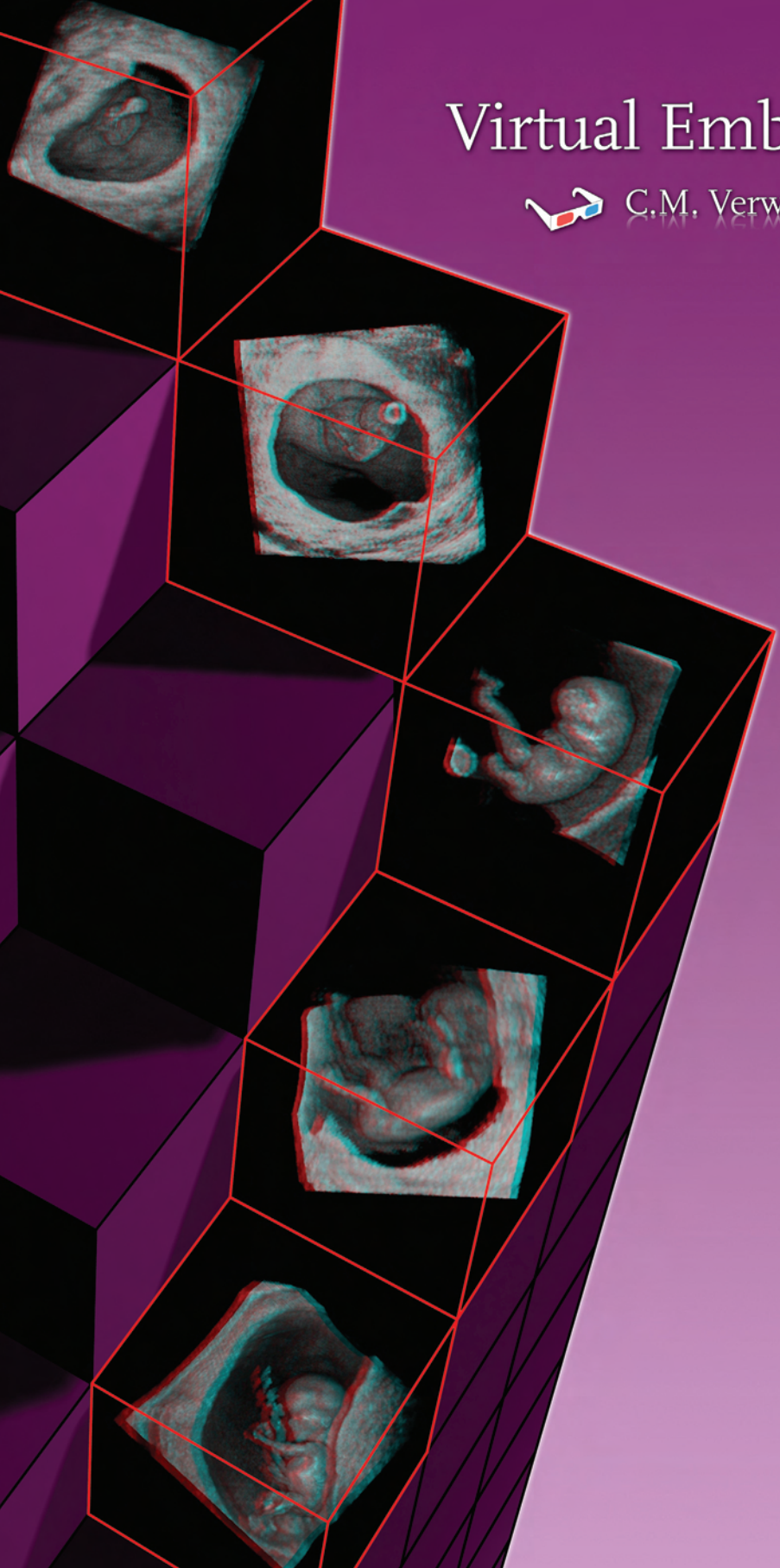
Virtual Embryoscopy

C.M. Verwoerd-Dikkeboom



VIRTUAL EMBRYOSCOPY

Christine Verwoerd-Dikkeboom

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Printing of this thesis was financially supported by the Department of Obstetrics and Gynaecology and the Department of Bioinformatics of the Erasmus MC, University Medical Centre and the Erasmus University Rotterdam. Additional support was kindly provided by BARCO, Schering-Plough, Crosslinks and GE Medical Systems.

Front cover: Lennard Goedknegt.
The embryo on the cover is of 7^{+2} wks, 8^{+0} wks, 9^{+2} wks, 10^{+2} wks and 11^{+2} wks gestational age respectively. It is the unborn child of the author of this thesis.

Layout and printing: Offsetdrukkerij Haveka Bv, Alblasserdam
ISBN: 978-90-9024251-4

VIRTUAL EMBRYOSCOPY

Virtuele embryoscopie

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE

ERASMUS UNIVERSITEIT ROTTERDAM

OP GEZAG VAN DE RECTOR MAGNIFICUS

PROF.DR. S.W.J. LAMBERTS

EN VOLGENS BESLUIT VAN HET COLLEGE VOOR PROMOTIES.

DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP

WOENSDAG 8 MEI 2009 OM 13.30 UUR

DOOR

CHRISTINE MARGARETHA VERWOERD-DIKKEBOOM

GEBOREN TE PAPENDRECHT

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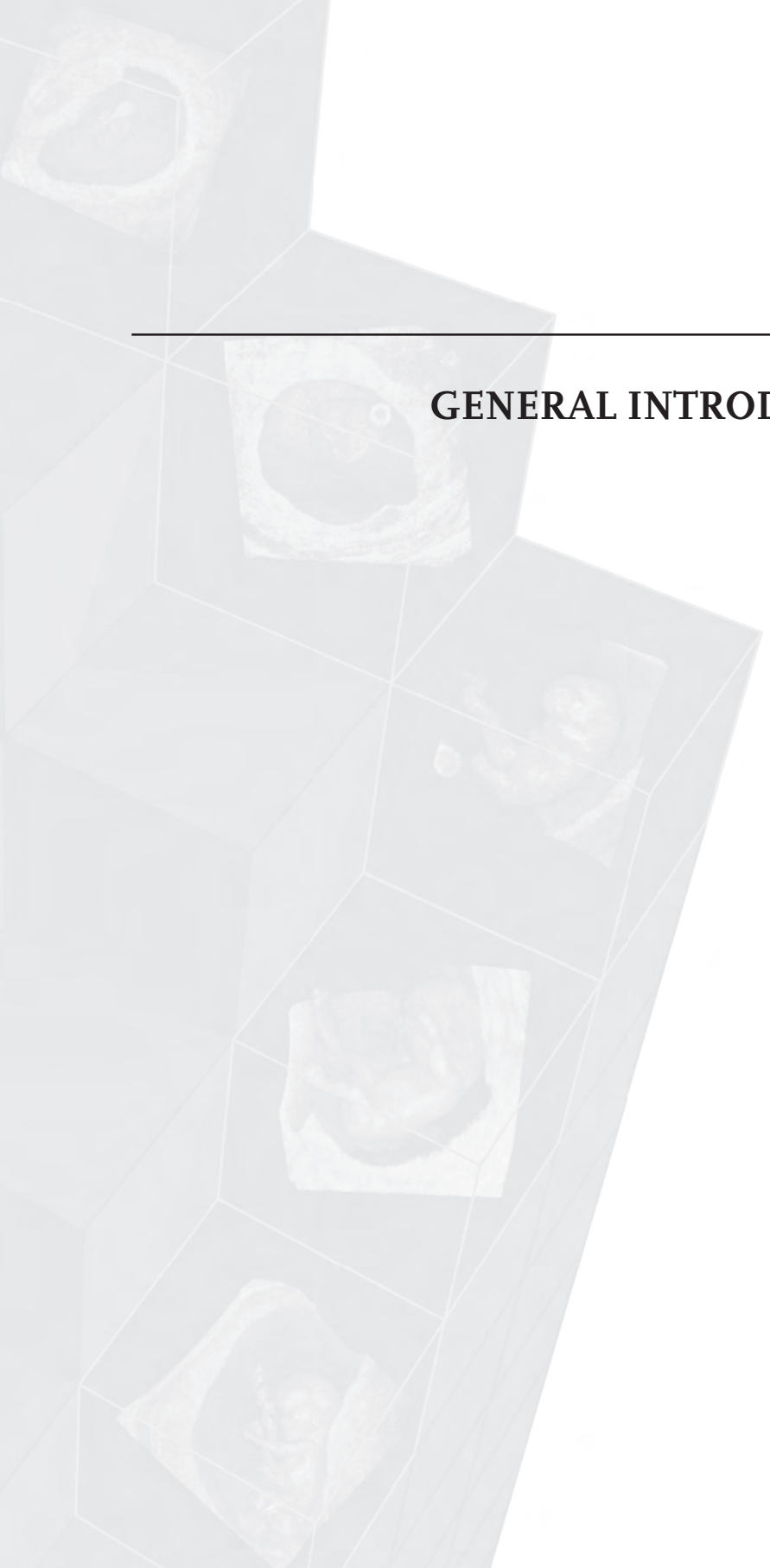
VOOR MIJN OUDERS

TABLE OF CONTENTS

Supplements:

- Appendix with colour illustrations
- Anaglyphic glasses



PART 1

GENERAL INTRODUCTION

CHAPTER 1.1

INTRODUCTION AND RESEARCH OBJECTIVES

The first 10 weeks of pregnancy are of great importance for the developing embryo: abnormal growth and / or development during these weeks are likely to have an impact on foetal growth in the 2nd and 3rd trimester of pregnancy and subsequent health of the newborn. It is therefore of major importance to define normal and abnormal embryonic growth and development. Solid comprehension of human embryogenesis will eventually allow for early detection of any abnormalities, for instance in the case of recurrent miscarriages or pregnancies with chromosomal abnormalities. Human embryology has been studied extensively during the last centuries. However, it is quite remarkable that the classical description of normal human embryonic growth and development is generally based on information of abnormal pregnancies like miscarriages and ectopic pregnancies.

The 'Vesalius of human embryology', Wilhelm His Senior (1831 - 1904) was the first to study human embryology systematically. His book 'Anatomie Menschlicher Embryonen' [1] described the anatomy of human embryos studied as a whole. Franklin Mall (1862 - 1917), founder of the Department of Embryology of the Carnegie Institution in Washington, first employed 'staging' in human embryology. The need for staging embryonic development is mainly found in accurately describing normal development and for utilization in experimental work. Stages are based on the apparent morphological state of development, and hence are not directly dependent on either chronological age or on size [2], although embryos of different stages do show a high degree of uniformity regarding their appearance and length [3].

Mall's successor, George Streeter (1873 – 1948) provided the definitive classification of human embryos into so-called 'horizons', based on both external and internal morphological state of development. Streeters classification was modified by Ronan O'Rahilly into the Carnegie Staging system that is still effective today [2,4,5]. This system describes approximately the first 10 weeks of pregnancy (gestational age) and the stages, numbered from 1 to 23, are based on internal and external physical characteristics of the embryo. At stage 23, all essential internal organ systems are present, therefore representing the end of the embryonic period.

Imaging was essential in the classical descriptions of human embryology. Whilst Franklin Mall used photographs to categorize the human embryos in his collection, Wilhelm His realised the need for magnified three dimensional (3D) imaging and the need for models of dissected objects. In his book 'Anatomie Menschlicher Embryonen' [1] he made 3D reconstructions from freehand drawings of histological slices. Reconstructions made of stacking wax plates of histological slices was the next step, as first described by Gustav Born (1850 - 1900) in 1876 [5,6] and brought to high standards by Osborne Heard (1890 – 1983), who made over 800 wax-based reconstructions in the Carnegie Institute.

After the visualisation of a foetus with ultrasonography by Ian Donald in 1957, Robinson was the first to publish an embryonic growth curve based on Crown-Rump Length (CRL) measurements using ultrasound in 1975 [7]. Drumm and O'Rahilly later compared the CRL data obtained by ultrasound with the data from the Carnegie Collection on embryonic length and adjusted the lengths of the staged embryos according to these CRL data [8]. Today, measuring the CRL of the developing embryo is considered to be standard prenatal care, and numerous growth charts have been devised.

It was in the early 1980s that the first attempts were made to construct 3D images from ultrasound recordings of foetuses [9]. During the last decades the advance in computer technology has opened up new possibilities for 3D reconstructions [9,10]. Nowadays the advantages of 3D ultrasound for foetal imaging are unequivocal; its use in the detection of foetal anomalies, especially for those of the face, limbs, thorax and spine is applied by numerous centres around the world [11]. Since the amount of amniotic fluid greatly influences the image quality of 3D ultrasound images, the embryonic period with its abundance of amniotic fluid is especially well visualized. The embryo can be viewed as a whole, from all three directions, allowing for a more defined demonstration of all anatomical planes. However, although these ultrasound datasets are three dimensional, they are still presented on flat 2D screens or paper, which implies that the information concerning the third dimension is not used optimally. To benefit from all three dimensions one can use a stereoscopic projection system, like the Barco I-Space. This virtual reality system immerses the user(s) in a three dimensional virtual environment that allows them to perceive depth and interact with

the volume rendered data in an intuitive manner.

In this thesis the use of the I-Space in the assessment of human embryonic growth and development is described. The research objectives were as follows:

1. To establish the reproducibility of embryonic biometric and volume measurements performed with this new visualisation technology.
2. To describe a staging system that takes into account both morphological features and biometric measurements to improve knowledge of embryogenesis in vivo.
3. To provide normative data relative to gestational age for standard and non-standard embryonic biometric measurements.
4. To evaluate the applicability of this technology in cases of embryonic and foetal malformations.

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CHAPTER 1.2

THE I-SPACE VIRTUAL REALITY SYSTEM

In 1833 Sir Charles Wheatstone invented the first stereoscopic display system and at the World's Fair in London in 1851 stereoscopes were a big hit with the enthusiastic attendees. The first three dimensional (3D) movie was demonstrated to the public in 1893. Two years later, Wilhelm Röntgen made his first X-ray photograph. However, it was not until 1971 that the "EMI-scanner", the first commercially available CT-scanner, made use of the third dimension for medical imaging.

Today, the use of two dimensional (2D) display technology is still common practice: almost all radiological examinations, including those of 3D modalities like CT and MRI are performed on 2D display systems and viewed from 2D slices. While it is certainly true that the modern medical profession can gather a wealth of information from these scans using only two dimensions, various studies have shown that humans are better in understanding 3D relations when viewed on 3D displays. Over the years, numerous / various techniques have been proposed for stereoscopic display systems for medical applications, but so far none have attained any widespread acceptance.

On March 24, 2005 the mayor of Rotterdam, Ivo Opstelten officially opened the Barco I-Space virtual environment at the Department of Bioinformatics of the Erasmus MC University Medical Centre Rotterdam. The I-Space is a CAVE™-like projection based virtual reality system and Erasmus MC was the first to install such a system in a university medical centre for both medical and research applications.

CAVE is the recursive acronym for Cave Automatic Virtual Environment (and also a reference to the Cave in Plato's Republic; perception, reality and illusion are contemplated, see next page). The first CAVE system was developed by the Electronic Visualization Laboratory at the University of Illinois in Chicago and demonstrated at the SIGGRAPH conference in 1992. It was developed as a multi-person alternative to the 'head mounted display' (VR-helmet). In addition to offering a multi-viewer experience, the CAVE technique has several advantages: besides offering high-resolution images, it also tends to prevent so-called 'simulation-sickness', i.e. nausea and/or dizziness similar to motion sickness, which many people experience while using a head mounted display.

THE ALLEGORY OF THE CAVE.

Plato (428 BC – 347 BC) (The Republic bk. VII, 516b-c; trans. Paul Shorey).

Imagine prisoners, who have been chained since their childhood deep inside a cave: not only are their limbs immobilized by the chains; their heads are chained in one direction as well so that their gaze is fixed on a wall.

Behind the prisoners is an enormous fire, and between the fire and the prisoners is a raised walkway, along which puppets of various animals, plants, and other things are moved along. The puppets cast shadows on the wall, and the prisoners watch these shadows. When one of the puppet-carriers speaks, an echo against the wall causes the prisoners to believe that the words come from the shadows.

The prisoners engage in what appears to us to be a game: naming the shapes as they come by. This, however, is the only reality that they know, even though they are seeing merely shadows of objects. They are thus conditioned to judge the quality of one another by their skill in quickly naming the shapes and dislike those who play poorly.

Suppose a prisoner is released and compelled to stand up and turn around. At that moment his eyes will be blinded by the sunlight coming into the cave from its entrance, and the shapes passing by will appear less real than their shadows. The last object he would be able to see is the sun, which, in time, he would learn to see as the object that provides the seasons and the courses of the year, presides over all things in the visible region, and is in some way the cause of all these things that he has seen.

Once enlightened, so to speak, the freed prisoner would not want to return to the cave to free "his fellow bondsmen," but would be compelled to do so. Another problem lies in the other prisoners not wanting to be freed: descending back into the cave would require that the freed prisoner's eyes adjust again, and for a time, he would be one of the ones identifying shapes on the wall. His eyes would be swamped by the darkness, and would take time to become acclimated. Therefore, he would not be able to identify the shapes on the wall as well as the other prisoners, making it seem as if his being taken to the surface completely ruined his eyesight.

Interpretation:

We are prisoners in our own body, our fellow humans are prisoners as we are and nobody is capable of knowing our own true self or of somebody else. We cannot see reality, only what is in our mind.

The technology behind the CAVE involves the creation of a lifelike visual display by projectors that are positioned outside the CAVE. Physical movements from a user inside the CAVE control the perspective of this visual display. The user wears a pair of lightweight glasses with circular polarizing lenses. A computer generates pairs of images, projected through polarizing filters. The principle behind this 'stereoscopic imaging' is illustrated in figure 1.2.1.

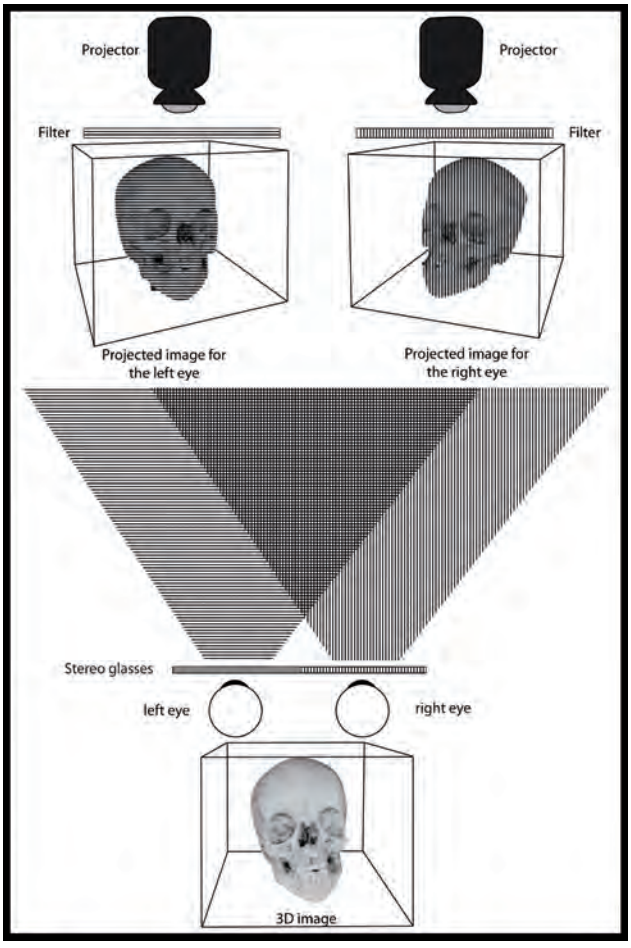


Figure 1.2.1 *Stereoscopic imaging. The image projected runs through filters. The glasses also have filters. With the right filter only the light from the right projector is visible, with the left filter only the light from the left projector is visible. This forces the eyes to see a different image with each eye. Since it is in fact the same image, but viewed in a slightly different angle, this results in depth perception. The I-Space uses left rotating and right rotating polarizing light, which has the same effect.*

The images of the Barco I-Space in Rotterdam are generated by a Silicon Graphics Prism® visualization system with 8 Intel® Itanium® 2 processors, 8 ATI® FireGl™ X3 graphics processors and 12 GB of memory. Eight Barco SIM4 projectors (1280x1024 pixels, 60Hz, 1500 lumen, 1300:1 contrast ratio) are installed behind or above the four 'projection screens' (2.60 x 2.08 meters) of the I-Space.

