An innovative virtual reality technique for automated human embryonic volume measurements

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BACKGROUND: The recent introduction of virtual reality (VR) enables us to use all three dimensions in a three-dimensional (3D) image. The aim of this prospective study was to evaluate an innovative VR technique for automated 3D volume measurements of the human embryo and yolk sac in first trimester pregnancies.

METHODS: We analysed 180 3D first trimester ultrasound scans of 42 pregnancies. Scans were transferred to an I-Space VR system and visualized as 3D ‘holograms’ with the V-Scope volume-rendering software. A semi-automatic segmentation algorithm was used to calculate the volumes. The logarithmically transformed outcomes were analysed using repeated measurements ANOVA. Interobserver and intraobserver agreement was established by calculating intraclass correlation coefficients (ICCs).

RESULTS: Eighty-eight embryonic volumes (EVs) and 118 yolk sac volumes (YSVs) were selected and measured between 5+5 and 12+6 weeks of gestational age (GA). EV ranged from 14 to 29,877 mm3 and YSV ranged from 33 to 424 mm3. ANOVA calculations showed that when the crown-rump length (CRL) doubles, the mean EV increases 6.5-fold and when the GA doubles, the mean EV increases 500-fold (P < 0.001). Furthermore, it was found that a doubling in GA results in a 3.8-fold increase of the YSV and when the CRL doubles, the YSV increases 1.5-fold (P < 0.001). Interobserver and intraobserver agreement were both excellent with ICCs of 0.99.

CONCLUSION: We measured the human EV and YSV in early pregnancy using a VR system. This innovative technique allows us to obtain unique information about the size of the embryo using all dimensions, which may be used to differentiate between normal and abnormal human development.

Key words: embryonic volume / fetal volume / virtual reality / 3D ultrasound / first trimester

Introduction

First trimester ultrasound is widely used for early pregnancy localization and assessment of viability. The crown-rump length (CRL) measurement is used for an accurate determination of gestational age (GA) (Robinson and Flemming, 1975). From recent studies on first trimester dating and growth, however, it is now known that a smaller than expected CRL may also be related to impaired embryonic growth (Bottomley and Bourne, 2009). The latter is not only associated with first trimester miscarriage (Mukri et al., 2008) and aneuploidy (Goldstein et al., 1996; Schemmer et al., 1997), but also with fetal growth restriction in the second and third trimester of pregnancy (Smith et al., 1998; Bukowski et al., 2007; Bottomley and Bourne, 2009). As a consequence there is a growing interest in tools for more precise measurement of embryonic growth.

A better assessment of embryonic growth by embryonic volume (EV) measurements in comparison with CRL measurements has been suggested in several studies (Aviram et al., 2004; Falcon et al., 2005a,b). Because of the irregular shape of the embryo, however, it is difficult to acquire accurate EV measurements. The first successful attempt was published by Blaas et al. (1998). The authors used vaginal three-dimensional (3D) ultrasound data and segmented the objects by manually drawing contours in several parallel two-dimensional (2D) slices. Polyhedrons were created, using specialized software, to define the surface and volume of these objects. This group reported that a significant proportion of the EV is represented
by the limbs (Blaas et al., 2006). Others used the Virtual Organ Computer-Aided Analysis (VOCAL®) method, which makes it possible to accomplish rotational volume measurements. These volume estimations are performed by drawing contours around the embryo in various rotational steps, without including the limbs, resulting in a fetal trunk and head volume only (Falcon et al., 2005a, b; Martins et al., 2008).

In a virtual reality (VR) system, such as the I-Space, high-resolution 3D ultrasound data sets can be visualized as holograms with optimal depth perception. We use the V-Scope volume-rendering application to benefit from all three dimensions in the immersive I-Space (Barco, Belgium) VR system (Fig. 1). The combination of V-Scope and the I-Space has already been successfully applied to 3D prenatal ultrasonography (Groenenberg et al., 2005; Verwoerd-Dikkeboom et al., 2008a,b,c; Rousian et al., 2009). Even the classical staging system of embryonic development, the Carnegie stages, could be determined by inspecting the embryo from different angles (Verwoerd-Dikkeboom et al., 2008b).

