First trimester brain ventricle fluid and embryonic volumes measured by three-dimensional ultrasound with the use of I-Space virtual reality

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STUDY QUESTION: Is it possible to evaluate first trimester brain ventricle development in human pregnancies using an innovative virtual reality (VR) application and to analyze the relation of the embryonic volume (EV) and brain ventricle fluid volume (BVVF) with gestational age (GA), crown-rump length (CRL) and the Carnegie stage?

SUMMARY ANSWER: Volumetry and staging of the human embryo using a VR application make it possible to obtain unique information about in-vivo embryonic normal and abnormal development and about the sizes of the ventricles and body.

WHAT IS KNOWN ALREADY: Human brain development is complex and has a rapidly changing anatomy during the first trimester of pregnancy. New insights will enable early detection of cerebral pathology.

STUDY DESIGN, SIZE, DURATION: In a prospective cohort study, we weekly performed three-dimensional (3D) ultrasound examinations in 112 uncomplicated pregnancies between 6 + 0 and 12 + 6 weeks GA.

MATERIALS, SETTING, METHODS: The examinations resulted in 696 3D ultrasound scans that were transferred to the I-Space VR system and analyzed using V-Scope volume rendering software. V-Scope is used to create a 'hologram' of the ultrasound image and allows depth perception and interaction with the rendered objects. The CRL measurements were performed with a tracing tool, and the volume measurements were automatically performed with a segmentation algorithm. The embryos were staged according to the internal and external characteristics of the Carnegie staging system. All longitudinal outcomes were analyzed using repeated measures ANOVA.

MAIN RESULTS AND THE ROLE OF CHANCE: CRL could be measured in 91% of the datasets and ranged from 2.5 to 79.0 mm. EV could be measured in 66% of the datasets and ranged from 2.4 to 23 812.0 mm³, whereas the BVVF could be measured in 38% of the datasets and ranged from 10.4 to 226.3 mm³. Finally, in 74% of the datasets, the embryos were staged according to the Carnegie criteria, starting as early as stage 12. Reference charts of volumes versus GA, CRL and stage were constructed. There was no significant relationship between the CRL or EV and the birthweight.

LIMITATIONS, REASONS FOR CAUTIONS: The low success rate is a limitation of this study that can be explained mainly by non-targeted scanning of the embryonic head.

WIDER IMPLICATIONS OF THE FINDINGS: The I-Space VR system and the V-Scope software enable automatic EV and BVVF measurements and 3D observations of embryonic development in the first trimester. This allows in-vivo staging of human embryos based on both internal and external morphological characteristics.

STUDY FUNDING, COMPETING INTERESTS: None.

Key words: brain ventricle / virtual reality / 3D ultrasound / embryo staging / first trimester
Introduction

Evaluating embryonic and fetal health during and at the end of the first trimester will become more and more important as a quality control in reproductive medicine. Innovative ultrasonographic reference data are essential for early in-vivo detection of abnormal development, structural defects and associated chromosomal abnormalities. Monitoring brain development during the first trimester of pregnancy is difficult because of a complex and rapidly changing anatomy. The introduction of transvaginal ultrasonography has contributed to some observational studies on brain development in the embryonic period (Blaas et al., 1994, 1995a,b). New insights into primitive brain ventricle appearance along a developmental timeline will enable early detection of cerebral pathology (Blaas et al., 2000; Timor-Tritsch et al., 2008).

The development of the embryonic brain can be visualized by measuring the volume of the brain ventricles (Blaas et al., 1995a, 1998; Kim et al., 2008). However, the methods involved either are time-consuming due to manual segmentation or do not allow quantification of the volume.

In 2005, the department of Bioinformatics at Erasmus University Medical Center introduced a Barco I-Space virtual reality (VR) system. With this system, a three-dimensional (3D) ultrasound dataset is presented as a free floating ‘hologram’ that allows the study of the morphology of the embryo in detail (Verwoerd-Dikkeboom et al., 2008a). In VR, several length measurements can be performed (Verwoerd-Dikkeboom et al., 2008b, 2010). Instant and semi-automated volume measurements can be performed on different embryonic structures, such as the brain ventricles, or on the total body (Rousian et al., 2009, 2010). Because this tool enables the user to visualize and quantify the brain ventricles, this aids with in-vivo staging of embryos based on both internal and external morphological characteristics (O’Rahilly and Muller, 1987, 2010). With the development of a low-budget desktop 3D VR system running on a personal computer, the new technique will become available for routine clinical use within the near future.