These screens make up the floor, left, right and front wall of the I-Space (figure 1.2.2). In every corner of this "room" an infrared camera (four in total) is installed for tracking purposes. The tracking system registers the position and orientation of the user's head and joystick.

The wireless 4-button plus hat-switch joystick, which emits a virtual pointer, is used for manipulation of and interaction with the hologram, as well as operation of the menu and other user-interface objects.

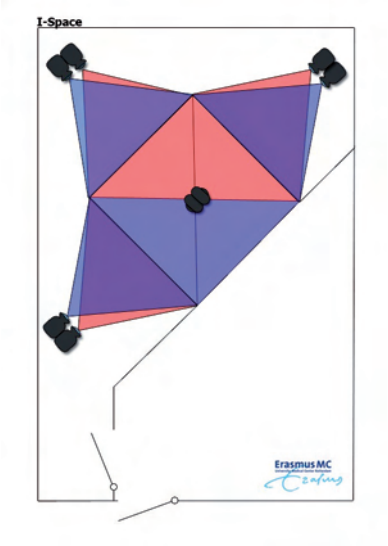


Figure 1.2.2. *Schematic overview of the I-Space with its different projectors.*

Anton Koning of the department of Bioinformatics at the Erasmus MC developed a specialized general-purpose volume rendering application (CAVORE), allowing visualization of medical volumetric data in the virtual environment of the I-Space. It uses a combination of direct manipulation of the data set with the joystick and a simple graphical user interface (GUI) with a drop-down menu and a widget (graphical object that can be manipulated by the user) to control the transfer function that assigns grey scale, color and opacity values to the data. A distance measuring tool has also been implemented in this system. All modifications of the volume are shown in real-time. Recently (2008), a redesigned version of this software program was launched and called V-Scope. One of the goals of the development of V-Scope was the ability to measure volumes. Therefore, a flexible and robust segmentation algorithm based on region growing was implemented, since this type of algorithm does not depend on 2D interaction, like others used for volume measurements. The algorithm of V-Scope

was not only developed for volume measurements in ultrasound data but also for efficient and reliable handling of CT, MRI and other volumetric data.

The use of CAVORE / V-Scope in the I-Space allows medical professionals to view and interact with their volumetric data in all three dimensions. V-Scope is able to handle very large datasets (limited by the computer's main memory) of most 3D medical imaging modalities (e.g. MRI, CT, PET, SPECT and 3D ultrasound). This provides clinicians with views much more alike those they will experience during surgery. Since its introduction in 2004 many departments of the Erasmus MC have used the I-Space either incidentally or as part of a larger project. Besides the research described in this thesis, the I-Space and CAVORE / V-Scope have been used for:

- Surgical planning (e.g. in plastic and reconstructive surgery) with CT and CTA data.
- Diagnosis of heart defects with 3D echocardiography data
- Neurosurgery (e.g. for brain tumour resection) with MRI data
- Post-mortem examinations by the department of pathology, using full-body CT scans.

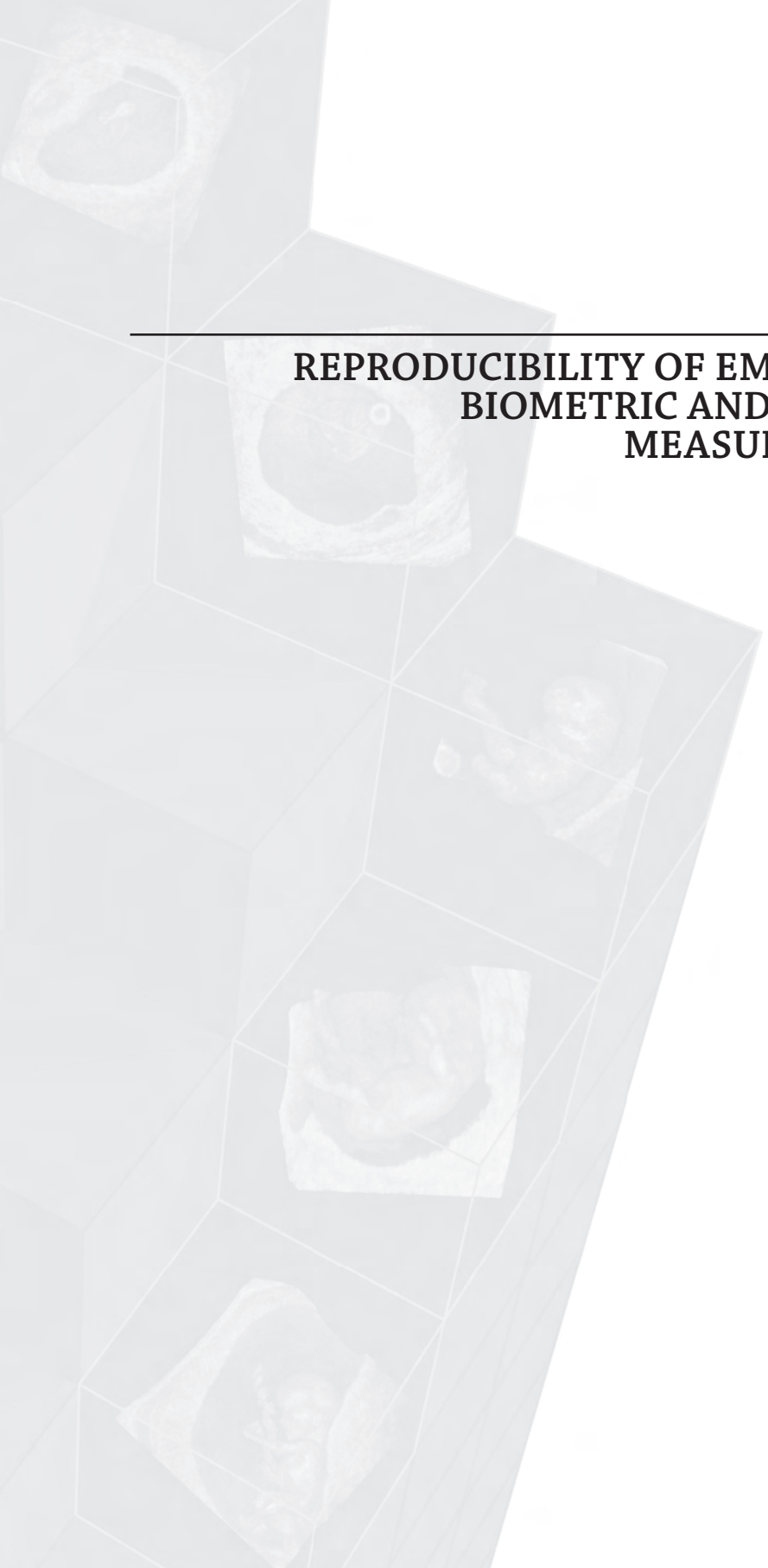
Future perspectives and general applicability

Many users of the I-Space have commented that they would like a similar system on their desk, which is why the department of Bioinformatics has built a system based on a 3D monitor. It consists of an auto-stereoscopic LCD monitor, a tracking system for the pointer device and a six degree-of-freedom 3D mouse. With V-Scope on a single screen configuration (desktop model), the user works with a 3D mouse and a pointer inside a stereoscopic virtual workspace. An interaction model such as this is very similar to that of the immersive I-Space system. A standard personal computer with a high performance graphics card is used to drive the system, with a normal second monitor for display of the user interface.

With research clearly showing the advantages of 3D displays and solutions based on off-the-shelf hardware now available, integration of 3D display technology into daily clinical practice is desirable and feasible.

PART 2

REPRODUCIBILITY OF EMBRYONIC BIOMETRIC AND VOLUME MEASUREMENTS



CHAPTER 2.1

RELIABILITY OF EARLY PREGNANCY MEASUREMENTS IN 3D USING VIRTUAL REALITY

Ultrasound Obstet Gynecol 2008; 32: 910-916

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ABSTRACT

Objective:

To establish the reliability of three dimensional (3D) ultrasound measurements in early pregnancy using a virtual reality system (the BARCO I-Space).

Methods:

The study included 28 pregnancies with gestational ages ranging from 6 to 14 weeks (median 10 weeks). 3D volumes were acquired and offline measurements were made, where possible, of the yolk sac diameter (YS), crown-rump length (CRL), biparietal diameter (BPD), head circumference (HC) and abdominal circumference (AC). The datasets were then transferred to the Barco I-Space, a virtual reality system that allows the observer to perceive depth and interact with volume-rendered (ultrasound) data. The 3D rendered volumes were measured using a virtual pointer, controlled by a wireless joystick.

For intraobserver variability, 3D and virtual reality volumes were measured twice by one operator. For interobserver variability, another operator performed the same measurements once. All measurements were repeated three times and their mean values were used for comparisons.

Results:

All intraclass correlation coefficients (ICCs) comparing 4D View measurements with I-Space measurements were > 0,97. Intra- and interobserver ICC's for the 4D view measurements were > 0,96 and for the I-Space ICC's were > 0,98, representing good agreement.

Conclusions:

The application of virtual reality is a novel method of visualising 3D ultrasound data. The perception of depth in the I-Space offers possibilities of measuring non-planar structures. We have demonstrated that early pregnancy measurements in the I-Space are reliable. New areas of embryonic and foetal biometry can now be explored using this technique we tentatively name 'Virtual Embryoscopy'.

INTRODUCTION

Biometry measurements in the second and third trimester (the foetal period) are generally based on the comparison of ultrasonographically measured values with predicted values derived from reference charts or equations obtained from normal populations. Although it is well known that ultrasound measurements are subject to systematic and random errors, studies investigating the intra- and interobserver reproducibility of foetal biometry have shown that these measurements are highly reliable [1,2]. Various authors have stressed the importance of choosing appropriate reference charts [3-5]. However, up-to-date charts for embryonic biometry (measurements until the 10th week of gestational age) and early foetal biometry are hard to find. Recently, new and reliable growth curves were presented from our department based on a large population-based study (Generation R) by Verburg et al [6]. As in most studies, transabdominal probes were used for crown-rump length (CRL) measurements [5,7]. The introduction of the vaginal probe has greatly improved the imaging of early pregnancy [8-10], and in general is well tolerated [11].

Recent developments in three dimensional (3D) sonographic imaging techniques have resulted in remarkable progress in the visualisation of the developing embryo/foetus. However, studies investigating the reliability of measurements in 3D generally focus on volume measurements of the placenta [12] and on volumetry of the embryo/foetus itself [13,14]. We aimed to demonstrate the reliability of biometry measurements in 3D volumes using a new visualisation technique, the BARCO I-Space (Barco N.V., Kortrijk, Belgium). This virtual reality system immerses the viewer(s) in a 3D virtual environment that allows them to perceive depth and interact with volume-rendered (ultrasound) data in a more natural and intuitive manner than is possible with 3D views displayed on a 2D screen. This technique offers new opportunities for embryonic and foetal biometry. We compared embryonic and foetal standard biometric measurements of 3D ultrasound images using the BARCO I-Space to those obtained using specialized 3D imaging software and established the intra- and interobserver reproducibility.

MATERIALS EN METHODS

This study included 28 pregnancies with gestational ages ranging from 6 to 14 (median 10) weeks. These women were the first 28 participating in another ongoing study to evaluate embryonic growth and development using novel imaging techniques and were seen weekly during the first trimester of their pregnancy. For this study we used only one examination of each patient to obtain a wide range of gestational ages. 3D ultrasound scanning was performed on a GE Voluson 730 Expert system (GE Medical Systems, Zipf, Austria). Using specialized 3D software (4DView, GE Medical Systems), we measured (when possible) the yolk sac diameter (YS), CRL, biparietal diameter (BPD), head circumference (HC) and abdominal circumference (AC). YS was measured from outer-to-outer border, in three orthogonal planes and the mean yolk sac diameter (MYS) was calculated. All measurements were performed offline and repeated three times. For each parameter, mean values of these 3 assessments were used for comparisons.

The 3D datasets were then saved as cartesian (rectangular) volumes and transferred to the BARCO I-Space at the department of Bioinformatics of the Erasmus MC. This is a 4-walled CAVE-like [15] virtual reality system. Observers are immersed in a virtual world, surrounded by computer-generated passive stereo images which are projected by four projectors onto three walls and the floor of a small 'room'. The CAVORE [16] (CAve VOlume REndering) volume rendering application is used to create a 'hologram' of the ultrasound volume that is being investigated [17], floating in space in front of the observer, which must be viewed through glasses with polarizing lenses to create the perception of depth. The hologram can be manipulated by means of a virtual pointer, controlled by a wireless joystick. This joystick also operates a measuring tool. Wireless tracking of the viewer's head allows the computer to provide the correct perspective and motion parallax, which in addition to the stereoscopic images, helps in discerning fine details and in the understanding of 3D structures in the volumes [17-19].

For our study the 3D volumes were resized (enlarged), rotated and cropped when necessary and grey scale and opacity values were adjusted for optimal image quality. Measurements were made using the wireless joystick (Figure 2.1.1 and 2.1.2). Again, all measurements were repeated three times and their mean value used for comparisons. Direct measurement of circumferences has not yet been implemented

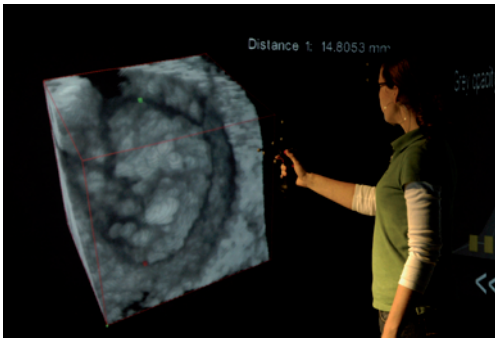


Figure 2.1.1 *Operator using the BARCO I-Space to examine an embryo of 7+4 weeks' gestation, with a crown-rump length of 14,8 mm.*

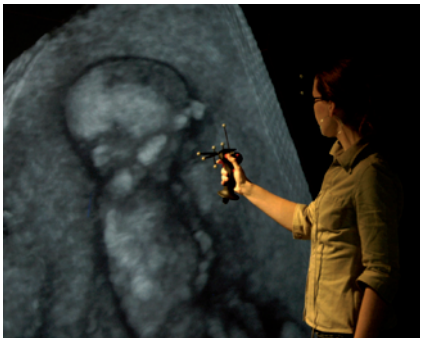


Figure 2.1.2 *Operator using the I-Space to examine an embryo of 12+5 weeks' of gestation.*

in the CAVORE system. Therefore, to obtain the HC in the I-Space, we measured the BPD and occipito-frontal diameter (OFD). From GE Medical Systems we obtained the HC formula used in the Voluson 730 Expert system and 4DView software program used to calculate the HC from the BPD and OFD. The same principle was applied to the AC calculation, using two diameters perpendicular to each other, one from right to left and one from back to front.

All measurements were performed twice by one operator (CV-D) en then repeated independently by another examiner (MR). The second series of measurements by CV-D was performed at least one week later than the first series to overcome recollection bias. An independent observer reported the results of the measurements, so the examiner was blinded to the earlier results. We quantified the agreement between and among these two examiners by calculation of Intraclass Correlation Coefficients (ICC), a value > 0.90 representing good agreement. Statistical analysis was performed using SPSS (SPSS Release 12.0.1 for Windows, SPSS Inc, Chicago, IL, USA) and SAS PROC MIXED (release 8.02, SAS Institute Inc, Cary, NC, USA). For general comparisons we also analysed the ICC's of 4D View measurements and I-Space measurements compared with standard two dimensional (2D) measurements, performed at the time of ultrasound examination in the clinical setting.

We created Bland-Altman [20] plots to assess the agreement between the measurements using the different methods and calculated limits of agreement (mean difference ± 2 SD). We also created Bland-Altman plots to asses the agreement between and among the two operators using the same measuring method. The difference between the measurements of both operators and the difference between

the measurements of the same operator at a later time were plotted against the mean of all measurements. We tested whether the variation depended on the level of measurements (i.e. on gestational age) using the Breisch-Pegan test.

RESULTS

Figure 2.1.3 shows 2D measurements of the biometric parameters (MYS, CRL, BPD, HC and AC) of the study subjects. A comparison of these measurements obtained by 4DView and I-Space is shown in figure 2.1.4 and Bland-Altman plots of the difference between the measurements are given in figure 2.1.5. The variation did not depend on the level of measurements in the Bland-Altman plots.