Recently a region-growing segmentation algorithm, which calculates the volume of selected structures of interest semi-automatically, has been implemented in V-Scope. The algorithm was validated by Rousian et al. (2009), both in vitro and in vivo, using balloons and yolk sacs. The next step is to test the V-Scope technique for complex structures like the embryo, including the limbs. Although the measurements were performed in the embryonic as well as in the early fetal period, in this manuscript we use the term embryo throughout.

The aim of this study was to evaluate the use of an automated VR application for volume measurements of first trimester human embryos, including the limbs. We also measured the human yolk sac volumes (YSVs) and studied the interobserver and intraobserver variability of the EV measurements.

**Patients and methods**

**Patient selection**

A group of 50 volunteering pregnant women, without any predisposing condition or medication use that could interfere with normal embryonic growth, was included in a longitudinal first trimester 3D ultrasound study (Verwoerd-Dikkeboom et al., 2008a,b). Written informed consent was obtained, and the regional committee for medical ethics approved the study. In the 25 spontaneous and 25 IVF/ICSI pregnancies, 3D ultrasound scans were performed serially from ~6–8 to 12 weeks of gestation. GA was calculated using the first day of the last menstrual period, and in cases of an unknown last menstrual period or a discrepancy of more than a week, the GA was determined by the CRL measurements performed in the first trimester. For the IVF/ICSI pregnancies, the GA was based on the date of oocyte retrieval. During the ultrasound examinations, three patients were found to have twin pregnancies and two patients were diagnosed with non-viable pregnancies. These patients were excluded during the examination period. One patient was diagnosed with a placental confined trisomy 16 mosaicism (Verwoerd-Dikkeboom et al., 2008c) and two patients developed severe placental insufficiency during the second half of pregnancy. We also excluded these ultrasound examinations. Data from 42 women, consisting of 21 spontaneous and 21 IVF/ICSI pregnancies, remained in our study cohort. In this group a total of 180 3D ultrasound scans were performed, with a mean of 4.3 ultrasound scans per patient (standard deviation (SD): 1.7 scans).

Not all 180 images could be visualized optimally in the I-Space and since this is the first study describing embryonic and yolk sac growth by using a VR application, we included the images with the best image quality only. Thus images were excluded because the embryonic features could not be recognized due to poor image quality or because they were lacking parts of the embryo or the yolk sac. Finally, 88 images of the embryos of 40 women and 118 images of the yolk sacs of 41 women remained for further study.

**Ultrasound measurements**

Our study was performed using a 3.7–9.3 MHz transvaginal probe of the GE Voluson 730 Expert system (GE, Zipf, Austria). The 3D ultrasound scans were acquired by one examiner (C.M.V.). Later, the scans were stored as Cartesian volumes using specialized 3D software (4DView, version 5.0, GE Medical Systems) and visualized using the I-Space, a so-called four-walled CAVE™-like (Cruz-Neira et al., 1993) (Cave Automatic Virtual Environment) VR system.

**I-Space measurements**

The V-Scope (Koning et al., 2009) application is used to create an interactive hologram of the ultrasound image that can be manipulated by means of a virtual pointer, controlled by a wireless joystick. To perform volume measurements, this application includes a flexible and robust segmentation algorithm that is based on a region-growing approach in combination with a neighbourhood variation threshold, as originally proposed for magnetic resonance imaging data by Myers and Brinkley (1995). 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 grey level data using a Gaussian blur. The user selects an upper and lower grey-level threshold and an upper threshold for the SD of the voxels’ neighbourhood. A seed point is placed and the algorithm will segment (grow) the region starting from the seed point. The SD threshold will stop the region growing when it reaches a tissue interface.

Prior to the volume measurement, the embryonic insertion of the umbilical cord and the vitelline duct insertion at the yolk sac have to be ‘brushed’ away with the eraser to avoid segmentation of other parts from the whole body or yolk sac. To measure the volume of the embryo, first the hyperechoic structures have to be segmented (Supplementary Movie S1), followed by the hypoechoic structures (by the brain ventricles). For the YSV measurement, first the hypoechoic interior and then the hyperechoic shell has to be segmented. V-Scope can automatically add the segments and calculate the total volume.