The aim of this study was to evaluate the embryonic development of the brain ventricles and Carnegie stages and to measure the crown-rump length (CRL) and embryonic volume (EV) in uncomplicated first

Figure 1  I-Space holograms of three embryos showing development of shape and size of the brain ventricles. (A) An embryo of 8 + 2 weeks GA (CRL of 15 mm and Carnegie stage 18). (B) An embryo of 8 + 6 weeks GA (CRL of 21 mm and Carnegie stage 20). (C) An embryo of 9 + 6 weeks GA (CRL of 28 mm and Carnegie stage 22). 1: Mid-sagittal plane of the embryo. The BVFV is segmented in magenta. 2. The axial view of the brain ventricles. 3. The sagittal view of the brain ventricles. The lateral ventricles, cavity of the mesencephalon, third ventricle (=cavity of diencephalon) and the fourth ventricle (=cavity of the rhombencephalon) are displayed.
trimester pregnancies using VR, to obtain a baseline for use in future research on abnormal brain development. A secondary aim was to study the predictive value of the first trimester CRL and embryonic body volume measurements on the outcome of pregnancy in terms of birthweight.

**Materials and Methods**

**Patients**

We recruited 141 volunteering pregnant women. These women were recruited via the Department of Obstetrics and Gynaecology at the Erasmus University Medical Center Rotterdam in The Netherlands. A small proportion (around 20%) of women were recruited from outside the university hospital, but still from the same area. All women gave written informed consent, and the regional committee for medical ethics approved the study.

After giving birth, data about the outcomes of pregnancy were collected. From the 141 women who participated in the study, a total of 29 patients were excluded because of multiple gestation, fetal complications or personal reasons. Of these, 3 carried a twin or triplet pregnancy, 16 had a first trimester miscarriage, 2 experienced an intrauterine fetal death, 3 carried a child with a structural congenital anomaly and 2 were diagnosed with a trisomy 21 pregnancy. Additionally, one patient discontinued the study because of her religious background and two discontinued because they were unable to participate in our research on a weekly basis.

The remaining 112 volunteers were scanned weekly from 6 to 7 weeks until 12 weeks of gestational age (GA). GA was calculated from the first day of the last menstrual period (LMP). In case of an unknown LMP or a discrepancy of a week or more, GA was determined using the CRL measurements performed in the first trimester [The American College of Obstetricians and Gynecologists (2009); ACOG practice bulletin nr. 101]. The CRL measurements performed in this study are the greatest length of the embryo and early fetus. For the IVF/ICSI pregnancies, the GA was based on the date of oocyte retrieval.

A total of 696 3D ultrasound scans were performed in these 112 women, although no specific attempt to capture the brain was made. When the brain ventricle fluid volumes (BVFVs) were measured, a selection was made by excluding images that lacked contrast or lacked parts of the brain cavities of the embryo due to shadowing or an unfavorable position of the embryo. Another reason for excluding images was movement artifacts. Before performing EV measurements, scans that lacked contrast or parts of the embryo were excluded.

In six women, GA adjustments were made due to a discrepancy of the CRL of seven gestational days or more. These women were excluded when evaluating various parameters in relation to GA.

**Ultrasound equipment**

The ultrasound examinations were performed using a 4.5–11.9 MHz vaginal probe of the GE Voluson E8 (GE, Zipf, Austria) by two different examiners. The scans were evaluated off-line using specialized 3D software (4D View, version 7.0, GE Medical Systems), and the included scans were stored as Cartesian volumes that were later transferred and visualized using the BARCO I-Space of the Department of Bioinformatics.