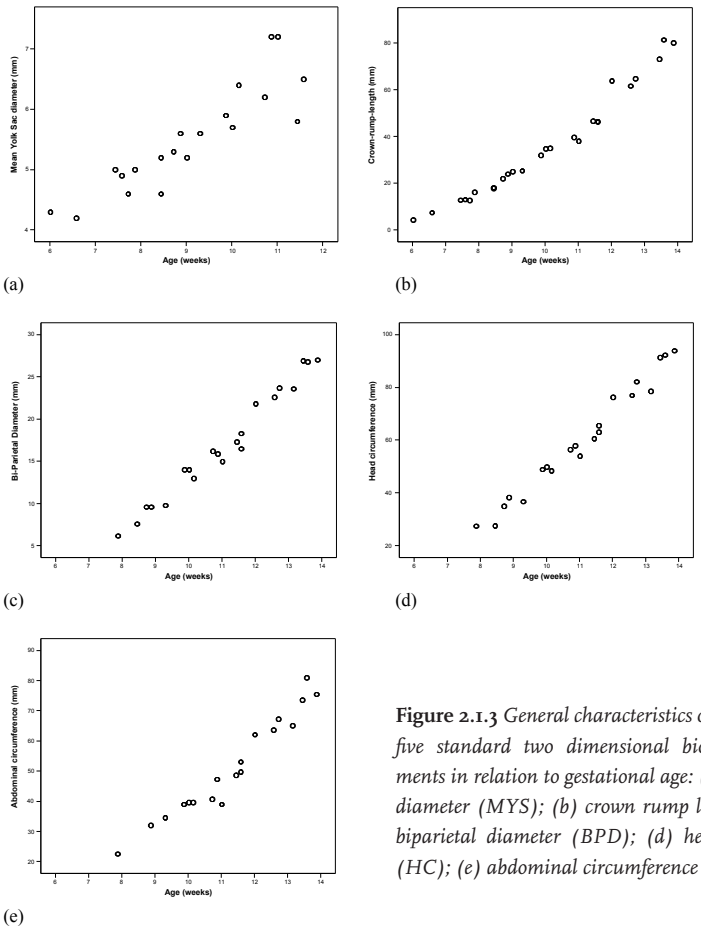
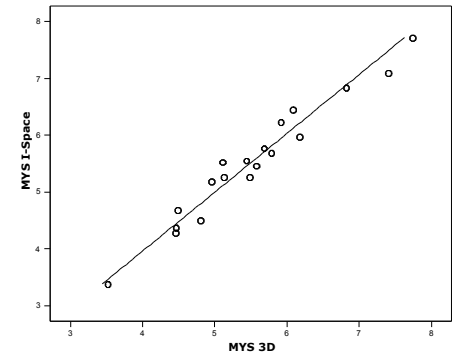


Figure 2.1.3 *General characteristics of the study group: five standard two dimensional biometric measurements in relation to gestational age: (a) mean yolk sac diameter (MYS); (b) crown rump length (CRL); (c) biparietal diameter (BPD); (d) head circumference (HC); (e) abdominal circumference (AC).*



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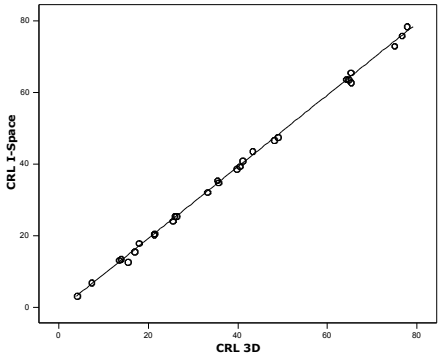

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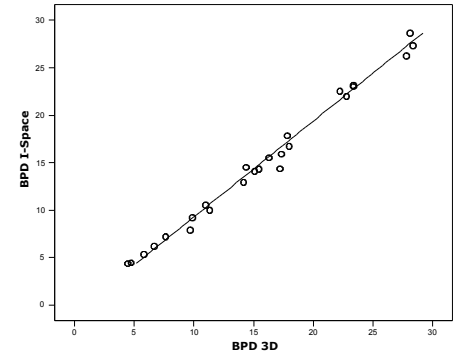
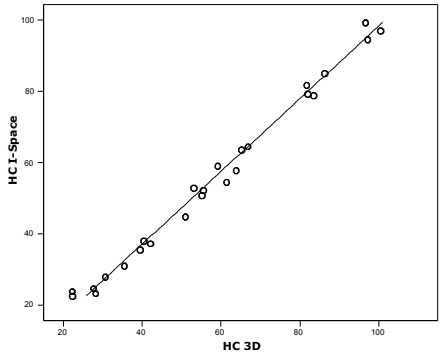
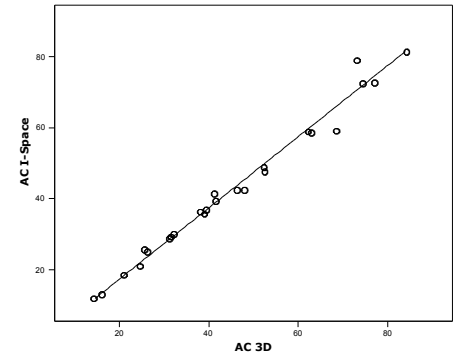


(c)

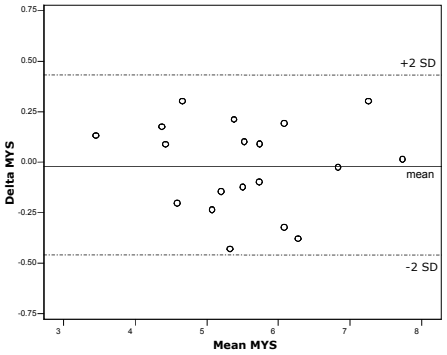


(d)

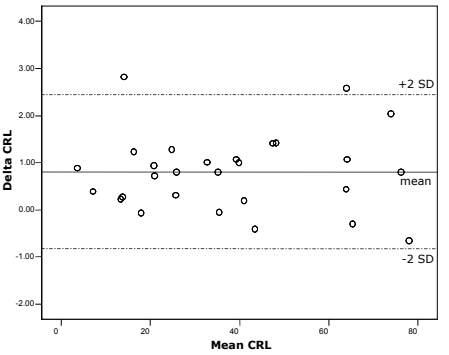


(e)

Figure 2.1.4 *Scatter plots comparing I-Space with 4DView measurements of five standard biometric parameters: (a) mean yolk sac diameter (MYS); (b) crown rump length (CRL); (c) biparietal diameter (BPD); (d) head circumference (HC); (e) abdominal circumference (AC). The line of equality is indicated.*



(a)



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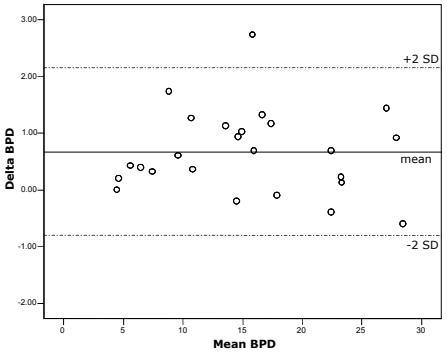

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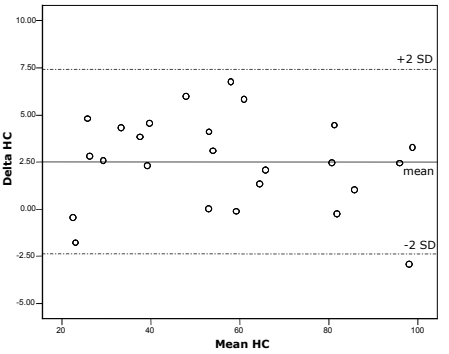

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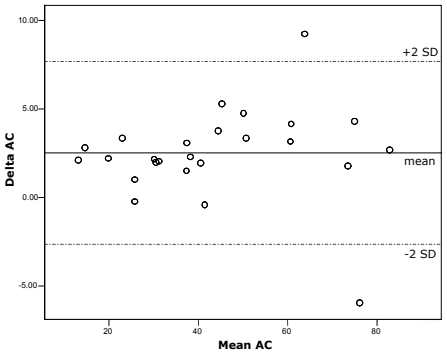

(e)

Figure 2.1.5. *Bland-Altman plots comparing I-Space with 4DView measurements of five standard biometric parameters: (a) mean yolk sac diameter (MYS); (b) crown rump length (CRL); (c) biparietal diameter (BPD); (d) head circumference (HC); (e) abdominal circumference (AC). The mean difference was calculated as 4DView minus I-Space. The solid line represents the mean difference and the broken lines represent the limits of agreement, calculated as mean difference ± 2SD.*

The corresponding mean differences between measurements made with 4DView and with I-Space, the limits of agreement and the ICC values are displayed in Table 2.1.1. For HC and AC measurements, the mean difference was statistically significant (one-sample t-test), but, as the ICC values show, this did not effect the agreement: all ICC values were > 0,97, indicating good agreement for all five parameters.

TABLE 2.1.1 *Agreement between three dimensional measurements using 4DView and I-Space of five standard biometric parameters.*

PARAMETER	N	MEAN DIFFERENCE *	95% CI FOR MEAN DIFFERENCE	LIMITS OF AGREEMENT #	ICC	95% CI
MYS	19	-0.02	-0.12 to 0.09	-0.46 to 0.43	0.977	0.942 to 0.991
CRL	28	0.80	0.48 to 1.11	-0.83 to 2.44	0.999	0.999 to 1.000
BPD	25	0.67	0.36 to 0.97	-0.81 to 2.15	0.995	0.989 to 0.998
HC	25	2.52	1.50 to 3.53	-2.39 to 7.43	0.995	0.989 to 0.998
AC	25	2.53	1.46 to 3.60	-2.64 to 7.71	0.992	0.981 to 0.996

N represents the number of patients in which both measurements could be performed
* *Mean difference = 4DView minus I-Space*
\# *Limits of agreement = mean difference ± 2 SD*

Table 2.1.2 shows the agreement between measurements by the same operator (intraobserver variation) and Table 2.1.3 shows that between two different operators (interobserver variation). The ICC's of the 4DView measurements are all > 0.96 and those of the I-Space measurements were > 0.98, indicating good agreement in all cases.

In the I-Space it was easier to view all the structures of interest, while when measuring the 3D volumes using the 4DView software it was sometimes difficult or even impossible to view all structures. For example, in some cases the yolk sac did not seem to be present in the volume evaluated in 4DView, whereas in the I-Space it was visualised very easily using exactly the same volume. As a result, more measurements could be performed in the I-Space than when using 4DView software.

Table 2.1.4 gives the ICC's of the 2D ultrasound measurements compared with 4DView measurements and I-Space measurements; all indicated good agreement.

TABLE 2.1.2 *Intraobserver agreement of three dimensional measurements using 4DView and I-Space of five standard biometric parameters.*

METHOD	PARAMETER	N	MEAN DIFFERENCE*	95% CI FOR MEAN DIFFERENCE	LIMITS OF AGREEMENT#	ICC	95% CI
4DView	MYS	19	-0.11	-0.25 to 0.02	-0.67 to 0.45	0.967	0.916 to 0.987
	CRL	28	-0.04	-0.34 to 0.26	-1.58 to 1.50	0.999	0.999 to 1.000
	BPD	25	-0.10	-0.30 to 0.10	-1.08 to 0.88	0.998	0.995 to 0.999
	HC	25	-0.89	-1.55 to –0.23	-4.10 to 2.31	0.998	0.995 to 0.999
	AC	25	-0.62	-1.50 to 0.27	-4.91 to 3.68	0.994	0.987 to 0.997
I-Space	MYS	20	0.03	-0.05 to 0.10	-0.29 to 0.35	0.988	0.971 to 0.995
	CRL	28	-0.07	-0.26 to 0.12	-1.04 to 0.90	1.000	0.999 to 1.000
	BPD	26	-0.10	-0.30 to 0.10	-1.12 to 0.92	0.998	0.995 to 0.999
	HC	26	-0.15	-0.97 to 0.67	-4.23 to 3.93	0.997	0.993 to 0.999
	AC	26	-0.03	-0.61 to 0.55	-4.91 to 3.68	0.997	0.994 to 0.999

N represents the number of patients in which both measurements could be performed
* *Mean difference = first measurement minus second measurement*
\# *Limits of agreement = mean difference ± 2 SD*

Table 2.1.3 *Interobserver agreement of three dimensional measurements using 4DView and I-Space of five standard biometric parameters.*

METHOD	PARAMETER	N	MEAN DIFFERENCE*	95% CI FOR MEAN DIFFERENCE	LIMITS OF AGREEMENT#	ICC	95% CI FOR ICC
4DView	MYS	19	-0.26	-0.34 to –0.18	-0.60 to 0.08	0.987	0.967 to 0.995
	CRL	27	0.63	0.24 to 1.01	-1.31 to 2.56	0.999	0.998 to 1.000
	BPD	25	-0.11	-0.35 to 0.12	-1.28 to 1.06	0.997	0.993 to 0.999
	HC	25	-0.23	-3.07 to 2.62	-14.03 to 13.58	0.963	0.918 to 0.984
	AC	25	0.01	-0.83 to 0.85	-4.06 to 4.08	0.995	0.988 to 0.998
I-Space	MYS	20	-0.04	-0.09 to 0.02	-0.29 to 0.22	0.993	0.982 to 0.997
	CRL	28	-0.07	-0.32 to 0.18	-1.37 to 1.22	1.000	0.999 to 1.000
	BPD	26	-0.47	-0.99 to 0.05	-3.06 to 2.11	0.987	0.971 to 0.994
	HC	26	-1.07	-2.18 to 0.04	-6.58 to 4.45	0.994	0.987 to 0.997
	AC	26	-0.49	-0.95 to –0.04	-2.76 to 1.77	0.998	0.997 to 0.999

N represents the number of patients in which both measurements could be performed
* *Mean difference = measurements of first examiner (CV-D) minus those of second examiner (MR)*
\# *Limits of agreement = mean difference ± 2 SD*

Table 2.1.4 *Agreement between standard two dimensional (2D) measurements and three dimensional measurements using 4DView/I-Space of five standard biometric parameters.*

METHOD	PARAMETER	N	MEAN DIFFERENCE*	95% CI FOR MEAN DIFFERENCE	LIMITS OF AGREEMENT#	ICC	95% CI
4DView	MYS	19	-0.02	-0.16 to 0.12	-0.61 to 0.58	0.953	0.883 to 0.982
	CRL	26	-0.50	-1.23 to 0.23	-4.14 to 3.13	0.997	0.993 to 0.999
	BPD	21	-0.39	-0.78 to 0.00	-2.11 to 1.33	0.992	0.979 to 0.997
	HC	21	-3.09	-4.24 to –1.94	-8.15 to 1.97	0.993	0.983 to 0.997
	AC	19	-0.42	-1.57 to 0.73	-5.20 to 4.35	0.990	0.975 to 0.996
I-Space	MYS	20	-0.01	-0.18 to 0.17	-0.76 to 0.75	0.922	0.813 to 0.968
	CRL	26	0.34	-0.31 to 0.99	-2.89 to 3.56	0.997	0.994 to 0.999
	BPD	21	0.32	-0.12 to 0.76	-1.62 to 2.25	0.990	0.974 to 0.996
	HC	21	-0.33	-1.90 to 1.25	-7.25 to 6.59	0.988	0.970 to 0.995
	AC	19	2.32	0.67 to 3.97	-4.53 to 9.16	0.980	0.949 to 0.992

N represents the number of patients in which both measurements could be performed.
* *Mean difference = 2D minus 4DView/I-Space measurement*
\# *Limits of agreement = mean difference ± 2 SD*

DISCUSSION

When a new visualisation technique is introduced, it must be demonstrated that measurements obtained with it can be reproduced reliably. We have demonstrated that measuring BPD, HC and AC using I-Space is reliable even in early pregnancy. 2D ultrasonography, especially using a transvaginal probe, is limited in that it does not always allow visualisation of all orthogonal planes. Using 3D ultrasound with specialized software has a major advantage: the plane that cannot be visualized with 2D ultrasound can be examined using the computer-generated reconstruction. However, extensive experience with 3D software programs is necessary to benefit fully from these multiplanar images, which are displayed on a 2D screen. The I-Space facilitates this examination since one can interact with the volume-rendered ultrasound data more intuitively than is possible on a 'flat' computer screen.

The proportion of total variance in measurements that can be explained by differences between the individuals examined is indicated by the intra- and interobserver ICC 21. Our results show that both measurements in 3D using 4DView and using I-Space are highly reliable, with ICC values of at least 0.98. Using exactly the same volume for 4DView and I-Space measurements allowed us to compare the measurements directly, with no possibility of interference from confounding factors such as movement of the embryo between measurements. Measurements in virtual reality are therefore at least as reliable as are routine 3D measurements using 4DView.

The perception of depth in the I-Space also offers the possibility of measuring non-planar structures, such as the umbilical cord. Since we have demonstrated the excellent reliability of the I-Space as a measuring tool, research should now be extended to assess this new technique, which we tentatively name 'Virtual Embryoscopy', in other areas of embryonic and foetal biometry.

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CHAPTER 2.2

EARLY PREGNANCY VOLUME MEASUREMENTS: VALIDATION OF ULTRASOUND TECHNIQUES AND NEW PERSPECTIVES

BJOG 2009; 116(2): 278-285

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ABSTRACT

Objective:

To investigate the accuracy and reliability of four different ultrasound related volume-measuring methods.

Methods:

Ten phantoms for *in vitro* measurements and twenty-eight pregnancies with gestational ages ranging from six to eleven weeks for *in vivo* measurements were included. Three dimensional (3D) ultrasound images of phantoms (with known variable contents) and yolk sacs were used to calculate volumes using four different methods; Virtual Organ Computed-Aided AnaLysis (VOCAL), Inversion mode, Sono Automatic Volume Calculation (SonoAVC) and V-Scope. V-Scope is a newly developed 3D volume visualization application using a Barco I-Space Virtual Reality system. Intra- and interobserver agreement was established by calculating intraclass correlation coefficients (ICC).

Results:

In the *in vitro* study volume measurements by VOCAL, Inversion mode and V-Scope proved to be accurate. SonoAVC measurements resulted in a substantial systematic underestimation. Correlation coefficients of measured versus true volumes were excellent in all four techniques. For all techniques an intra- and interobserver agreement of at least 0.91 was found. Yolk sac measurements by the different techniques proved to be highly correlated (ICCs > 0.91).

Conclusions:

We demonstrated that VOCAL, Inversion mode and V-Scope can all be used to measure volumes of hypoechoic structures. The newly introduced V-Scope application proved to be accurate and reliable.

INTRODUCTION

Until the late nineties, mathematical formulae were used to estimate volumes using two dimensional (2D) ultrasound images. For instance, the prolate ellipsoid or trapezoid formula is used for measuring ovarian volumes [1] and the ellipsoid formula for foetal bladder volume calculations [2]. In such volume calculations, a certain regularity of shape of the structure is assumed and correction for surface irregularities is not possible.

The introduction of three dimensional (3D) ultrasound allows visualisation of planes that can not be obtained using 2D ultrasound. In this way, volumetric measurements without geometric assumptions as well as corrections or assessments of surface irregularities are obtainable.

Computer software programs have been developed for volume measurements, either incorporated into the ultrasound equipment or for off-line evaluation on personal computers. Conventional volume measurements involve the delineation of the object of interest in one plane of the multiplanar display. Several *in vitro* [3] and *in vivo* [4] studies for validation of volume measurements have demonstrated this to be an accurate and reliable technique. The operator can conduct as many serial slices as needed so that less regularly shaped or larger objects can also be measured [5-7].

The introduction of the Virtual Organ Computer-Aided AnaLysis (VOCAL™) imaging program makes it possible to measure volumes by rotation around a central axis. Raine-Fenning et al [8] demonstrated that this rotational technique is better than the conventional measuring method. After several *in vitro* [8] and *in vivo* [9-11] experiments, the VOCAL imaging application is now considered to be the 'gold standard' for volume measurements in ultrasound imaging.

In this paper we evaluate three other volume-measuring techniques that use grey level information instead of delineation. Inversion mode is a thresholding algorithm that has been available for several years. It makes visualisation and volume calculation of fluid-filled structures possible in 3D and four dimensional (4D) ultrasound images [12-14].

In 2008 GE Medical Systems introduced the Sono Automatic Volume Calculation (SonoAVC) technique on the Voluson E8 ultrasound system. This new algorithm allows semi-automatic measurements of volumes, mean diameters and

absolute dimensions of hypoechoic regions in a 3D ultrasound dataset [11,15].

The latest technique uses a Virtual Reality (VR) system to benefit from all three dimensions offered by 3D ultrasound datasets. In this study we will use the V-Scope application in a Barco I-Space, a system that uses stereo projection on three screens and the floor to immerse viewers in a 3D world. This application has already been successfully applied to 3D prenatal ultrasonography [16-20]. A region-growing segmentation algorithm has been implemented in this program that calculates the volume of selected structures of interest semi-automatically.