If the volume measurement is incomplete, the user can manually grow (or shrink) the segmented region and a spherical, free hand ‘paint brush’ can be used to add voxels or to delete voxels from the segmented structure when necessary. All embryonic (Figs. 2 and 3) and YSVs were measured three times and the mean of these three assessments was used in the analysis. EV measurements were performed by an examiner (M.R.), and in a randomly chosen subset of 20 embryos these measurements were repeated independently, at a different time, by another examiner (R.v.O.). Both examiners were blinded to each other’s volume measurements. M.R. was an experienced examiner and R.v.O. was a non-experienced examiner, but the level of experience is less important when there is a large contribution of automatic procedures, which is the case in volume measurements performed by using the V-Scope application. The duration of the off-line V-Scope EV measurement ranged between 5 and 10 min. In a previous study by Rousian et al. (2009), the duration of the YSV measurement with V-Scope had a mean of 61 s. V-Scope CRL measurements, already validated by Verwoerd-Dikkeboom et al. (2008b), were performed three times by the examiner M.R. and the mean values of these were used for analysis.
EVs of IVF/ICSI and spontaneous pregnancies were analysed separately and tested for differences.

**Statistical analysis**

Data analysis was performed using SPSS (SPSS release 15.0 for Windows) and SAS PROC MIXED (release 8.02; SAS Institute Inc. Cary, NC, USA). To analyse the longitudinal measurements, we used repeated measurements ANOVA (random coefficient models). To analyse the EV and YSV versus the CRL, we used the equation: \[ \log_{10}(EV \text{ or } YSV) = a + b \times \log_{10}(CRL) \]. The same model equation was used for the analysis of GA by replacing CRL with GA.

Intraclass correlation coefficient (ICC) was used to quantify the interobserver and intraobserver reliability of the volume measurements. For a good agreement, the ICC has to be 0.90 or higher.

Figure 1 Image of the I-Space VR system. The data sets are projected on the floor and three walls by eight different projectors. An embryo of 10 weeks and 4 days GA is projected on the walls.
Results

The 88 EV measurements ranged from 14 to 29 877 mm³ (median: 2214 mm³) and are presented in Fig. 4 and grouped together per completed gestational week in Table I. The GA ranged from 42 to 90 days (mean: 66 days; SD: 11 days). The CRL ranged from 3.0 to 68.0 mm (median: 25.7 mm).

In our study, the mean EV measurements can be presented by the following equations:

\[
\log_{10}(\text{Embryonic volume (cm}^3)) = -3.56 + 2.72 \times \log_{10}(\text{CRL (mm)}),
\]

\[
\log_{10}(\text{Embryonic volume (cm}^3)) = -16.50 + 9.03 \times \log_{10}(\text{GA (days)}).
\]

From these equations, it can be calculated that when the CRL doubles, the mean EV increases 6.5-fold (95% confidence interval (CI): 6.2–7.0; \(P < 0.001\)). For each doubling of GA, the mean EV increases \(\approx 500\)-fold (95% CI: 403–680; \(P < 0.001\)).

These two equations did not statistically significantly differ between IVF/ICSI and spontaneous pregnancies (both \(P > 0.5\) for the slopes; both \(P > 0.5\) for the intercepts).

Of the 42 included women, the GA at delivery ranged from 35±0 to 42±0 weeks (mean: 39±12 weeks; SD: 10 days). Birthweight ranged from 2175 to 4750 g (mean: 3346 g; SD: 588 g). Post-natally all 22 girls and 20 boys were healthy.

The YSV measurements are shown in Table II.
The resulting mean YSV can be represented by the following equations:

\[
\log_{10}(Yolk \text{ sac volume (cm}^3)) = -1.75 + 0.57 \times \log_{10}(CRL \text{ (mm)}),
\]

\[
\log_{10}(Yolk \text{ sac volume (cm}^3)) = -4.44 + 1.92 \times \log_{10}(GA \text{ (days)}).
\]

In Fig. 5 and Table II, YSVs are plotted against the CRL and GA. The YSV increases on average 3.8-fold (95% CI: 2.8–5.1; \(P, 0.001\)) for each doubling of gestational days. When the CRL doubles, the YSV increases 1.5-fold (95% CI: 1.4–1.6; \(P, 0.001\)).