**Figure 2** I-Space holograms of four different embryos are displayed. Especially, the development of the limbs and the curvature of the embryo are used as external morphological criteria when the embryos were staged. These I-Space holograms are displayed as 2D images and cannot capture the 3D aspect of the I-Space system.
Length and volume measurements

The I-Space is a so-called four-walled CAVE Automatic Virtual Environment (Cruz-Neira et al., 1993). A special volume rendering application called V-Scope was written to create an interactive hologram of the ultrasound image (Koning et al., 2009). This hologram can be manipulated by means of a virtual pointer that is controlled by a wireless joystick. Two different integrated functions were used in our study: the length measuring tool and the volume measuring tool. As described in detail by Verwoerd-Dikkeboom et al. (2008b), a trace application makes it possible to measure the lengths of the structures of interest. This tool was used to measure the CRL of the serially scanned embryos (O’Rahilly and Muller, 2000). The volume measuring tool is based on a region-growing approach (Rousian et al., 2009). The algorithm has been modified to handle the speckles in ultrasound data by simplifying some of the parameters of the original algorithm and smoothing the gray-level data. The user selects an upper and lower gray-level threshold and an upper threshold for the standard deviation (SD) of the voxel’s neighborhood. A seed point is placed, and the algorithm grows the region starting from the seed point. The SD threshold stops the region growing when it reaches a tissue interface. Post-processing tools are available to correct for incomplete segmentations. The user can manually grow or shrink the segmented region, and a spherical, freehand ‘paint brush’ can be used to add voxels to, or to delete voxels from the segmented structure when necessary. The reliability and accuracy of the volume measuring tool have been proven in both in-vivo and in-vitro settings. Embryonic body, yolk sac and phantom volumes were measured in these studies, including both regular and irregular shaped structures (Rousian et al., 2009, 2010). We used this tool to establish the embryonic BVFV (see Fig. 1) and the embryonic body. To measure the BVFV, the hypoechoic structures of the embryo have to be segmented as well (Rousian et al., 2010). Before the EV is measured, the embryonic insertion of the umbilical cord and its connections with the placenta have to be ‘brushed’ away with the eraser function to avoid segmentation of other parts than the whole body.

All BVFV and CRL measurements were performed three times, and mean values of these three assessments were used for the analysis.

The duration of an off-line V-Scope BVFV measurement was approximately 1 min (Supplementary data Movie S1). The duration of an EV measurement ranged between 5 and 10 min. Given the time required and the very good inter-observer variability score obtained in our previous study, only one-third of all EV measurements were performed three times.

Embryonic staging

The embryos were staged based on their internal and external morphological characteristics using the Carnegie criteria (O’Rahilly and Muller, 1987, 2010). The Carnegie system is based on 23 stages covering the first 8 post-fertilization weeks of development (O’Rahilly and Muller, 2010). Embryonic staging is, therefore, only possible up to gestational week 10 + 1 of pregnancy and generally takes minutes to perform. Verwoerd-Dikkeboom et al. (2008a) staged the embryos in VR using only the limb development criteria. In our study, both external and internal morphological criteria were used. As external morphological characteristics, the Carnegie criteria for the limb development and curvature of the developing embryo were used (see Fig. 2). For the internal morphological development, the criteria for the brain ventricle development were primarily used.

Statistical analysis

Data analysis was performed using SPSS (SPSS Release 17.0 for Windows) and SAS PROC MIXED (release 9.2; SAS Institute Inc, Cary, NC, USA).

To analyze the longitudinal measurements, we used repeated measures ANOVA (random coefficient models).

To analyze the relation between the BVFV and the CRL or GA, or between the EV and the Carnegie stage, we used the equation: \[ \log_2(\text{BVFV or EV}) = a + b \times \log_2(\text{CRL or GA or stage}) \] because the logarithmic transformation of both axes results in an approximate linear relationship. For the relationship between the EV and the GA or CRL, we used the equation: \[ \log_2(\text{EV}) = a + b \times \log_2(\text{CRL or GA}) + c \times \log_2(\text{CRL or GA})^2 \]. The following equation was used for the analysis of the relation between the BVFV or CRL and the Carnegie stage: \[ \sqrt{\text{brain ventricle volume or CRL}} = a + b \times \text{stage} \]. Finally, a linear relationship fitted best when the GA was related to the Carnegie stage: \[ \text{GA} = a + b \times \text{stage} \].