The aim of this study was to investigate the accuracy and reliability of currently available volume-measuring methods and of the two newly introduced techniques, SonoAVC and V-scope, both *in vitro* and *in vivo* settings. Robust establishment of accuracy and reliability is needed as a validation before these techniques can be applied in daily clinical practice.

METHODS

Our study was performed using a 3.7-9.3 MHz transvaginal probe of the GE Voluson 730 Expert system (GE Medical Systems, Zipf, Austria) for the *in vivo* part and a 1.9-7.8 MHz abdominal probe of the GE Voluson E8 (GE Medical Systems, Zipf, Austria) for the *in vitro* part. 4DView software (version 5.0 and 7.0, GE Medical Systems) was used to explore and visualize the datasets and to measure volumes using VOCAL, Inversion mode and SonoAVC. The fourth, innovative, application used in our study, V-scope, is not available on ultrasound machines or personal computers. Since 2005 the department of Bioinformatics of the Erasmus MC in The Netherlands operates a BARCO I-Space. The I-Space is a so-called 4-walled CAVE-like [21] (Cave Automatic Virtual Environment) virtual reality system, allowing depth perception and interaction with the rendered objects in an intuitive manner. We use an in-house developed volume rendering application [19] (CAVORE, recently renamed to V-Scope).

VOCAL

Virtual Organ Computer-Aided Analysis (VOCAL™) is a volume-measuring algorithm based on 2D segmentations around a central rotational axis. The user can specify the number of rotational steps. Kusanovic et al [12] described this volume-measuring method in detail. The user is able to define the rotation step and the mode of interest. The software calculates the volumes of the structures automatically, which are expressed in cubic centimetres. A 'manual mode' can be chosen, allowing drawing around the object of interest with the use of a computer mouse in the A, B or C plane. Measurements can then be performed in various degrees of rotational steps.

Inversion mode

This segmentation algorithm uses grey scale voxels (3D pixels or volume elements) in the 3D dataset for volumetric measurements [22]. Only hypoechoic regions can be estimated with the inversion mode, because it uses a single upper threshold after inverting the grey value. Kusanovic et al [12] already provided a detailed description and manual of this system. As inversion mode is a global operator, working on the entire dataset, it is necessary to erase incorrectly segmented areas, before calculating the segmented volume. Inversion mode can also be used in combination with VOCAL, where the contour serves as a delineation of the 'volume of interest'.

SonoAVC

Sono Automatic Volume Calculation (AVC) is a new algorithm that identifies and quantifies hypoechoic regions within a 3D dataset and provides automatic estimation of their absolute dimensions (x, y, z diameters), mean diameter (relaxed sphere diameter) and volume [15]. It was originally developed for measurements of follicle volumes and therefore can produce multiple volumes as a result of the segmentation. SonoAVC works only within a specified region of interest (ROI), and only inner volumes can be estimated. Similar to Inversion mode, SonoAVC calculates volumes by counting all volume elements (voxels) within hypoechoic regions and converting them to a standard unit (cubic centimetres). Post-processing is available for correction by allowing the user to add or delete incorrectly segmented areas. If there are aberrances in the region of interest, the 'growth' or 'separation' function can also be used [15].

V-Scope

The V-Scope application is used to create a 'hologram' of the ultrasound image that can be manipulated by means of a virtual pointer, controlled by a wireless joystick. 3D ultrasound datasets can be transferred to the BARCO I-Space after transformation to cartesian (rectangular) volumes. For use in the V-Scope application, we have implemented a flexible and robust segmentation algorithm that does not depend on 2D interaction, like the VOCAL algorithm. The algorithm is based on a region-growing approach in combination with a neighbourhood variation threshold, as proposed for magnetic resonance imaging (MRI) data by Myers and Brinkley [23]. the algorithm has been modified to accommodate the 'noisy nature' of ultrasound data: in addition to simplifying some of the parameters of the original algorithm, the grey level and variation thresholds are applied to a 'blurred version' of the original data, to average out most of the noise [24].

The user selects an upper and lower grey level threshold and an upper threshold for the standard deviation based on the characteristics of the target area. A seed point in the area and the algorithm will segment out (grow) the region starting from the seed point. The standard deviation threshold will stop the region growing when it reaches a tissue interface.

When evaluating the yolk sac, the tissue interface (the 'skin' of the yolk sac) is recorded much wider than it really is. In addition, should the region have grown outside the actual object (for instance as a result of noise or drop outs) or part of the object have been skipped (due to artefacts), a spherical, free hand 'paint brush' can be used to add voxels to or delete voxels from the segmented object.

In vitro study

For the *in vitro* part of our study, we placed a water-filled object in an ultrasound test reservoir. This reservoir contained a tissue-mimicking fluid medium, created by a suspension of graphite particles in sterile water and glycerine. We used simple structured objects; water balloons filled with different volumes of sterile water. A total of ten balloons were filled with 1.0, 1.3, 1.5, 1.8, 2.0, 2.2, 2.3, 2.5, 2.8 and 3.0 millilitre of volume respectively. One observer acquired all of the different ultrasound datasets of the objects using the Voluson E8. Dynamic range was set at 12, harmonics was set on low and 3D volumes were acquired at maximum quality. All images were stored

for off-line evaluation. Another observer evaluated the quality and completeness of the datasets, and for each balloon, the best image was selected.

Volumes of the balloons were measured with all four techniques (figure 2.2.1). For VOCAL, we used rotational angles of 15° and 30°. The inversion mode technique was applied without the VOCAL option. In the SonoAVC application the 'growth' function was set to maximum and the 'separation' function to minimum.

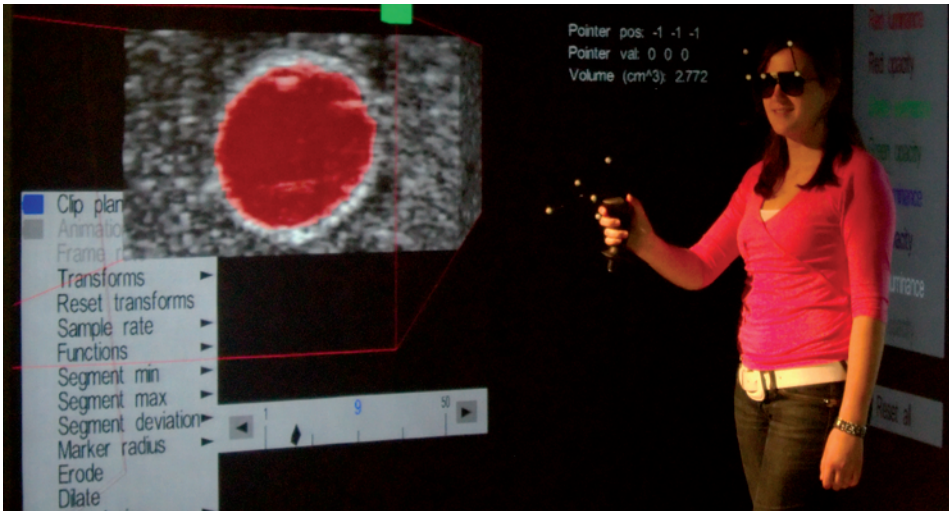

Figure 2.2.1 *I-Space image of a water balloon. The dark-grey colour of the balloon marks the segmented volume. The true volume of the water balloon was 2.8 millilitres.*

All volume measurements were repeated three times; mean values were used for comparison of the techniques. The observer was blinded for the true volume of the balloons. All measurements were performed twice by one observer (MR) and repeated independently by another (CV-D). The second series of measurements by MR were performed at least two weeks after the first series to prevent recollection bias. The observers were blinded to each other's results.

The duration of time required to obtain all measurements was registered by observer one, to evaluate applicability in daily clinical practice. Timing was started when the data were loaded and ended when the final estimated volume was shown.

In vivo study

To evaluate the use of the four different techniques in daily clinical practice, 24

pregnancies were examined at a median gestational age of 9 weeks (range 6-11 weeks) to visualize the yolk sac in 3D. These data were obtaining in another study to evaluate embryonic growth and development by Verwoerd et al [18]. We used the yolk sac as our object of interest, since it is an easy to identify, well-defined, fluid-filled structure. All measurements using the four different techniques were performed off-line (figure 2.2.2) as described in the *in vitro* part and repeated three times; mean values of these three assessments were used for comparison. The duration of time was monitored as described in the *in vitro* section.

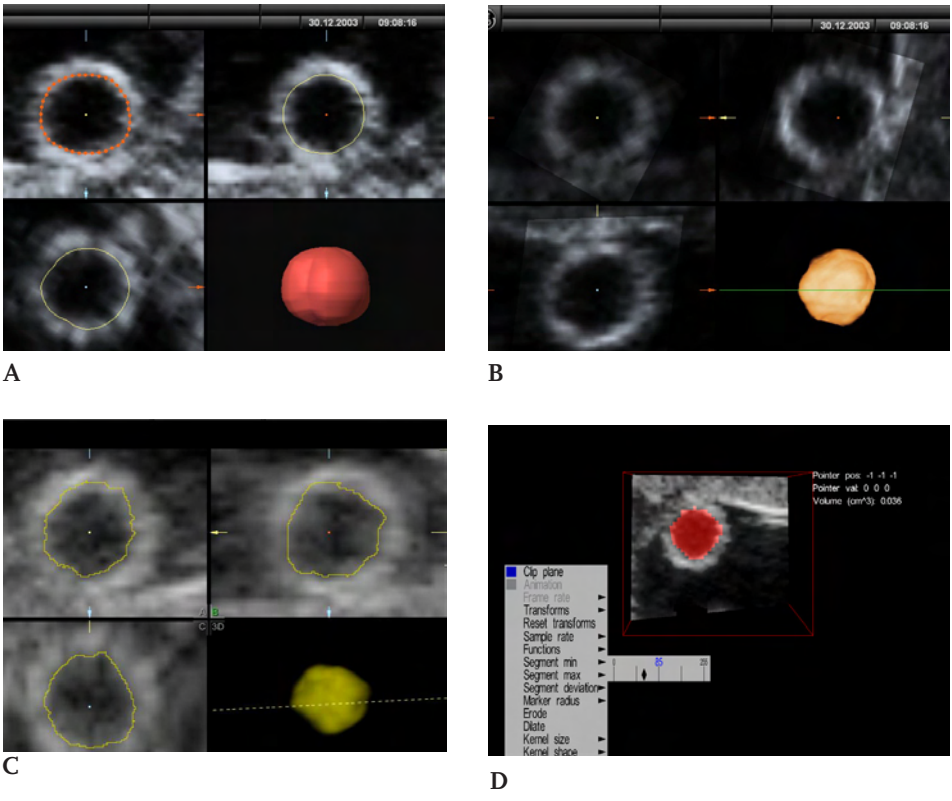

Figure 2.2.2 *Images of yolk sac (66 days of gestational age) volume measurements obtained by four different techniques. (A) VOCAL. The balloon is traced in the A plane, and the estimated volume is 0.039 cm. (B) Inversion mode. The estimated volume is 0.04 cm. (C) SonoAVC. The 3D image shows an estimated volume of 0.04 cm. (D) V-Scope. The segmented volume is normally marked with a colour (in this picture it is dark-grey). The volume of the yolk sac is 0.036 cm.*

Statistical analysis

Data analysis was performed using SPSS (SPSS Release 12.0.1 for Windows, SPSS Inc, Chicago, IL, USA). In the *in vitro* part of the study the accuracy and reliability

were investigated. To assess the accuracy of the different techniques, differences between measured volumes and true volumes were calculated and compared with the paired *t*-test. A P-value of 0.05 (two sided) was considered the limit of significance. In addition, Pearson correlation coefficients were calculated of measured against true volumes.

Reliability is the extent to which we can assume that it will yield the same result if repeated a second time. To assess this, intraclass correlation coefficients (ICC) were used. In addition, we tested both intra- and interobserver reliability for all four measuring methods. For good agreement, ICC has to be 0.90 or higher.

In the *in vivo* part of the study, ICCs were calculated for all four measuring methods as described for the *in vitro* part. We further calculated the mean difference and limits of agreement (mean difference ± 1.96SD) as described by Bland-Altman [25] to show the agreement between measurements when different methods are used. The mean time needed to perform volume measurements, the associated standard deviations and the time ranges were calculated for the *in vitro* and *in vivo* part.

RESULTS

In vitro study

Of the original ten water-filled balloons, in seven balloons, image quality was good enough to perform volume measurements with all four different techniques. These seven balloons contained 1.3, 2.0, 2.2, 2.3, 2.5, 2.8 and 3.0 millilitre of sterile water respectively.

Table 2.2.1 shows the mean differences between the measured volumes and the real volumes, with the corresponding significance. For VOCAL, Inversion mode and V-Scope, there are no significant differences. SonoAVC, however, demonstrates a systematic underestimation (mean difference of –0.63 for both observers) of the measured volume compared with the true volume (both $P < 0.001$). All measured volumes correlate well with the true volume (all correlation coefficients > 0.91, both for observer one and observer two).

Table 2.2.1. *Evaluation of accuracy of the in vitro study using the different techniques of volume estimation.*

OBSERVER	TECHNIQUE	MEAN DIFFERENCE (CM)	SD OF DIFFERENCES (CM)	95% CI OF THE DIFFERENCE (CM)	P-VALUE
Observer 1					
	VOCAL 30°	0.019	0.063	-0.039 to 0.780	0.449
	VOCAL 15°	0.020	0.055	-0.031 to 0.071	0.371
	Inversion	0.011	0.063	-0.047 to 0.069	0.662
	SonoAVC	-0.632	0.240	-0.854 to -0.410	< 0.001
	V-Scope	0.011	0.035	-0.022 to 0.043	0.449
Observer 2					
	VOCAL 30°	0.023	0.112	-0.081 to 0.127	0.605
	VOCAL 15°	0.015	0.105	-0.082 to 0.113	0.716
	Inversion	-0.007	0.080	-0.081 to 0.067	0.822
	SonoAVC	-0.626	0.243	-0.851 to -0.402	< 0.001
	V-Scope	-0.010	0.006	-0.025 to 0.004	0.135

The mean difference between measured volumes minus the real volumes of the balloons with the associated standard deviation and 95% confidence interval (CI) are displayed. The results of both observers are displayed.

Table 2.2.2 shows the agreement between measurements performed by the same observer (intraobserver variability) and the agreement between two different observers (interobserver variability). ICCs are all greater than 0.99, representing excellent agreement in all cases.

Table 2.2.2. *Intraobserver (ICC-A) and interobserver (ICC-B) correlation coefficients of the in vitro study.*

TECHNIQUE	ICC-A	95% CI ICC-A	ICC-B	95% CI ICC-B
VOCAL 30º	0.998	0.990 to > 0.999	0.995	0.971 to 0.999
VOCAL 15º	0.999	0.996 to > 0.999	0.993	0.962 to 0.999
Inversion	0.998	0.988 to > 0.999	0.995	0.973 to 0.999
SonoAVC	0.999	0.994 to > 0.999	0.996	0.980 to 0.999
V-Scope	0.998	0.992 to > 0.999	0.997	0.982 to 0.999

In vivo study

Yolk sac volumes could be measured with VOCAL, Inversion mode and V-Scope in all 24 ultrasound scans. With SonoAVC we were not able to measure the volume of four yolk sacs. All techniques were compared to each other and ICCs were calculated. Results of these comparisons are displayed in Table 2.2.3. In all cases, the ICC is at least 0.91, suggesting good reliability. The best ICC with narrowest confidence interval is demonstrated between V-Scope and VOCAL. The mean difference and 95% limits of agreement are displayed in Table 2.2.3.

Table 2.2.3. *Evaluation of reliability of the yolk sac volume measurements.*

TECHNIQUE	N	MEAN DIFFERENCE* (CM)	95% CI OF MEAN DIFFERENCE (CM)	LIMITS OF AGREEMENT# (CM)	ICC	95% CI OF ICC
VOCAL 30° vs. VOCAL 15°	24	-0.001	-0.003 to 0.003	-0.004 to 0.002	0.996	0.990 to 0.998
VOCAL 30° vs. Inversion	24	-0.004	-0.013 to 0.001	-0.010 to 0.003	0.963	0.715 to 0.989
VOCAL 30° vs. SonoAVC	20	0.002	-0.008 to 0.012	-0.007 to 0.012	0.958	0.884 to 0.984
VOCAL 30° vs. V-Scope	24	-0.000	-0.004 to 0.004	-0.005 to 0.004	0.992	0.981 to 0.996
Inversion vs. SonoAVC	20	0.006	-0.003 to 0.020	-0.004 to 0.016	0.909	0.340 to 0.975
Inversion vs. V-Scope	24	0.003	-0.004 to 0.016	-0.006 to 0.012	0.943	0.777 to 0.981
SonoAVC vs. V-Scope	20	-0.002	-0.012 to 0.006	-0.012 to 0.007	0.956	0.879 to 0.983

N is the number of yolk sacs that could be compared.
* *Mean difference as technique 1 minus technique 2.*
\# *Limits of agreement mean difference ± 1.96 SD.*

Results of the time required for each separate volume calculation are shown in Table 2.2.4. In the *in vitro* study, VOCAL is fastest and V-Scope is slowest when compared with the other techniques. All the comparisons result in statistically significant differences ($P < 0.001$). However, in the *in vivo* study, V-Scope is faster than the other techniques (for all comparisons: $P < 0.004$) and has the smallest time range. VOCAL 15° takes the most time (for all comparisons: $P < 0.001$).

Table 2.2.4. *Time required to perform one volume measurement using the different techniques.*

STUDY	TECHNIQUE	MEAN TIME ± SD (SECONDS)	RANGE (SECONDS)
in vitro			
	VOCAL 30°	66 ± 9	51 to 86
	VOCAL 15°	110 ± 12	89 to 129
	Inversion mode	78 ± 17	50 to 118
	SonoAVC	99 ± 25	40 to 142
	V-Scope	123 ± 78	40 to 281
in vivo			
	VOCAL 30°	96 ± 29	56 to 160
	VOCAL 15°	159 ± 24	89 to 208
	Inversion mode	112 ± 28	65 to 197
	SonoAVC	81 ± 23	41 to 134
	V-Scope	61 ± 22	29 to 117

DISCUSSION

In this study we tested the accuracy and reliability of four different 3D ultrasound volume-measuring methods in both an *in vitro* and an *in vivo* setting. Clinical use of volume measurements using 3D ultrasound is likely to become increasingly important in the study of human reproduction and embryogenesis. In recent years, new techniques have been developed for estimating volumes in 3D. This is the first study comparing four of these techniques; VOCAL, Inversion mode, SonoAVC and V-Scope.

We have limited our research to measurements of hypoechoic structures since only these structures can be measured by all four techniques. However, hyperechoic

structures, such as the foetal body and placenta, can also be measured using VOCAL and V-Scope.