Interobserver variability was calculated by comparing 20 EV measurements of examiner M.R. with the measurements of examiner R.v.O., which resulted in an ICC of 0.999 (95% CI: 0.997–0.999), representing excellent agreement. The intraobserver variability was calculated by comparing the EV measurements of M.R., which resulted in an ICC of 0.999 (95% CI: 0.998–0.999).

### Discussion

In this prospective study based on longitudinally collected ultrasound data, we show, for the first time, that first trimester human embryonic whole-body volumes and YSVs can be measured using an instant and automated VR system.

The development of 3D ultrasound at the end of the 1980s was a step forward for volume-based growth and weight estimations. Since then several studies have been published on reliability and accuracy of 3D volume measurements (Blaas et al., 1998; Berg et al., 2000; Raine-Fenning et al., 2003; Cheong et al., 2009; Rousian et al., 2009). Subsequently, specialized software used by Blaas et al. (1998, 2006) and Berg et al. (2000), the multiplanar method (Cheong et al., 2009) and VOCAL software (Figueras et al., 2003; Aviram et al., 2004; Falcon et al., 2005a,b; Martins et al., 2008; Rolo et al., 2008; Bagratee et al., 2009, Cheong et al., 2009) have been used for the estimation of EVs and YSVs. In all studies the examiner had to place contours around the structure of interest on a 2D screen, which is subject to individual variation. Blaas et al. (1998, 2000) showed that the examiner usually draws the segmentation line slightly away from the real surface, resulting in larger volumes with outer surfaces and smaller volumes in the case of cavities with inner surfaces. The I-Space offers depth perception and subsequently the data set can be examined in all dimensions and from all the different sides. This makes it easy for an examiner to measure volumes, and to prevent incomplete segmentations. The V-Scope software enables the measurement of volumes in real 3D semi-automatically, and proves to be less sensitive to individual variation (Rousian et al., 2009), because there is no need to draw contours around the structure of interest.

Another advantage of the I-Space is that this algorithm allows one to compute the whole-body volume, including the limbs, while with

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**Table I** Mean EV and CRL estimations with the corresponding SD, number (N) per complete gestational week and/or range.

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>N</th>
<th>Mean EV (mm³)</th>
<th>SD (mm³)</th>
<th>Range (mm³)</th>
<th>Mean CRL (mm)</th>
<th>SD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>3</td>
<td>42</td>
<td>34</td>
<td>14–80</td>
<td>6.5</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>310</td>
<td>137</td>
<td>90–543</td>
<td>13.2</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>1017</td>
<td>352</td>
<td>489–1800</td>
<td>20.3</td>
<td>2.2</td>
</tr>
<tr>
<td>9</td>
<td>19</td>
<td>2447</td>
<td>785</td>
<td>1469–4619</td>
<td>27.2</td>
<td>3.6</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>5348</td>
<td>1412</td>
<td>2478–7761</td>
<td>36.7</td>
<td>4.4</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>9709</td>
<td>2579</td>
<td>5232–12 927</td>
<td>47.0</td>
<td>4.8</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>22 195</td>
<td>5693</td>
<td>10 911–29 877</td>
<td>63.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

CRL, crown-rump length; EV, embryonic volume, SD, standard deviation.

**Table II** Mean YSV and CRL estimations with the corresponding SD, number (N) per complete gestational week and/or range.

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>N</th>
<th>Mean YSV (mm³)</th>
<th>SD (mm³)</th>
<th>Range (mm³)</th>
<th>Mean CRL (mm)</th>
<th>SD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 and &lt;6</td>
<td>8</td>
<td>48</td>
<td>13</td>
<td>33–72</td>
<td>7.0</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>79</td>
<td>23</td>
<td>40–139</td>
<td>13.3</td>
<td>2.0</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>100</td>
<td>39</td>
<td>49–245</td>
<td>19.9</td>
<td>2.6</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>122</td>
<td>42</td>
<td>63–206</td>
<td>26.1</td>
<td>2.8</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>147</td>
<td>37</td>
<td>99–228</td>
<td>37.0</td>
<td>4.5</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>180</td>
<td>104</td>
<td>75–424</td>
<td>48.3</td>
<td>5.8</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>116</td>
<td>43</td>
<td>76–160</td>
<td>61.8</td>
<td>6.0</td>
</tr>
</tbody>
</table>

CRL, crown-rump length; SD, standard deviation; YSV, yolk sac volume.
VOCAL only a head and trunk volume can be measured, resulting in a substantial underestimation (Falcon et al., 2005a,b; Martins et al., 2008; Bagratee et al., 2009). Blaas et al. (2006) showed that the volume of the limbs, as a percentage of the total EV, increases from 5% at 7 weeks’ GA to 10% at the end of the first trimester.