In the charts relating the GA or CRL to the Carnegie stage, the range (minimum and maximum values) of the GA and CRL according to O’Rahilly and Muller (2010) were plotted.

When the parameters were related to the GA, the six patients with un-reliable GA according to the CRL measurements performed in the first trimester were excluded.

The birthweight SD score (SDS) of the study population was measured using the formulae of a large birth cohort, the Generation R study (Mook-Kanamori et al., 2010). The calculated SDSs are adjusted for gender and for GA at birth. The correlation between the birthweight SDS with the first trimester CRL or EV was measured with linear regression analysis. Finally, \( P = 0.05 \) (two sided) was considered the limit of significance.

Results

Data from a total group of 112 women were evaluated. Table I shows the characteristics of these patients and their newborns.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range) or percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers (( n = 112 ))</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>32.9 (18.9–42.7)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>62.5%</td>
</tr>
<tr>
<td>1</td>
<td>27.7%</td>
</tr>
<tr>
<td>( \geq 2 )</td>
<td>9.8%</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1–10)</td>
</tr>
<tr>
<td>Miscarriages ( \geq 2 )</td>
<td>25.9%</td>
</tr>
<tr>
<td>Conception mode</td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>70.5%</td>
</tr>
<tr>
<td>IVF/ICSI</td>
<td>27.7%</td>
</tr>
<tr>
<td>IUI</td>
<td>1.8%</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>5.4%</td>
</tr>
<tr>
<td>Hypertensive disorder</td>
<td>8.9%</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>3.6%</td>
</tr>
<tr>
<td>Newborns (( n = 112 ))</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>52.7%</td>
</tr>
<tr>
<td>Birthweight (grams)</td>
<td>3390 (450–4700)</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>39 + 4 (26 + 4–42 + 0)</td>
</tr>
</tbody>
</table>

Data shown are median (range) or percentages of patients.
A total of 696 3D ultrasound scans were performed, with a median of 6 scans per patient (range: 4–8 scans). The median GA was 68 days and ranged between 42 and 90 days. CRL measurements could be performed in 636 (91.4%) scans (median: 28.1 mm; range: 2.5–79.0 mm) and EV measurements could be performed in 458 (65.8%) scans (median: 1657.0 mm³; range: 2.4–23812.0 mm³). A total of 696 3D ultrasound scans were performed, with a median of 6 scans per patient (range: 4–8 scans). The median GA was 68 days and ranged between 42 and 90 days. CRL measurements could be performed in 636 (91.4%) scans (median: 28.1 mm; range: 2.5–79.0 mm) and EV measurements could be performed in 458 (65.8%) scans (median: 1657.0 mm³; range: 2.4–23812.0 mm³).

Table II  Numbers and percentages of successful CRL, EV, BVFV and Carnegie stage measurements per gestational week.

<table>
<thead>
<tr>
<th>Gestational week</th>
<th>n</th>
<th>CRL</th>
<th>n</th>
<th>EV</th>
<th>n</th>
<th>BVFV</th>
<th>n</th>
<th>Carnegie</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>66</td>
<td>53 (80.3%)</td>
<td>66</td>
<td>37 (56.1%)</td>
<td>--</td>
<td>--</td>
<td>66</td>
<td>38 (57.6%)</td>
</tr>
<tr>
<td>7</td>
<td>102</td>
<td>89 (87.3%)</td>
<td>102</td>
<td>77 (75.5%)</td>
<td>102</td>
<td>31 (30.4%)</td>
<td>102</td>
<td>73 (71.6%)</td>
</tr>
<tr>
<td>8</td>
<td>103</td>
<td>97 (94.2%)</td>
<td>103</td>
<td>81 (78.6%)</td>
<td>103</td>
<td>54 (52.4%)</td>
<td>103</td>
<td>82 (79.6%)</td>
</tr>
<tr>
<td>9</td>
<td>107</td>
<td>103 (96.3%)</td>
<td>107</td>
<td>86 (80.4%)</td>
<td>107</td>
<td>54 (50.5%)</td>
<td>107</td>
<td>86 (80.4%)</td>
</tr>
<tr>
<td>10</td>
<td>109</td>
<td>106 (97.2%)</td>
<td>109</td>
<td>75 (68.8%)</td>
<td>109</td>
<td>22 (20.2%)</td>
<td>27</td>
<td>20 (74.1%)</td>
</tr>
<tr>
<td>11</td>
<td>108</td>
<td>103 (95.4%)</td>
<td>108</td>
<td>64 (59.3%)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>101</td>
<td>85 (84.2%)</td>
<td>101</td>
<td>38 (37.6%)</td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>