VOCAL is considered to be an important development in volume measurements in 3D ultrasound images. The *in vitro* study by Raine-Fenning et al [8] demonstrated that VOCAL is more reliable and accurate in calculating volumes than conventional methods. Using an angle of 30° for performing the volume measurements showed good accuracy, especially when regularly shaped structures were measured. In our study we used both the 30° and 15° rotational angles to verify that their conclusion also applies to very small structures. We did not find significant differences between the two volume angles. Although VOCAL is generally recognized as the gold standard for performing volumetric measurements, it does have some limitations. The *in vitro* study performed by Raine-Fenning et al [8] showed that VOCAL has a tendency to overestimate true volumes. In our study, we do not find a tendency of overestimation, neither *in vitro* nor *in vivo*. Another limitation is the time required for measuring the volume of interest. Different studies illustrate that the time needed to perform the volumetric measurements can range between one and ten minutes [8,11,12,26]. The greater the angle is, the shorter the measuring time. In our study, mean time for VOCAL measurements was shorter than usually reported in the literature. This can probably be explained by the fact that we used only regularly shaped structures. This also means that, using the VOCAL approach, possible inaccuracies when measuring very irregularly shaped volumes cannot be excluded. Finally, it can be difficult to determine the boundaries of some structures because of shadows, other structures [26] or dispersion of the boundaries in women with limited image quality.

Inversion mode was mainly developed to generate information about the anatomical and pathological characteristics of fluid-filled structures; for instance, the visualisation of abnormal systemic venous connections [14]. In addition to visualisation, volume measurements can be performed [13] with a high degree of reliability when compared with other techniques like VOCAL [12]. Kusanovic et al [12] concluded that inversion mode had larger volume measurements than VOCAL, which they contributed to the fact that relatively high threshold levels were chosen [8]. A limitation of their study was that accuracy could not be evaluated since the true volumes were not known. They also concluded that inversion mode is a slightly faster technique than VOCAL. We found this to be true only for the rotational angle of 15°. We show with the *in vitro*

part of our study that the accuracy and reliability of volume measurements performed with the inversion mode are very high. Like VOCAL, image quality is very important when using inversion mode. Adjustments in contrast, threshold and transparency may improve image quality. In our study, the combination of the 'surface smooth' and 'gradient light' filters produced the best 3D images, although visualisation problems still persisted. Especially dispersion of the borders from the structures of interest was found to affect the outcome of the volume estimation.

In 2008, SonoAVC has become available as a semi-automatic volume-measuring application, which has the potential to remove observer bias and to reduce the time needed for measurements [15]. Studies have shown that SonoAVC is able to provide highly accurate automatic follicular volume measurements in a short time, especially when image quality is high [15,27]. Again, image quality greatly influences the ability to measure volumes. If noise speckles are present in the dataset, SonoAVC will measure the volume of interest without these speckles (including an area surrounding each speckle), producing a substantial underestimation. In some cases, the post-processing tools in SonoAVC can be used to improve the value of the measurement, but this is at the cost of extending the time required for measuring the volumes. In the *in vitro* part of our study, we found that SonoAVC gives a substantial systematic underestimation of the phantoms. All speckles visible within the phantom images were not included into the volume calculation, and this led to underestimation of the true volumes. This underestimation persisted even after the use of the post-processing tools. For the *in vivo* study, in 4 of the 24 yolk sacs, SonoAVC could not be applied due to image quality problems. The remaining 20 yolk sac volumes were in concordance with the other measuring techniques. We therefore conclude that SonoAVC is a reliable measuring method but very dependent on image quality and with a tendency to underestimate the true volume. Since post-processing tools had to be used so often, we do not consider this technique to be 'automatic' in a true sense.

The I-Space uses stereo projection, which makes it possible to visualise a 'real' 3D ultrasound image. Studies using this virtual reality technology have demonstrated that it provides additional insights [16-20], especially where structures that need depth for accurate interpretation of size and position are concerned. Verwoerd et al [18] already demonstrated the reliability of this system in measuring standard human embryonic biometry. The volume-measuring tool of V-Scope is a new development. Our study

reveals that accuracy and reliability are very good. In the *in vitro* part of this study, V-Scope measurements took the longest time. This can be attributed to the low image quality of two of the balloons. In these two objects, ultrasound fall-out created an apparent gap in the shell of the balloons, and therefore, voxels outside of the balloons were also included. Erasing these voxels is a time-consuming process. Disregarding these balloons brings the time required in line with the other methods.

The basis of every measuring method, accuracy, was tested in the *in vitro* part of this study. VOCAL and V-Scope have the best correlation coefficients when measured volumes are compared with the true volumes. Both applications measure the volumes without a statistically significant difference. SonoAVC gives a substantial systematic underestimation of the volumes. Inter- and intraobserver variability revealed very good results (ICC > 0.90) for all four techniques. The *in vivo* part of this study demonstrates that for yolk sac volumes, all four techniques are capable of making precise measurements.

Feasibility, an important aspect for implementation in daily clinical practice, is good in general for all four systems. In the *in vitro* setting VOCAL 30° and in the *in vivo* study V-Scope are the fastest techniques, but time alone is not the most important factor. Image quality greatly influences the time needed to measure a volume and may be related to several factors such as maternal obesity and oligohydramnios. Especially for the (semi-) automatic volume-measuring methods (SonoAVC and V-scope), good image quality greatly reduces of measuring time. In cases of poor image quality, SonoAVC is very labour intensive, mainly as a result of the post-processing required. Although V-Scope is currently only available in the Erasmus MC I-Space, it is being adapted to run on a desktop system using a 3D auto-stereoscopic computer monitor. The advantage of this type of display is that it can be used without any viewing aids, that is without 3D polarizing glasses. These low cost virtual reality displays may well become available in daily clinical practice in the near future.

As V-Scope performs at least as well as the established VOCAL technique, it can be used in research on human embryogenesis. Measurements can be made of any volume such as the entire embryo, the amniotic sac or the embryonic brain. After obtaining reference values of early pregnancy volumes, associations can be studied with clinical outcomes such as miscarriage, congenital abnormalities and intrauterine growth restriction. This technique, tentatively called 'Virtual Embryoscopy', may help to improve our knowledge on embryonic growth and development.

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PART 3

IMPROVING KNOWLEDGE OF EMBRYOGENESIS IN VIVO BY USE OF A STAGING SYSTEM TAKING INTO ACCOUNT BOTH MORPHOLOGICAL FEATURES AND BIOMETRIC MEASUREMENTS

CHAPTER 3.1

EMBRYONIC STAGING USING A 3D VIRTUAL REALITY SYSTEM

Hum Reprod 2008; 23(7): 1479-1484

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ABSTRACT

Background:

The aim of this study was to demonstrate that Carnegie Stages could be assigned to embryos visualized with a 3D virtual reality system.

Methods:

We analyzed 48 3D ultrasound scans of 19 IVF/ICSI pregnancies at 7-10 weeks' gestation. These datasets were visualized as 3D 'holograms' in the BARCO I-Space virtual reality system. Embryos were staged according to external morphological features (i.e. mainly limb development). After staging, the crown-rump length (CRL) was measured. Stage and CRL were compared with gestational age based on the date of oocyte retrieval and with the classical data on embryology from the Carnegie Collection.

Results:

Embryonic staging was relatively easy because the I-Space allows depth perception, which helps in the estimation of size and position. The presumed stages corresponded well with the measured CRL. However, in 28 out of 48 cases stages seemed to have been reached earlier than previously described for the Carnegie Collection.

Conclusions:

The I-Space, tentatively named Virtual Embryoscopy, is a promising non-invasive tool for early pregnancy evaluation. Combining embryonic growth with embryonic development opens a new area to study the relationship between embryonic growth, development and morphology as well as second and third trimester pregnancy complications.

INTRODUCTION

Adequate staging of embryonic development is important for an accurate description of normal development and provides insight in abnormal embryonic growth and development. Developmental embryonic staging was first employed in human embryology by Franklin Mall (1914), founder of the Department of Embryology of the Carnegie Institution in Washington. The Carnegie Stages system describes approximately the first nine weeks of pregnancy and the stages, numbered from 1 to 23, are based on internal and external physical characteristics of the embryo. At stage 23, all essential internal organ systems are present and this stage therefore represents the end of the embryonic period.

Blaas et al [1] have already described that embryonic development visualized by ultrasound is in good agreement with the 'developmental time schedule' of human embryos, as described in the Carnegie Staging system. Although O'Rahilly and Müller [2] stated that there are variations in embryonic age as well as in embryonic size, Blaas et al [3] have shown that longitudinal ultrasound studies of normal embryos demonstrate virtually identical growth velocities for embryos and their associated structures. In their most recent study 3D ultrasound was used, calculating volumes of human embryos and young foetuses [4].

Wilhelm His [5] was the first who acknowledged the importance of three dimensional (3D) reconstructions of human embryos, making freehand drawings of histological slices. In the last decennia, development of computer technology has opened new possibilities for 3D reconstructions. The advantages of 3D ultrasound for foetal imaging in second and third trimester are unequivocal. The use of 3D ultrasound in the detection of foetal anomalies, especially for anomalies of face, limbs, thorax and spine is applied by numerous centres around the world [6]. The use of 3D and four dimensional (4D) ultrasound in early pregnancy assessment was recently summarized by Zanforlin Filho et al [7].

However, although these ultrasound datasets are three dimensional, they are presented on flat two dimensional (2D) screens or paper, which implies that information concerning the third dimension, is not used optimally.
To benefit from all three dimensions we used a three dimensional projection system, the I-Space. This virtual reality system immerses the viewer(s) in a three dimensional

virtual environment that allows the users to perceive depth and interact with the volume rendered data in an intuitive manner [8].

Being able to accurately determine embryonic stages in the first trimester would provide a promising non-invasive tool for early pregnancy evaluation of embryonic growth and development. The aim of this study is to demonstrate that Carnegie Stages based on external morphological features can be assigned to embryos visualized with 3D ultrasound using this novel 3D virtual reality system. We tentatively name this technique 'Virtual Embryoscopy'.

MATERIALS AND METHODS

Patients

We analyzed 3D ultrasound scans of 19 IVF/ICSI pregnancies. A total of 20 patients from the department of Reproductive Medicine in our hospital volunteered, of whom 19 had ongoing pregnancies and one miscarried before six weeks gestational age. This patient was therefore excluded from our study. All patients were in good health, without any predisposing conditions or use of medication that could interfere with normal embryonic growth. Serial 3D ultrasound scans were made starting at ~26 days after oocyte retrieval until ~84 days after oocyte retrieval (corresponding with ~6 weeks gestational age until fourteen weeks gestational age). This resulted in a total of 93 scans, varying from 3 scans to 9 scans per patient, with a median of 5 scans. A short analysis of the scans revealed that before day 36 (corresponding with ~7 weeks gestational age), it was almost impossible to obtain images with high enough resolution to discern the fine details we needed for staging purposes. Since the Carnegie Staging System ends at Stage 23, which corresponds with day 57 according to O'Rahilly, we excluded all ultrasound scans made after the 57th day (corresponding with ~10 weeks gestational age). This resulted in a total of 48 ultrasound scans from 36 to 57 days after oocyte retrieval.

Ultrasound

Ultrasound scanning was performed on a GE Voluson 730 Expert system (GE Medical Systems, Zipf, Austria). The 3D volumes were transferred to a personal computer

for offline evaluation using specialized 3D software (4Dview, GE Medical Systems). These data were then saved as cartesian (rectangular) volumes and transferred to the BARCO I-Space at the department of Bioinformatics of the Erasmus MC. This is a 4-walled CAVE-like [9] virtual reality system that uses passive stereo to immerse viewers in a virtual world. The images are generated by an SGI Prism visualization system with 8 graphics cards and are projected on three walls and the floor of a small 'room'. The images need to be viewed through glasses with polarizing lenses in order to perceive depth. The CAVORE [10] volume rendering application is used to create a 'hologram' of the ultrasound volume that is being investigated, which can then be manipulated by means of a virtual pointer, controlled by a wireless joystick [8]. Wireless tracking of the viewer's head allows the computer to provide a correct perspective and motion parallax which, in addition to the stereoscopic images, helps in discerning fine details and understanding of three dimensional structures in the volumes.

The 48 volumes we obtained with 3D ultrasound were visualized in the I-Space as 3D holograms. Volumes were resized, turned and clipped to provide an unobstructed view of the embryo and grey scale and opacity were adjusted for optimal image quality.

Staging

Embryos were staged according to the description of the external morphological features, mainly limb development, of the Carnegie Stages illustrated and described by O'Rahilly and Müller [2]. After staging, the crown-rump length (CRL) was measured. In the classical description, the CRL is better known as the greatest length of the embryo [11,12]. Stage and CRL were compared with gestational age based on the date of oocyte retrieval and with the classical data on embryology described by O'Rahilly and Müller (Table 3.1.1).

Statistical analysis was performed using SAS PROC MIXED (release 8.02, SAS Institute Inc, Cary, NC, USA). For analysis of the longitudinal measurements we used repeated measurements ANOVA (random coefficient model).

RESULTS

In all 48 ultrasound scans, we were able to determine the Carnegie Stage of the embryo easily (Figures 3.1.1 + 3.1.2). Curvature of the elbow for instance, which distinguishes stage 19 from stage 20, was quite obvious, as was the position of the limb buds or hands and feet. In 12 out of 48 cases we believed the Carnegie Stage to be in between two stages, for instance at stage 17-18.

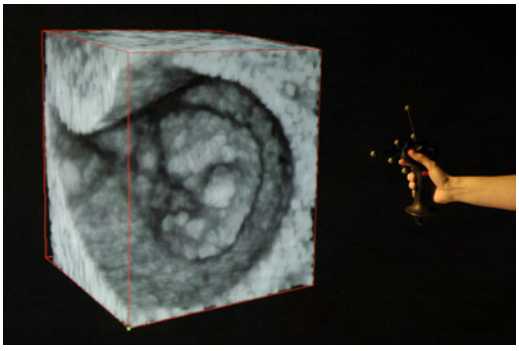

Figure 3.1.1. *I-Space picture of an embryo, age 39 days since oocyte retrieval. The presumed stage is Stage 17.*

Figure 3.1.2. *Picture of an embryo in the I-Space, age 53 days since oocyte retrieval. The presumed stage is Stage 22-23.*

The measured greatest lengths (Table 3.1.2) show a high degree of uniformity with the length reported for that stage by O'Rahilly.

TABLE 3.1.1. *Characteristics of Carnegie Stages 16 to 23 as described by O'Rahilly and Müller (1987)*

CARNEGIE STAGES	AGE (DAYS)	GREATEST LENGTH (MM)
16	37	8-11
17	41	11-14
18	44	13-17
19	47-48	17-20
20	50-51	21-23
21	52	22-24
22	54	25-27
23	56-57	23-32 (28-30)

Figure 3.1.3 shows the comparisons of the individual length measurements in the I-Space with the lengths for the corresponding stage that O'Rahilly reported. From this figure, it is clear that for stage 23 the measured lengths are in all cases substantially larger than reported. Two CRL measurements (stages 17-18 and 19 respectively), representing one patient (nr 18 in Table 3.1.2), are clearly above the 95th percentile. After birth no abnormalities were found and birth weight was at the 50th percentile. Therefore, these findings remain unexplained.

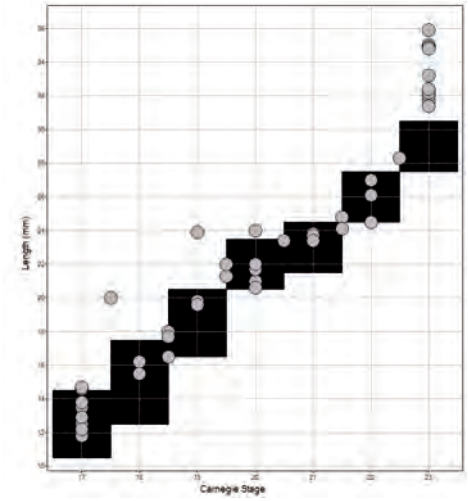

Figure 3.1.3. *Comparisons of the lengths of the individual measurements of our study group in the I-Space (grey dots) with the range of lengths O'Rahilly reported for the different stages (in black).*

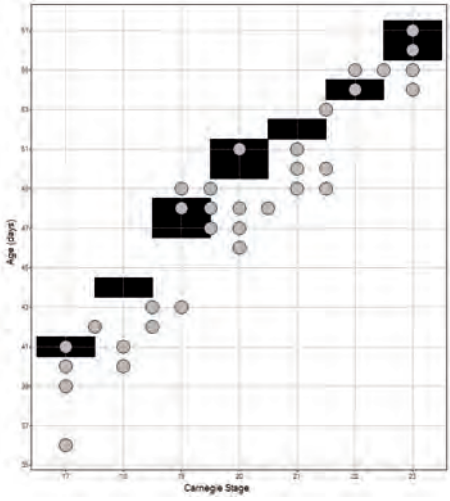

Figure 3.1.4. *Comparisons of the days after oocyte retrieval of our study group in the I-Space (grey dots) with the age reported by O'Rahilly for the different stages (in black).*

TABLE 3.1.2. *Individual measurements and staging details of all examinations in 19 patients* ⟶

PATIENT	GESTATIONAL AGE (WKS+DAYS)	DAYS AFTER OOCYTE RETRIEVAL	I-SPACE CARNEGIE STAGE	GREATEST LENGTH (MM)
1	8+5	47	20	20.7
	9+5	54	22	27
2	7+5	40	17	14.6
	8+6	48	20-21	23.4
	9+6	55	23	31.7
3	7+6	41	17	12.5
	8+6	48	19	19.7
	9+6	55	22-23	28.3
4	7+4	39	17	14.7
	8+4	46	20	21
	9+1	50	21-22	24.8
	10+1	57	23	35
5	8+1	43	19	19.8
	9+0	49	21-22	24.8
	10+0	56	23	34.9
6	7+5	40	17	13.5
	8+5	47	20	20.6
	9+5	54	22	26.1
7	7+6	41	17	13.8
	8+6	48	19-20	22
	9+6	55	23	32
8	8+1	43	18-19	18
	9+1	50	21	23.8
	10+0	56	23	32.2
9	7+5	40	17	12.9
	9+0	49	19-20	21.3
	9+4	53	21-22	24.1
10	8+1	43	18-19	16.5
	9+2	51	21	23.7
11	8+5	47	19-20	22
	9+5	54	23	33.2
12	8+0	42	18-19	17.7
	9+0	49	21	23.4
	10+0	56	23	34.8
13	7+5	40	18	16.2
	8+4	46	20	21.7
	9+5	54	23	32.4
14	7+6	41	18	15.5
	8+6	48	20	22
	9+5	54	23	31.4
15	7+6	41	17	11.8
	9+6	55	22	24.5
16	8+6	48	19	19.6
17	9+2	51	20	24.0
18	7+1	36	17	12.2
19	8+0	42	17-18	20
	9+0	49	19	23.9
	10+0	56	23	35.9

We also found that in our study, the age determined by the date of oocyte retrieval was younger than that which O'Rahilly reported for the corresponding Carnegie Stage. This is demonstrated in figure 3.1.4, which shows the comparison of embryonic age according to the date of oocyte retrieval with the embryonic age reported by O'Rahilly for that stage. Of 48 ultrasound scans, only 18 cases had a Carnegie Stage with an age that corresponded with O'Rahilly's report. In two cases, the age was one day older than according to the Carnegie Stage. In 28 cases the age was younger than the lowest value for that stage given by O'Rahilly, varying from 1 to 5 days younger. The individual patterns of growth per embryo are displayed in figure 3.1.5. ANOVA showed an average daily increase in length of 1.08 mm (0.04 SEM).