Each volume measurement took 5–10 min starting from the moment a data set was loaded, including all post-processing time. The more experienced one gets, the faster it goes, which makes it also useful in a clinical setting. Compared with the other techniques, the VR volume measurement is less time consuming, due to its segmentation algorithm (Rousian et al., 2009). Clinical applicability will be tested in the near future. Our main goal in this translational research was to provide new insights into embryonic growth and development. Furthermore, the interobserver and intraobserver agreement for yolk sac (Rousian et al., 2009) and EV measurements appeared to be very good.

We measured the embryonic body volume in 49% of the data sets. In this translational research setting, we only included these data sets with a very high image quality, as our main goal was to provide new insights into embryonic growth and development using automated analysis. New studies will be conducted with regard to applications in a clinical setting.

In comparison with the cross-sectional EV data of Blaas et al. (2006), we found, on average, 23% higher volumes above a CRL of 30 mm and comparable volumes below a CRL of 30 mm. The relatively small number of women studied, however, is a limitation of both studies. It has been suggested that reduced embryonic growth might be better assessed by EV than by CRL measurement (Aviram et al., 2004; Falcon et al., 2005a,b), because the increment of EV between the seventh and twelfth gestational week is much larger than the respective increment of the CRL, as was also demonstrated in our study. Between 111/2 and 131/6 weeks of gestation, the fetal head and trunk volume has also been shown to be ~10–15% lower in trisomy 21 and monosomy X fetuses and ~45% lower in trisomy 18 and triploidy fetuses (Falcon et al., 2005a,b) when compared with chromosomally normal fetuses. A generalized disturbance in growth of these embryos was illustrated by the fact that the volumes were smaller, even after correction for CRL.

As we know that there is a relation between abnormal first trimester growth and adverse obstetric outcomes from CRL studies (Smith et al., 1998; Bukowski et al., 2007; Mukri et al., 2008, Mook-Kanamori et al., 2010), it can be expected that EV measurements may be even more accurate in prediction models. EV measurements may therefore be implemented in routine clinical practice in the near future. However, more research is needed to validate the assumption that abnormal volume measurements can be successfully used to predict a miscarriage or low birthweight.

The yolk sac, being an embryonic structure with biosynthetic, hematopoietic and absorptive functions, plays a critical role in embryonic development. From the first sonographic analysis of the human yolk sac by Mantoni et al. (1979), researchers tried to find a relation between the yolk sac appearance and size, and the pregnancy outcome. Kupesic et al. (1999) were the first to study YSVs by using the planimetric 3D ultrasound method. In recent publications, VOCAL was used for YSV measurements (Figueras et al., 2003; Rolo et al., 2008). The volumes correlated with GA even more than the diameter of the yolk sac (Figueras et al., 2003; Rolo et al., 2008). Associations between an abnormal YSV and pregnancy outcome were noted in some (Figueras et al., 2003; Cho et al., 2006), but not in all studies (Babinski et al., 2001).

In the I-Space, these volumes can be measured automatically in 1 min (Rousian et al., 2009). These very high reproducible and accurate measurements can therefore be performed for research and may also be implemented in a clinical setting to study the predictive value of the YSV for an abnormal pregnancy outcome.

In conclusion, we have demonstrated that an innovative VR system can be used for automated first trimester measurement of human embryonic and YSV. This 3D VR approach allows us to obtain unique information about the size of the embryo using all dimensions, which opens a new area to study embryonic growth and development. This innovative technique may improve the differentiation between normal and abnormal human development in early pregnancies.

**Supplementary Data**

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

M.R. contributed to study design, data analysis and writing of the manuscript. A.H.J.K. developed the V-Scope software and revised the manuscript. R.H.F.v.O. and C.M.V.-D. were involved in the data collection and revised the manuscript. W.C.H. is a professional statistician who advised and supervised us on the statistical methods used in this study. 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.

References