*n* is the total number of datasets available per gestational week.

Figure 3  EV related to the CRL (A) and GA (B). The P50 curve is indicated by the solid line, and the 95% prediction interval is indicated by the dotted lines.

Figure 4  BVFV related to the GA (A) and CRL (B), with the corresponding P50 line (solid line) and 95% prediction interval (dotted lines).
total of 421 scans were performed between 7 + 0 and 10 + 6 weeks GA (median per patient: 4; range: 2–5), being the period for the BVFV measurements. BVFV was measured in 161 (38.2%) datasets (median: 45.1 mm³; range: 10.4–226.3 mm³). The embryos were staged until 10 + 1 weeks GA. Between 6 + 0 and 10 + 1 weeks GA, 405 scans (median per patient: 4; range: 1–5) were performed, and staging was possible in 299 (73.8%) scans (median 19; range: 12–23). Table II shows the total number of CRL, EV, BVFV and Carnegie staging scans per gestational week. The total number of datasets used and the total number of measurements performed are also shown.

Fig. 3 shows the relation between the EV and the GA or CRL. The relation between the brain ventricle volume and the GA or CRL is shown in Fig. 4. Fig. 5 illustrates the relation between the EV or brain ventricle volume and the Carnegie stage.

The Carnegie stages are related to the GA and CRL, as shown in Fig. 6. The original data from O’Rahilly and Muller (2010) are also plotted in these graphs (red boxes). A better fit is observed when the Carnegie stage is related to the CRL. The GA has a wider range, but still fits well. Especially in Carnegie stage 22 and 23 embryos, the CRL measured in the I-Space is larger in comparison to the CRL measured by O’Rahilly and Muller.

Table III shows the formulae of the relations calculated in this study, with the corresponding variance.

The mean birthweight SDS was 0.05 and was normally distributed ($P = 0.57$). Separate analysis of CRL and EV data for each week of gestation for their relation with birthweight SDS using regression analysis did not show a significant correlation (all $r^2 < 0.033$) for any week between 6 and 12 weeks’ GA. Categorizing birthweight SDS into categories less than –1, –1 to +1 and greater than +1 also did not show differences between these groups for longitudinal profiles of CRL or EV along advancing GA.