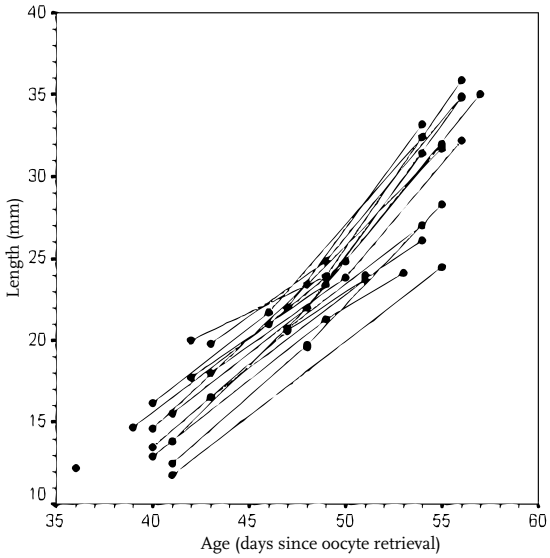

Figure 3.1.5. *Individual growth patterns per embryo.*

DISCUSSION

With this study, we successfully demonstrate that embryonic growth and development can be classified into Carnegie Stages using innovative imaging techniques. Until now, growth and development during the embryonic period is commonly only defined by its age and/or length exclusively. The embryonic period is generally believed to demonstrate uniform growth [1] and therefore biometry measurements are generally based on the comparison of measured values with predicted values derived from

reference charts or equations from normal populations. However, differences in growth and development in normal embryos have been described in several studies [13-16]. Recently Bukowski et al. [14] described the relationship between foetal growth in early pregnancy and the risk of delivering a low birth weight infant. Numerous articles have been published about measuring and estimating human embryonic and foetal age [1,11,12,17-22]. Apart from pregnancies from assisted fertility programs exact gestational age is difficult to establish. IVF/ICSI pregnancies however create new dilemmas such as the question whether embryonic growth in these pregnancies is similar to growth in normally conceived pregnancies [13,20,23-25].

For many species, growth and development is classified into stages based on the morphological state of development. The Carnegie Staging System has proven its value in the classification of human embryos for decennia. It does, however, have some limitations. For instance it is important to remember that embryologists generally use embryos obtained following spontaneous miscarriage (the embryos may have died in utero a few days before the miscarriage) and that these embryos are fixated. Hence it is not known how well they represent normal development [26]. In 1977, Drumm and O'Rahilly [27] assessed prenatal age from the crown-rump length determined ultrasonically in vivo and in utero in cases with 'known post-ovulatory age'. In this study, the CRL determined ultrasonically agreed well with those in length/age tables in embryologic literature. However, for a given age the ultrasonic lengths were 1 to 5 mm longer than those in fixed specimens. With this knowledge, Drumm and O'Rahilly adjusted the ages of the embryos in the Carnegie Collection to the ultrasonic findings.

Using 2D ultrasound it is very difficult to exactly determine morphologic features during embryonic period and thus only a few articles have been published about the use of the Carnegie Staging System in the evaluation of embryos using ultrasonography [12,15,28]. 3D ultrasound offers a better view of these features; it does however require a skilled sonographer with up to date knowledge of the 3D ultrasound software to optimize the use of the entire data set. We present a new imaging technique, using volume renderings in virtual reality. It offers an easy to interpret 3D image with depth perception and one can interact with volume rendered (ultrasound) data in an intuitive manner.

This study compares *in vivo* observations of human embryos, with well-established fertilisation dates, using high-resolution ultrasound imaging, with data on miscarried and fixated embryos with know post-menstrual ages. The embryos in our study seemed to reach a Carnegie Stage at an earlier gestational age than O'Rahilly described. In our study the assignment of a Carnegie Stage is purely based on external morphological features and completely neglects the inner features. Still, we are confident that the assignment is accurate. A morphological study using terminated pregnancies by Harkness and Baird [29] showed, that although they used more than one parameter for definite classification, identification of stages 14 to 23 primarily based on limb development is feasible. Therefore we are reluctant to explain the age difference this way. A possible explanation may be that the original ages of the Carnegie Collection and the ages after the adjustments made by Drumm and O'Rahilly are all based on menstrual history and basal body temperature, which are not completely reliable. Since we used pregnancies of assisted fertility programs, this could also explain the age discrepancy.

We conclude that the I-Space offers an impressive new way of looking at growth and development during embryogenesis. We emphasize that measuring size alone does not adequately reflect embryonic growth and development. The Carnegie Staging system is a well-established method that enables focusing on morphological features. Hence, combining length measurements with viewing developmental features using virtual reality techniques, will greatly improve knowledge of normal and abnormal embryonic growth, development and morphology. 'Virtual Embryoscopy' opens the way for studying the relationship between embryonic growth, development and morphology as well as second and third trimester pregnancy complications.

Acknowledgements

We would like to thank Dr. Joop Laven for his constructive comments on the manuscript and the staff of the department of Reproductive Medicine at the Erasmus MC for their help in recruiting the patients. We would also like to thank Wim Hop of the department of Biostatistics at the Erasmus MC for his help with the statistical analysis.

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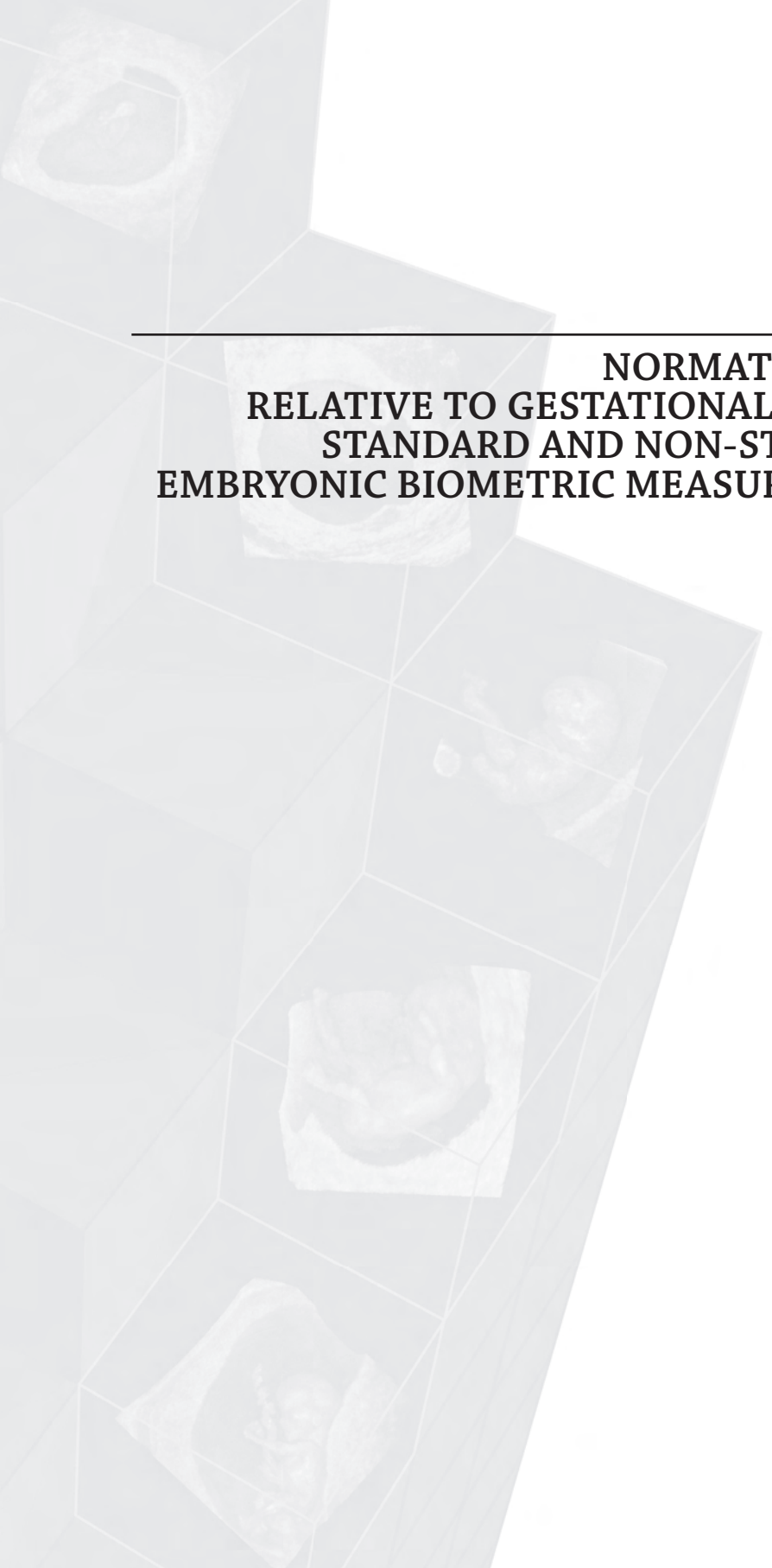
PART 4

NORMATIVE DATA RELATIVE TO GESTATIONAL AGE FOR STANDARD AND NON-STANDARD EMBRYONIC BIOMETRIC MEASUREMENTS



CHAPTER 4.1

FIRST TRIMESTER GROWTH CHARTS DERIVED FROM VIRTUAL REALITY MEASUREMENTS

Submitted for publication

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ABSTRACT

Background:

Innovative imaging techniques using up-to-date ultrasonic equipment, necessitates improved biometry charts. The aim of this study was, using a virtual reality (VR) technique, to comprise longitudinal charts of several embryonic biometry parameters.

Methods:

In a longitudinal study three dimensional (3D) measurements were performed from 6 to 14 weeks gestational age in 32 pregnancies. A total of 125 3D volumes were analyzed in the I-Space VR system, which allows binocular depth perception, providing a realistic 3D illusion. Crown-rump length (CRL), biparietal diameter (BPD), occipito-frontal diameter (OFD), head circumference (HC) and abdominal circumference (AC) were measured. Arm length, shoulder width, elbow width, hip width, and knee width could be measured as well.

Results:

CRL, BPD, OFD and HC could be measured in more than 96% of patients, AC in 78%. Shoulder width, elbow width, hip width and knee width could be measured in more than 95% of cases, arm length in 82% of cases. Growth charts were constructed for all variables. The CRL growth chart perfectly matched the curve from Robinson and Fleming from 1975.

Conclusions:

This study provides a detailed, longitudinal description of normal human embryonic growth, facilitated by a virtual reality system. New charts were created for embryonic biometry of the CRL, BPD, HC and AC early in pregnancy and also of several 'new' biometric measurements. Applying 'Virtual Embryoscopy' will enable us to diagnose growth and / or developmental delay earlier and more accurate. This is especially important for pregnancies at risk for severe complications like recurrent late miscarriage and early growth restriction.

INTRODUCTION

The importance of accurate foetal growth curves to describe normal foetal growth and to identify growth abnormalities is generally recognized and has recently been stressed by Verburg et al [1] and many other authors [2-4]. The first 10 weeks of pregnancy, the embryonic period, may be of even greater importance, since abnormal growth or development are likely to have an impact on foetal growth in the second and third trimester of pregnancy and subsequent health of the newborn [5-8].

Following the introduction of transvaginal sonography [9], three dimensional (3D) ultrasound has had major impact on visualization in early pregnancy [10-13]. However, 3D imaging is still used by means of a two dimensional (2D) medium, which is unable to provide all the information offered by the 3D volume. The Erasmus MC in Rotterdam operates an innovative Virtual Reality (VR) system, called the Barco I-Space. This VR system allows depth perception and offers viewers a complete 3D experience. We have already demonstrated the advantage of this so-called 'Virtual Embryoscopy' in the assignment of developmental Carnegie Stages during embryonic life [14] and its use in the evaluation of complex embryonic and foetal abnormalities [15-17]. The measuring tool of the CAVORE [18] software used in the I-Space has proven its reliability and reproducibility [16,19].

The aim of our study was, using virtual reality techniques, to comprise longitudinal charts of several embryonic biometry parameters, which had not been available up to now. Results on detailed embryonic biometry will contribute to a new field of study of abnormal human embryonic growth and morphogenesis.

MATERIALS AND METHODS

Patient selection

From January 2006 till July 2006 we included a total of 47 female volunteers for longitudinal 3D ultrasound evaluation of early pregnancy. The medical ethics review board approved this study. All patients were recruited from our outpatient's clinic. Twenty-two patients were pregnant after in-vitro fertilization (IVF) or intra-cytoplasmatic

sperm injection (ICSI) treatment. Two patients became pregnant with intra uterine insemination (IUI). Twenty-three patients conceived spontaneously. Ultrasound (US) scans were performed, when possible weekly, from about 6 weeks gestational age till the 14th week of gestation. Written consent was obtained from all patients. Gestational age for IVF/ICSI/IUI pregnancies was based on the date of oocyte retrieval or intra uterine insemination. Gestational age for the patients who conceived spontaneously was based on the last menstrual period, verified by US measurements.

To provide growth charts for normal uncomplicated singleton pregnancies we excluded 15 patients. Two patients were diagnosed with non-viable pregnancies on first examination (6 weeks of gestational age). Three patients carried twin pregnancies. One patient was diagnosed with placental confined trisomy 16 mosaicism, complicated by severe intra-uterine growth restriction [17]. Two patients developed severe placental malfunction: in one case the pregnancy was terminated because of severe growth retardation at 20 weeks of gestation. The other case resulted in intra-uterine death at 22 weeks of gestation due to placental abruption. Four patients who conceived spontaneously were excluded because gestational age based on last menstrual period did not agree (range of 8 days) with ultrasonographic measurements. A total of 11 ultrasound volumes, in which embryonic features could not be recognized due to poor quality of the data, were excluded. This resulted in the exclusion of three patients out of the IVF/ICSI group. Therefore, 32 patients with normal uncomplicated singleton pregnancies remained for further analyses, with a total of 125 3D volumes.

Materials

3D ultrasound scanning was performed using a GE Voluson 730 Expert system (GE, Zipf, Austria). These 3D datasets were then saved as cartesian (rectangular) volumes and transferred to the BARCO I-Space at the department of Bioinformatics of the Erasmus MC. This is a 4-walled CAVE-like [20] virtual reality system. Passive stereo is used to immerse viewers in a virtual world. The images are projected on three walls and the floor of a small 'room'. The images are viewed through glasses with polarizing lenses in order to perceive depth. A "hologram" of the (ultrasound) volume is created by the CAVORE [18]/V-Scope [19] volume rendering application. The implementation of CAVORE/V-Scope in the I-Space allows medical professionals to view and interact with their volumetric data in all three dimensions. V-Scope is able to handle very large

datasets (limited by the computer's main memory) of most 3D medical imaging modalities (e.g. MRI, CT, PET, SPECT and 3D ultrasound). This provides clinicians with views much more alike those they will experience during surgery [21,22]. Manipulation of the volume is possible using a virtual pointer, controlled by a wireless joystick. The computer provides a correct perspective and motion parallax through wireless tracking of the viewer's head. For this study, 3D volumes are resized, turned and clipped to provide an unobstructed view of the embryo in the I-Space and optimal image quality is obtained by adjusting grey scale and opacity (figure 4.1.1 and 4.1.2). Of course, these pictures of the I-Space are seen from a 2D medium (paper or computer screen), thus the depth perception is lost. Therefore, pictures such as these do notreflect the I-Space.

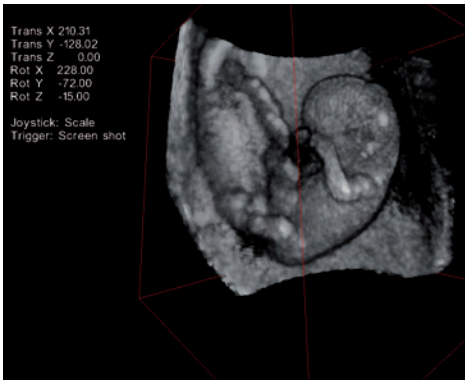


Figure 4.1.1: *Screen shot of an embryo of 10 weeks gestational age in the I-Space VR system. The embryo is hovering in space, the arms are in front of the face. Details of the arm as the shoulder, elbow en fingertips are clearly visible.*

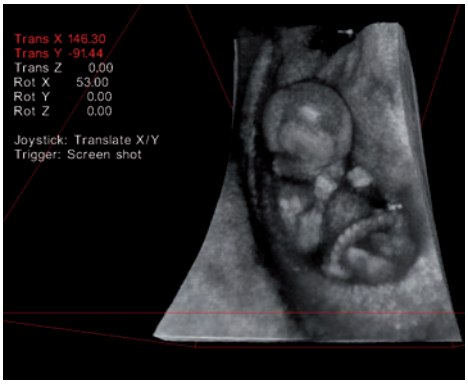


Figure 4.1.2: *Screen shot of a foetus of 13 weeks and 4 days gestational age.*

Measurements

All measurements were performed by a single operator, written down by a second operator and repeated three times. For each parameter, the mean value of the 3 assessments was used in all further calculations. We measured the following standard biometry parameters: crown-rump length (CRL), biparietal diameter (BPD), occipitofrontal diameter (OFD) and the abdominal diameter (AD1 – AD2) in two directions. For CRL measurements the callipers were placed from crown to caudal rump in a straight line. The embryo 'hologram' was turned to verify correct position of the callipers

in the midsagittal plane. BPD and OFD measurements were made in a transverse section of the head with both lateral ventricles (when visible) in view with a horizontal midline. Both measurements were made from outer-to-outer border of the skull, BPD perpendicular to the midline. Abdominal diameters were measured in a transverse section through the abdomen, just above the umbilical cord insertion or preferably in a section in which the stomach was visible. Ellipsoid figures were calculated from two perpendicular diameters, plotted in a mathematical formula. From GE Medical Systems we obtained the HC formula used in the 4DView software program and calculated the head circumference (HC) from the BPD and OFD using this formula.

$$[HC = \pi (1.5 (\frac{BPD + OFD}{2}) - \sqrt{(\frac{BPD * OFD}{2})}]$$

The two abdominal diameters were used to calculate the abdominal circumference (AC) using the same formula.

For non-standard biometry measurements we measured the following variables: arm length, shoulder width, elbow width, hip width, knee width, ear length and foot length.

The length of the arm of the embryo was measured, using the tracing function of the I-Space, starting at the highest and most distinct part of the shoulder, going to the outside of the elbow, then to the wrist, following the curvature of the hand towards the index finger. In the earlier stages, the maximum length of the limb bud was measured. The hologram of the embryo / foetus was then turned to visualize its back. We measured the width of the shoulders from outer to outer part of the shoulder. The width of the elbows was measured from outer to outer part as well. When the elbows could not be visualized, the maximum width of the limb buds was measured.

The width of the hips was measured just below the limb buds. The width of the knees was measured from outer to outer part. When only limb buds were present, the maximum width of the limb buds was measured. The hologram was then turned in either direction to check the position of the callipers to verify that all callipers were placed at their maximum width. When visible, the maximum length of the ear was measured from the superior to the inferior border of the external ear. The maximum length of the foot was measured when both heel and toes were recognizable. We

assumed that development of right and left side of the embryo was symmetrical and therefore measured the side that was best visualized.

