of young human embryos. *Am J Obstet Gynecol* 1966; **96**: 868–71.
- 25 Drumm JE, O'Rahilly R. The assessment of prenatal age from the crown-rump length determined ultrasonically. *Am J Anat* 1977; **148**: 555–60.
- 26 His W. Die Entwicklung des menschlichen Rautenhirns vom Ende des ersten bis zum Beginn des dritten Monats. *Abhandl KS Gesellsch Wissensch* 1890; **29**: 3–74.
- 27 Kostovic I. Zentralnervensystem. In: Hinrichsen KV, ed. Humanembryologie. Berlin: Springer-Verlag, 1990: 381–448.
- 28 Jenkins GB. Relative weight and volume of the component parts of the brain of the human embryo at different stages of development. *Contr Embryol Carneg Instn* 1921; **13**: 41–60.