**Discussion**

By measuring the BVFV automatically using transvaginal 3D ultrasound data in a VR application, we were able to systematically evaluate in-vivo
Table III  Mean and SD formula for the relation between various parameters.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>n</th>
<th>Line</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVFV versus GA</td>
<td>158</td>
<td>Mean</td>
<td>( \log_2(\text{BVFV}) = -31.0782 + 6.1658 \times \log_2(\text{GA}) )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 14.95151 - 4.6654 \times \log_2(\text{GA}) + 0.3674 \times \log_2(\text{GA})^2 )</td>
</tr>
<tr>
<td>BVFV versus CRL</td>
<td>161</td>
<td>Mean</td>
<td>( \log_2(\text{BVFV}) = -3.4261 + 2.0485 \times \log_2(\text{CRL}) )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 0.71027 - 0.3044 \times \log_2(\text{CRL}) + 0.03702 \times \log_2(\text{CRL})^2 )</td>
</tr>
<tr>
<td>EV versus GA</td>
<td>432</td>
<td>Mean</td>
<td>( \log_2(\text{EV}) = -11.3174 + 1.81004 \times \log_2(\text{GA}-40) )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 0.3044 \times \log_2(\text{GA}-40) + 0.03702 \times \log_2(\text{GA}-40)^2 )</td>
</tr>
<tr>
<td>EV versus CRL</td>
<td>458</td>
<td>Mean</td>
<td>( \log_2(\text{EV}) = -8.72 + 1.9812 \times \log_2(\text{CRL}-4) )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 0.3400 \times \log_2(\text{CRL}-4) + 0.00695 \times (\text{CRL}-4)^4 )</td>
</tr>
<tr>
<td>CRL versus Carnegie</td>
<td>299</td>
<td>Mean</td>
<td>( \sqrt{\text{CRL}} = -1.7375 + 0.3160 \times \text{stage} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 0.09498 + 2 \times \text{stage} - 0.00374 + 0.000201 \times \text{stage}^2 )</td>
</tr>
<tr>
<td>GA versus Carnegie</td>
<td>281</td>
<td>Mean</td>
<td>( \log_2(\text{GA}) = 10.3612 + 2.5530 \times \text{stage} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 19.0351 + 0.5038 \times \text{stage} + 0.01605 \times \text{stage}^2 )</td>
</tr>
<tr>
<td>EV versus Carnegie</td>
<td>295</td>
<td>Mean</td>
<td>( \log_2(\text{EV}) = -39.2655 + 9.0549 \times \log_2(\text{stage}) )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 13.514 - 5.9324 \times \log_2(\text{stage}) + 0.6573 \times \log_2(\text{stage})^2 )</td>
</tr>
<tr>
<td>BVFV versus Carnegie</td>
<td>146</td>
<td>Mean</td>
<td>( \sqrt{\text{BVFV}} = -12.3021 + 0.9662 \times \text{stage} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 1.6102 + 2 \times \text{stage} - 0.1050 + 0.007735 \times \text{stage}^2 )</td>
</tr>
</tbody>
</table>

EV and BVFV units are mm³, GA is measured in days and the CRL in mm. n represents the total number of data points on which the formulae are based.

The SDs of the individual data point around the fitted curves are obtained by taking the square root of the variance.
first trimester brain ventricle development for the first time. In addition, we measured the CRL and EV and determined the Carnegie stage based on the internal and external morphological criteria.

In various studies, Blaas et al. (Blaas et al., 1995a; Blaas et al., 1998) used specialized software to measure the size of the brain ventricles (including the choroid plexuses), outlining them by hand in different two-dimensional (2D) slices of the 3D image. In our own study, we measured the total fluid volume of the BVFV (excluding the choroid plexus). As the ventricle volume measurements are automated in the I-Space, any visible connection between the ventricles can be used as a medium through which the adjacent ventricle can be measured.

Post-processing, to obtain a correct and complete segmentation of the brain ventricles, was only necessary in a limited number of cases. Additionally, BVFV measurement usually takes less than 1 min, whereas measuring by manual segmentation is much more time-consuming, and as a previous study on validation of VR volume measurements showed, also less precise (Rousian et al., 2009).

To date, only Blaas et al. (Blaas et al., 1995a; Blaas et al., 1998) have succeeded in measuring embryonic brain ventricle volumes in a cross-sectional setting using a specially developed annular array 3D transvaginal probe. They manually drew the contours of the ventricles in several parallel 2D slices of the 3D image, which was very time-consuming. Two other 3D ultrasound visualization methods have been used to visualize the human embryonic brain ventricles semi-automatically (Timor-Tritsch et al., 2008; Pistorius et al., 2009). These cross-sectional case studies followed the embryonic brain development in only a few patients. Although these studies showed an improvement of the visualization of the human embryonic brain ventricles, quantitative volume measurements were not made due to the small size of these structures.