At the Erasmus MC, the CRL formulae from Robinson and Fleming [23] and the BPD, HC, AC and femur length (FL) formulae from Kustermann et al [24] are used as references for pregnancies with a maximum gestational age of 13^{+6} weeks. The charts derived from the data in this study were therefore compared to these biometric growth charts.

Validity, reliability and reproducibility of the I-Space measurement tool were tested in two different studies [16,19], demonstrating that both biometric and volumetric measurements are at least as reliable as present 2D and 3D techniques.

Statistical analysis was performed using SPSS (SPSS Release 12.0.1 for Windows, SPSS Inc, Chicago, IL, USA) and SAS PROC MIXED (release 8.02, SAS Institute Inc, Cary, NC, USA). Patient characteristics of the different groups were tested with Pearson's Chi-Square test, Fisher's exact test and the Mann-Whitney test for non-parametric testing. A p-value < 0,05 was considered to be statistically significant. The growth data were analyzed using linear mixed models (after square-root transformation of measurements). Although designed for cross-sectional studies on foetal size, we used the 'Checklist for studies of foetal size' from Altman and Chitty [2] and therefore constructed smoothed centiles when possible.

RESULTS

Apart from women with assisted fertility treatment being more often nulliparous, there were no significant differences in general characteristics between that group (N = 16) and those who conceived spontaneously (N = 16). These general characteristics are displayed in Table 4.1.1. All pregnancies resulted in the birth of a healthy child.

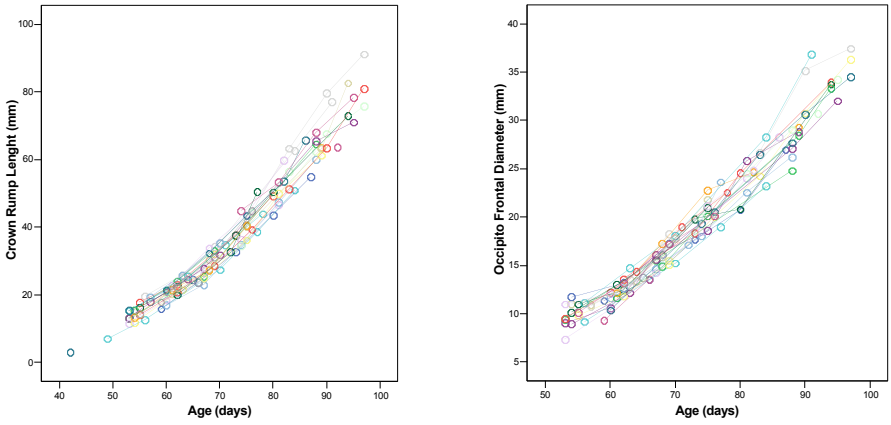
Table 4.1.1. *Patient characteristics of the study group.*

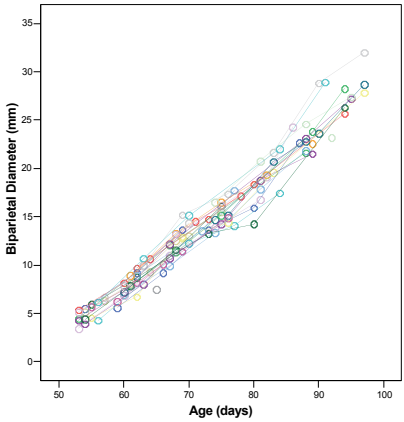
Patient characteristics	Spontaneous N = 16	IVF/icsi N = 16	P value
Mean age (years)	32 (21 - 40)	33 (24 – 43)	0.651
Mean BMI (kg/length2)	24.6 (19.7 - 34.9)	24.1 (19 – 34.3)	0.516
Mean pregnancy duration (weeks+days)	39+5 (37+3 – 41+4)	39+2 (35+0 – 42+0)	0.836
Mean birth weight (grams)	3410 (2690 - 4170)	3191 (2175 – 4370)	0.122
Mean number of US examinations	3.62 (1-6)	4.19 (1 – 7)	0.577

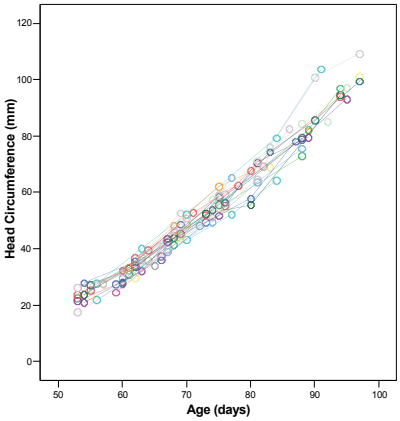
** p value < 0,05 is statistically significant (Mann-Whitney test)*
For each characteristic the mean value is given and the range.

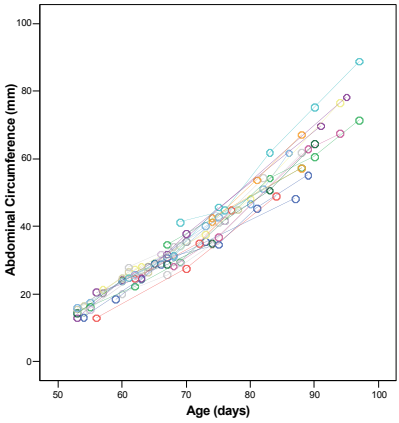
Differences in slope of the curves were calculated as the difference between the spontaneously conceived pregnancies and the pregnancies after assisted fertility treatment. No significant differences were found for the slope of the growth curves, indicating that growth rate is the same for both groups.
Figure 4.1.3 displays the individual growth charts for all biometry measurements.

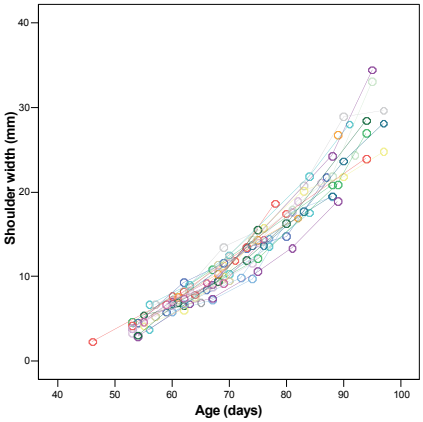
Figure 4.1.3. *Growth charts of all biometric measurements.*

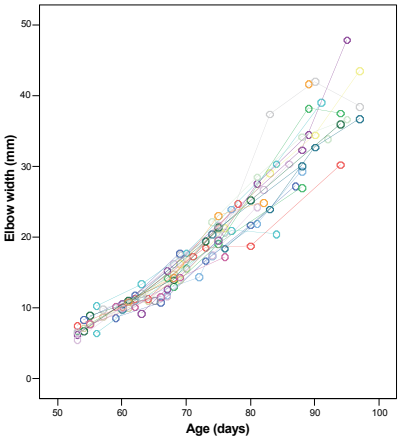


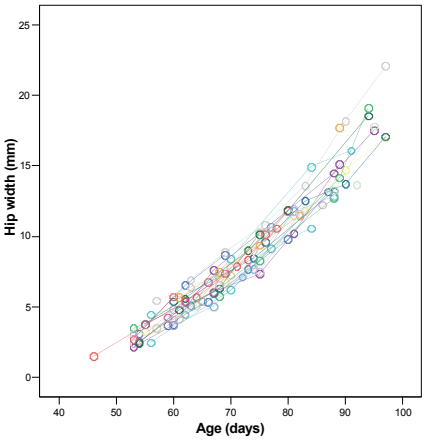

Biparietal Diameter (mm)
0
5
10
15
20
25
30
35
50
60
70
80
90
100
Age (days)


Head Circumference (mm)
0
20
40
60
80
100
120
50
60
70
80
90
100
Age (days)


Abdominal Circumference (mm)
0
20
40
60
80
100
50
60
70
80
90
100
Age (days)


Shoulder width (mm)
0
10
20
30
40
40
50
60
70
80
90
100
Age (days)


Elbow width (mm)
0
10
20
30
40
50
50
60
70
80
90
100
Age (days)


Hip width (mm)
0
5
10
15
20
25
40
50
60
70
80
90
100
Age (days)

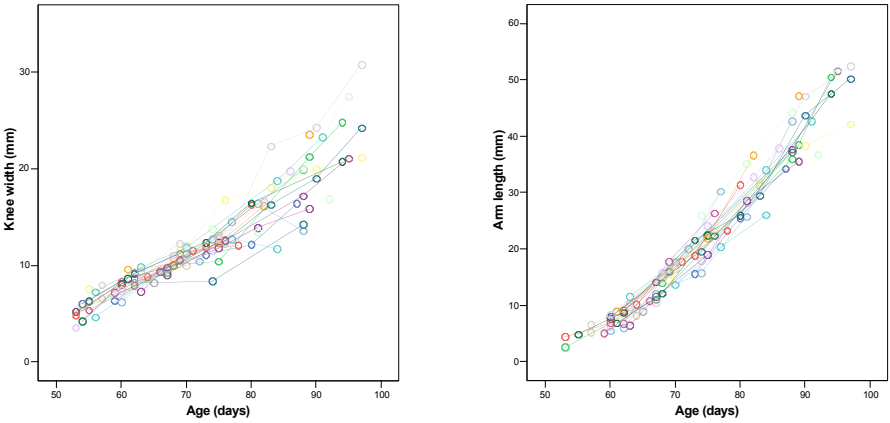


Table 4.1.2 displays in how many of the 125 included 3D volumes the measurements could be performed, and the minimum and maximum gestational age at which it was feasible to obtain measurements of a particular body part. As this table shows, CRL, BPD, OFD and HC could be measured in almost all patients. For the AC, measurements were not possible in 22%, mostly in the earlier gestational ages. Shoulder width, elbow width, hip width and knee width could be measured in more than 95% of cases. Arm length was a little bit more difficult to measure (82.4 %), especially in the earlier gestational ages. Ear size could only be measured in 28% and foot length in 38% of cases. Because of the limited data obtained and the observation that ear length and foot length could only be measured at the very end of the embryonic period (gestational age of 9^{+4} and 9^{+5} weeks respectively) we have not included these items in our final analyses.

Table 4.1.2 *Ability of performing the measurements for all different variables and the minimum and maximum age at which the measurement could be performed.*

Variable	N=125	%	% missing	Age min		Age max	
				wks	days	wks	days
CRL	124	99.2	0.8	6+0	42	13+6	97
BPD	121	96.8	3.2	7+4	53	13+6	97
OFD	121	96.8	3.2	7+4	53	13+6	97
HC	121	96.8	3.2	7+4	53	13+6	97
AC	98	78.4	21.6	7+4	53	13+6	97
Shoulder width	121	96.8	3.2	6+4	46	13+6	97
Elbow width	119	95.2	4.8	7+4	53	13+6	97
Hip width	120	96.0	4.0	6+4	46	13+6	97
Knee width	117	93.6	6.4	7+4	53	13+6	97
Arm length	103	82.4	17.6	7+4	53	13+6	97

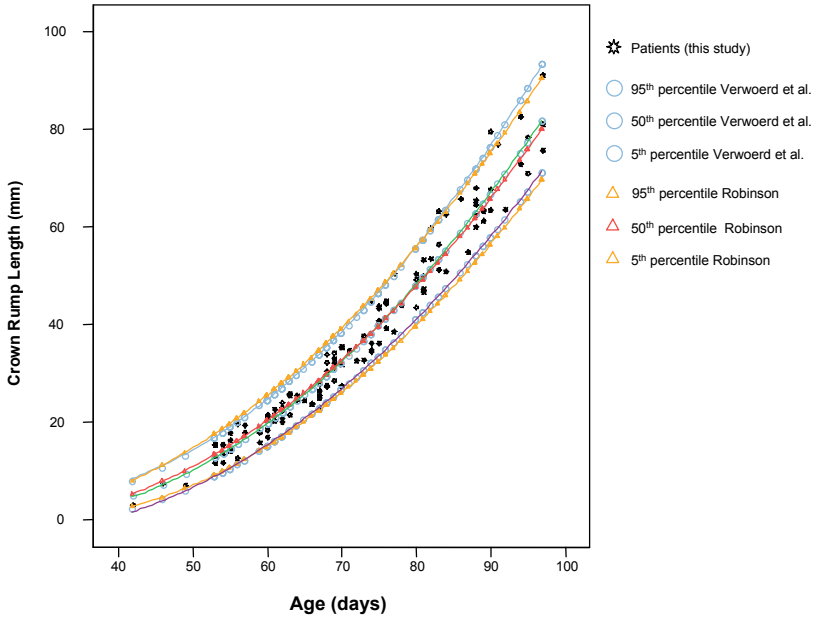
Equations for the fit-lines and standard deviations for all charts are given in Table 4.1.3.

Table 4.1.3 *Equations of fit lines of all variables (Y) and corresponding standard deviations (SD(Y)).*

Variable	N=125	Equations (Y)	SD (Y)
CRL	124	0.015508X2 - 0.751165X + 9.096256	0.000043X2 – 0.006087X + 0.687331
BPD	121	0.005463X2 - 0.248737X + 2.831479	0.03526
OFD	121	0.004574X2 - 0.082143X + 0.368813	0.000016X2 – 0.002056X + 0.088934
HC	121	0.015203X2 - 0.449330X + 3.320048	0.000015X2 – 0.001622 + 0.106270
AC	98	0.012217X2 - 0.418422X + 3.582692	0.0786
Shoulder width	121	0.006082X2 - 0.333766X + 4.578744	0.000013X2 – 0.001116 + 0.059450
Elbow width	119	0.007513X2 - 0.353013X + 4.146518	0.000033X2 – 0.002630 + 0.084090
Hip width	120	0.003592X2 - 0.176877X + 2.177690	0.000021X2 – 0.002896 + 0.126460
Knee width	117	0.003145X2 - 0.070650X + 0.396774	0.000049X2 – 0.005292 + 0.176490
Arm length	103	0.016205X2 - 1.256476X + 24.355212	0.000185X2 – 0.025390 + 0.938240

Figure 4.1.4 demonstrates a comparison of our data with the 5th, 50th and 95th percentiles form the Robinson and Fleming curve.

Figure 4.1.4. *Comparison of the Robinson and Fleming CRL growth curve from 197523 (triangles) with the data from this study (round dots).*



DISCUSSION

This study is unique as far as we know since it provides a detailed, longitudinal description of normal human embryonic growth, facilitated by a virtual reality system. We have been able to construct new charts for embryonic biometry. The fact that the slope of the curves of the patients who conceived spontaneously was not statistically different from those of pregnancies after assisted fertility treatment indicates that growth rate is the same for both groups.

Our newly created CRL chart perfectly matched the Robinson and Fleming curve from 1975 [23]. As figure 4.1.2 shows, both median, 5th and 95th percentile of our curve are similar to Robinson and Flemings' curve. Kustermann [24] and other authors [25] have also found their curve to be in concordance with the Robinson curve. However,

although Kustermann did demonstrate a resemblance for the median of the curve, the 5th and 95th percentile are considerably different. Kustermann et al [24] constructed charts with transvaginal ultrasonography for CRL, BPD, HC, AC, femur length (FL) and foot length measurements in pregnancies between 6 - 15+3 weeks of gestational age. They did not take the gestational age into account when calculating the standard deviation. This explains the large differences between the 5th and 95th percentiles especially in the lower gestational ages, visible in figure 4.1.5.

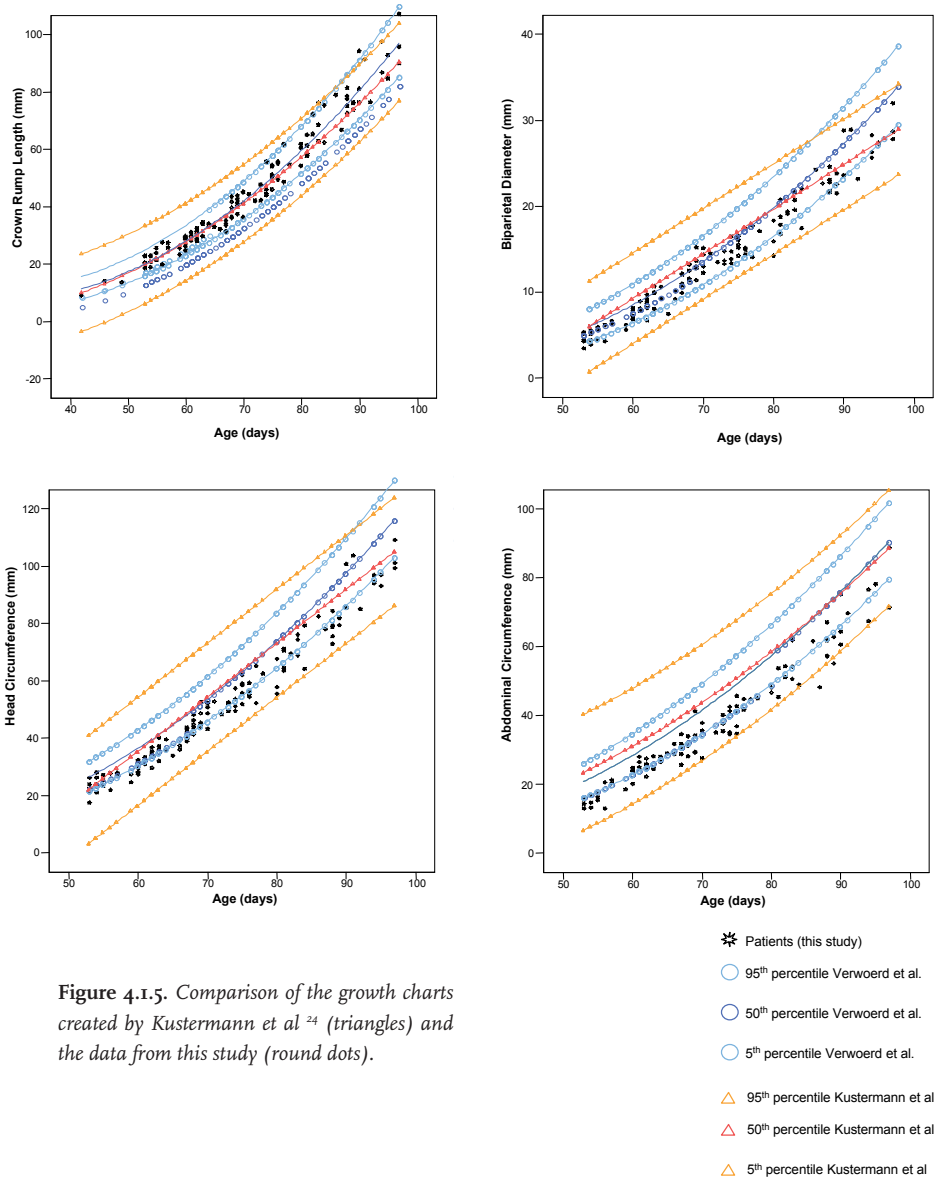


Figure 4.1.5. *Comparison of the growth charts created by Kustermann et al [24] (triangles) and the data from this study (round dots).*

In 1990 Timor-Tritsch et al [9] introduced 'sonoembryology'. They used a high-frequency vaginal probe to image early pregnancies. For limb development a cephalo-caudal maturity was described. The tail section protrudes caudally and exceeds the lower limbs. At 7 tot 8 weeks of gestational age the soles of the feet were found to face each other. At approximately 10 weeks, the knee rotates ventrally. At 12 to 13 weeks of gestation the knees rotated ventrally and the legs crossed. Our results are in accordance with theirs, and confirms our previous findings that these developing processes can be very well described according to the Carnegie Staging system [14].