Enlarged brain cavities are important diagnostic markers for central nervous system disorders in early pregnancy (Matsunaga and Shiota, 1977; Becker et al., 2000; Blaas et al., 2000; Machado et al., 2005; Timor-Tritsch et al., 2008). The reference charts constructed in this study provide accurate quantitative information and may help to differentiate between normal and abnormal early embryonic development. In contrast with regular 3D ultrasound, where the 3D image is evaluated on 2D media (screen or on paper), the I-Space allows us to use the third dimension of the 3D ultrasound image to its fullest extent and provides a better impression of the developing embryo. As this is essential for in-vivo staging according to the classical Carnegie stage system, it provides us with unique information about the state of development that, together with age and size of the embryo, is an independent parameter (O’Rahilly and Muller, 1987, 2010).

Staging gives information on embryonic development additional to embryonic growth and is of importance in the understanding of abnormal embryogenesis. The stages were related to CRL, BVFV and EV, resulting in smaller prediction intervals in comparison to GA alone. However, it is important to remember that this staging system is based not only on morphological characteristics but also on histologic ones that cannot be determined in the I-Space (O’Rahilly and Muller, 2010). Secondly, we should remember that in-vitro verification was not possible in this study.

Low birthweight babies are at increased risk of perinatal and infant morbidity and mortality and of cardiovascular and metabolic diseases in later life (Kramer, 1987; Barker, 1990; Gluckman et al., 2008; Mook-Kanamori et al., 2010). Whereas some studies have correlated delayed first trimester CRL growth with a low birthweight (Smith et al., 1998; Bukowski et al., 2007; Leung et al., 2008), another study has not (Habayeb et al., 2010). Antsaklis et al., (2011) concluded that the fetal volume correlates better with birthweight than the CRL between 11 + 0 and 13 + 6 weeks’ GA. Neither the CRL nor the EV measured in our study between 6 and 12 weeks GA was associated with birth-weight SDS. The SDS formulae used in this study are based on women living in Rotterdam that, given the normally distributed birthweight SDS, fit well with our population. There may be a power problem due to the limited number of measurements included. Almost 30% of women had a certain GA due to assisted reproductive therapy. An explanation may be that in the remaining patients, a relatively wide range (± 7 gestational days) of variance in the CRL was accepted as dating was based on their LMP.

In case of the brain ventricle segmentation, a clear delineation between fluid and the tissue interface is needed to measure the BVFV. We performed off-line BVFV measurements on previously recorded 3D datasets. These datasets were collected without specifically targeting the brain ventricles, which may explain the low overall success rate. Unfortunately, lack of depth penetration (due to BMI or an intermediate position of the uterus), movements and acoustic shadows will also affect the visualization quality and negatively influence the measurement abilities. In gestational weeks 8 and 9, approximately 50% of the VR BVFV measurements are adversely affected by these factors, meaning that the highest quality images are obtained in this period. As the entire embryo was targeted during scanning, the resulting much larger volume makes it less sensitive to small movements and acoustic artifacts. For these reasons, the success rate for EV measurements was higher. When the measurements of the BVFV are performed after targeted scanning of the brain cavities, the success rate is expected to be higher.

We are aware of the fact that most other hospitals currently do not have access to VR equipment. A desktop version running the same software as the I-Space has now been developed and is being tested for routine clinical use at our department. This low-cost VR desktop system will become available in the near future and will enable other hospitals to obtain the most information from their 3D images, as we were able to do in our study.

In conclusion, the I-Space VR system and the V-Scope software enable us to measure EV and BVFV automatically and to observe the embryonic development in the first trimester by visualizing all three dimensions. This approach makes it possible to perform in-vivo staging of human embryos based on both internal and external morphological characteristics.

The next step in our research will be to study abnormal development of the embryo and early fetus in VR systematically. This will provide us with new insights into abnormal development in vivo, to assist in the early diagnosis of abnormal embryogenesis.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors’ roles

M.R. contributed to study design, data collection, data analyses and writing of the manuscript. W.C.H. is a professional statistician and
supervised the statistical methods used in this study and revised the manuscript. A.H.J.K. developed the V-Scope software and revised the manuscript. P.J.v.d.S., N.E. and E.A.P.S supervised the study and revised the draft version of the manuscript. E.A.P.S. initiated the study.

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**Conflict of interest**

None declared.

**References**