Width of both elbows and knees depends on movement and position of the embryo and this is reflected in their growth pattern. Shoulder width and hip width demonstrate normal growth patterns. As figure 4.1.4 shows, the length of the arm also shows a good relation with gestational age. Length of the arm is a parameter that can only be measured with use of depth perception, since you need to visualize the shoulder as well as the elbow, wrist and fingers, which is almost impossible in one single plane. Conventional 3D ultrasound does not yet offer a tracing function that allows measurements in a 3D rendered image. Since many genetic disorders affect limb development, arm length measurements could be a very useful tool in the early detection of such disorders. The charts that are the result of our study using the I-Space may serve as new reference material for this purpose.

In this study we demonstrate the use of an innovative tool for the evaluation of developmental embryonic and early foetal morphology. The I-Space VR system is unique in its ability to view and measure the third dimension. Its tracing function allows for complex measurements. As far as we know, the Erasmus MC is the only centre in the world to use such a system in combination with the necessary computer software to study medical images. The fully immersive system can also be reduced to a desktop version, offering the same benefits at a fraction of the cost. We foresee a future where 3D display technology is as common as 2D displays are today. In this study new charts were established for standard biometry measurements such as CRL, BPD, HC and AC early in pregnancy. We also provide charts for new biometric measurements; arm length, shoulder width, elbow width, knee width and hip width. Combining these measurements with a description of morphological features such as limb development

provides a more solid comprehension of the developing embryo. Applying Virtual Embryoscopy will enable us to diagnose growth and/or developmental delay earlier and more accurate. This is especially important for pregnancies at risk for severe complications like recurrent late miscarriage and early growth restriction.

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CHAPTER 4.2

FIRST-TRIMESTER UMBILICAL CORD AND VITELLINE DUCT MEASUREMENTS USING VIRTUAL REALITY

Submitted for publication

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ABSTRACT

Introduction:

The aim of the study is to construct growth charts for the umbilical cord length and vitelline duct length in embryonic (first 10 weeks of gestation) and early foetal life (> 10 weeks of gestation) using a virtual reality technique (I-Space) to benefit from all three dimensions of 'standard' three dimensional (3D) ultrasound images.

Methods:

In a longitudinal study 3D measurements were performed from 6 to 14 weeks gestational age in 32 pregnancies. A total of 125 3D volumes were analyzed in the I-Space VR system. The measuring tool of the I-Space also exhibits a tracing function, allowing measurements of structures that are looped or curved. Total length of the umbilical cord was measured as was the length of the vitelline duct. The position of the yolk sac in relation to the embryo was described and its change in time.

Results:

Umbilical cord length could be measured in 55% of cases. There was a clear relationship between length of the umbilical cord and advancing gestational age. Vitelline duct length could be measured in 42% of cases. No relation was found between length of the vitelline duct and gestational age.

Conclusions:

The present study is the first to provide an *in vivo* longitudinal description of normal embryonic growth of the human umbilical cord and vitelline duct, facilitated by a virtual reality system. Although the clinical relevance of the length of the umbilical cord and vitelline duct is not clear to us at this moment, further studies will reveal whether these parameters can be used in the detection of any anomalies in growth or development of the embryo.

INTRODUCTION

First trimester ultrasound evaluation is predominantly used for establishment of normal embryonic development or verification of gestational age. Several biometric measurements are performed, crown-rump length (CRL) being the most important. Others may include mean gestational sac diameter (MGS), mean yolk sac diameter (MYS), biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and less often femur length (FL).

Structures such as the umbilical cord and vitelline duct however are rarely the subject of first trimester ultrasound evaluation, although their function is of vital importance for embryogenesis. This can easily be explained by the fact that these structures are hard or even impossible to evaluate from two dimensional media due to their complex shape and morphology. The Erasmus MC in Rotterdam operates an innovative Virtual Reality system, called the Barco I-Space. This system allows depth perception and creates holograms from three dimensional (3D) datasets. We have already demonstrated its use in the assignment of Carnegie Stages during embryonic life [1], its use in the demonstration of embryonic developmental delay [2] and in complex anatomical foetal malformations [3,4]. The reliability of the measurement tool of the I-Space has been established before [5,6]. Depth perception enables measurements of structures that have not been measured routinely before mainly due to technical limitations.

The aim of the study is to construct growth charts for the umbilical cord length and vitelline duct length in embryonic (first 10 weeks of gestation) and early foetal life (> 10 weeks of gestation). These growth charts are based on normal, uncomplicated singleton pregnancies and will serve as a basis for normal growth and development of these structures. Although we do not know the clinical implications of abnormal growth of these structures yet, structures such as the umbilical cord are essential for the developing embryo and are thus of vital importance. Growth charts of these structures may therefore eventually also serve as a background to detect any abnormalities.

MATERIALS AND METHODS

Patient selection

From January 2006 till July 2006 a total of 47 female volunteers were included for longitudinal 3D ultrasound evaluation of early pregnancy. The medical ethics review board approved this study and written consent was obtained from all patients. Twenty-four volunteers were recruited from the Department of Reproductive Medicine of our Department. Two of these patients became pregnant with intra uterine insemination (IUI) and 22 patients were pregnant after in-vitro fertilization (IVF) / intra-cytoplasmatic sperm injection (ICSI) treatment. The other 23 patients all conceived spontaneously. Ultrasound (US) scans were performed, when possible weekly, from about 6 weeks gestational age till the 14th week of gestation. Gestational age for IVF/ICSI/IUI pregnancies was based on the date of oocyte retrieval or intra-uterine-insemination. Gestational age for the patients who conceived spontaneously was based on the last menstrual period, verified by US measurements.

To provide growth charts for normal uncomplicated singleton pregnancies we excluded 15 patients. Two patients were diagnosed with non-viable pregnancies on first examination (6 weeks of gestational age). Three patients carried twin pregnancies. One patient was diagnosed with placental confined trisomy 16 mosaicism, complicated by severe intra-uterine growth restriction [2]. Two patients developed severe placental malfunction: in one of these cases the pregnancy was terminated because of severe growth retardation at 20 weeks of gestation and the other case resulted in intra-uterine death at 22 weeks of gestation due to placental abruption. Four patients who conceived spontaneously were excluded because gestational age based on last menstrual period did not agree (range of 8 days) with ultrasonographic measurements. A total of 11 ultrasound volumes were excluded because embryonic features could not be recognized due to poor image quality of the data. The latter resulted in the exclusion of three patients out of the IVF/ICSI group.

Thirty-two patients with normal uncomplicated singleton pregnancies remained for further analyses, with a total of 125 3D volumes.

Materials

3D ultrasound scanning was performed using a GE Voluson 730 Expert system (GE, Zipf, Austria). These 3D datasets were then saved as cartesian (rectangular) volumes and transferred to the BARCO I-Space at the department of Bioinformatics of the Erasmus MC. This 4-walled CAVE-like [7] virtual reality system uses passive stereo to immerse viewers in a virtual world. The images are projected on three walls and the floor of a small 'room'. Images are viewed through glasses with polarizing lenses in order to perceive depth. A 'hologram' of the (ultrasound) volume is created by the CAVORE [8] / V-Scope [5] volume rendering application. A virtual pointer allows for manipulation of the volume, which is controlled by a wireless joystick. The computer provides a correct perspective and motion parallax through wireless tracking of the viewer's head. In the I-Space, volumes are resized, turned and clipped to provide an unobstructed view of the embryo and optimal image quality is obtained by adjusting grey scale and opacity.

Measurements

All measurements were performed by the first author, documented by another operator and repeated three times. For each parameter, the mean value of the 3 assessments was calculated. We measured the crown-rump length (CRL) by placing the callipers from crown to caudal rump in a straight line. The embryo 'hologram' was turned to verify a correct position of the callipers in the mid-sagittal plane. The measuring tool of the I-Space also exhibits a tracing function. We could therefore measure the total length of the umbilical cord, even when looped. The total length was measured tracing it from the abdominal insertion of the cord to the amniotic membrane. We followed the midline of the umbilical cord whenever looping was present. We also measured the distance between the amniotic membrane and the placental insertion of the umbilical cord. We called this the AP (amniotic-placental) distance. The mean diameter of the umbilical cord was measured in two directions, at amniotic level as well as at the insertion into the abdomen. When a physiological herniation was present, the diameter was measured directly distal from the herniation. We also measured the length of the vitelline duct. This length was measured from the outside border of the yolk sac towards the part where the vitelline duct joins the umbilical cord at amniotic level. Mean diameter of the vitelline duct was measured in two directions both at yolk

sac side and umbilical cord side. Figure 4.2.1 is a picture of an embryo at 10+5 weeks gestational age, with measurements of both umbilical cord and vitelline duct length.

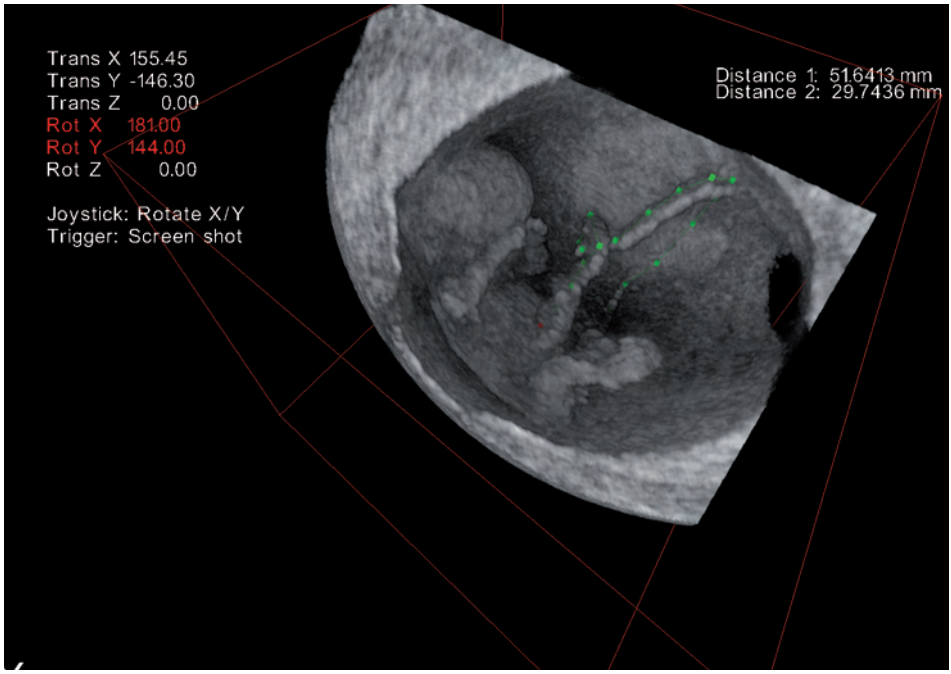

Figure 4.2.1. *Picture of an embryo of 10 weeks and 5 days gestational age. The umbilical cord length was measured (51.64 mm) en the length of the vitelline duct (29.74 mm).*

We described the position of the yolk sac in relation to the embryo after placing the embryo in an upright position facing the operator. The yolk sac was either reported right or left, below or above, in front or at the back of the embryo.

Statistical analysis was performed using SPSS (SPSS Release 12.0.1 for Windows, SPSS Inc, Chicago, IL, USA) and SAS PROC MIXED (release 8.02, SAS Institute Inc, Cary, NC, USA).

RESULTS

All pregnancies resulted in the birth of a healthy child. There were no significant differences in general characteristics between the group with assisted fertility treatment (N=16) and the group who conceived spontaneously (N=16).

Table 4.2.1 displays how often the parameters could be measured and the minimum and maximum age at which a parameter could be measured.

Table 4.2.1. *Number of measurements that could be performed for the different variables and the minimum and maximum age at which a measurement could be performed. (AP = amnion-placenta UC = umbilical cord VD = vitalline duct YS = yolk sac)*

Variable	N=125	%	% missing	Age min		Age max	
				wks	days	wks	days
Umbilical cord length	69	55.2	44.8	7+4	53	13+6	97
Vitalline duct length	52	41.6	58.4	6+4	46	11+4	81
AP Distance	44	35.2	64.8	7+4	53	12+4	88
Mean diameter UC-abdomen	105	84.0	16.0	7+4	53	13+6	97
Mean diameter UC-amnion	72	57.6	42.4	7+4	53	13+6	97
Mean diameter VD-YS	55	44.0	56.0	6+4	46	11+4	81
Mean diameter VD- amnion / UC insertion	36	28.8	71.2	7+4	53	11+4	81

Umbilical cord length could be measured in 55% of cases. Most problems in measuring the total length were encountered in the advanced gestational ages (> 75 days). In advanced gestational ages the cord often contained too many loops for measurements to be performed. Figure 4.2.2(a) clearly demonstrates the relationship between lengths of the umbilical cord en gestational age.

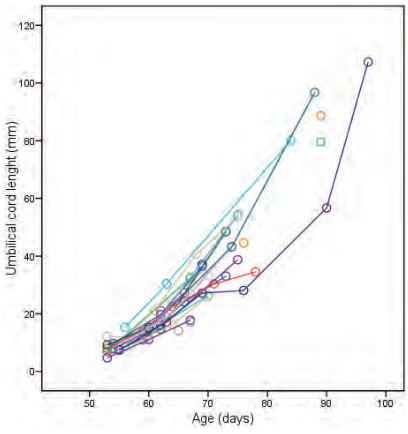

Figure 4.2.2(a). *Relation between umbilical cord length and gestational age.*

Vitelline duct length could be measured

in 42% of cases. In advanced gestational ages the yolk sac and thus end of the vitelline duct could no longer be recognized. The yolk sac could be visualized in 84 out of 125 cases (67%). Figure 4.2.2(b) demonstrates that no relation was found between length of the vitelline duct and gestational age.

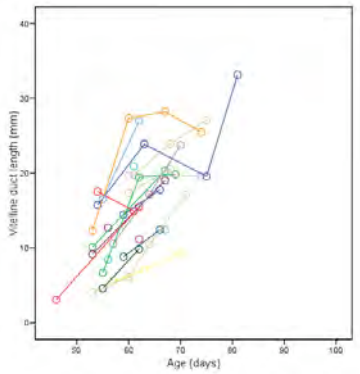

Figure 4.2.2(b). *Relation between vitelline duct length and gestational age.*

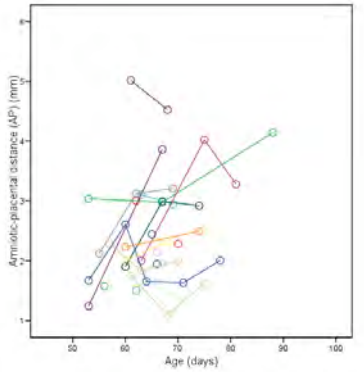

Figure 4.2.2(c). *Relation between AP distance and gestational age.*

AP distance was very dependent on image quality (the ability to distinguish enough detail) and could only be performed in 35% of cases. Figure 4.2.2(c) demonstrates the relation between the AP distance and gestational age.

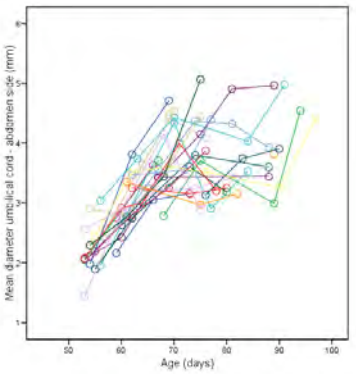

Figure 4.2.2(d). *Relation between mean diameter of the umbilical cord (abdominal side) and gestational age.*

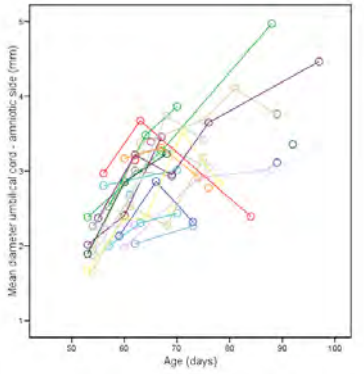

Figure 4.2.2(e). *Relation between mean diameter of the umbilical cord (amniotic side) and gestational age.*

Mean diameters of the umbilical cord could be measured in 84% (abdominal side) and 58% (yolk sac side) of cases (figure 4.2.2(d+e). Whenever the umbilical

cord contained too many loops, measurements of the total length of the cord were not possible, whereas mean diameters could be established. Mean diameter of the vitelline duct at the yolk sac side could be measured in 44%, but at the side of the umbilical cord it was only possible in 29% of cases (figure 4.2.2(f+g). In two cases it was not possible to measure the length of the vitelline duct, even though width of the vitelline duct at yolk sac side could be established.

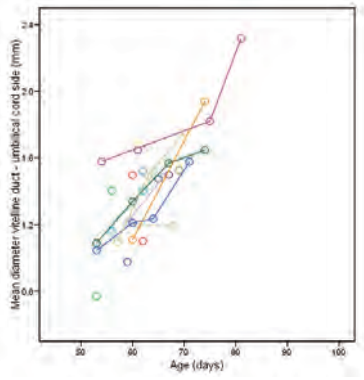

Figure 4.2.2(g). *Relation between mean diameter of the vitelline duct (umbilical cord side) and gestational age.*

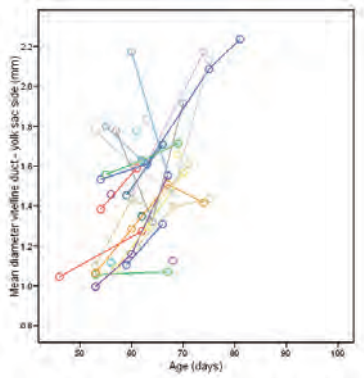

Figure 4.2.2(f). *Relation between mean diameter of the vitelline duct (yolk sac side) and gestational age.*

Figure 4.2.3 demonstrates the position of the yolk sac in relation to the embryo in a 3D graph. Lines are drawn to connect the individual patients, indicating that the position of the yolk sac changes in time.

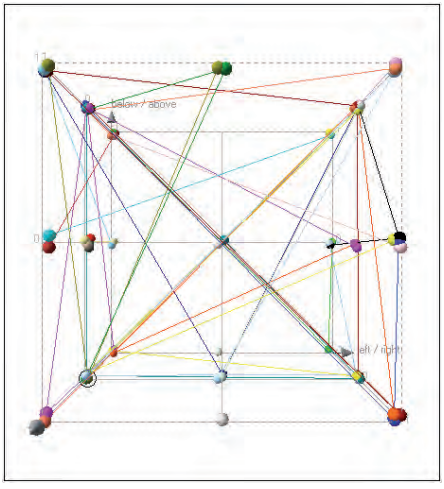

Figure 4.2.3. *Position of yolk sac in relation to the embryo (centre of this figure)*

DISCUSSION

The present study is the first to provide an *in vivo* longitudinal description of normal embryonic growth of the human umbilical cord and vitelline duct, facilitated by a virtual reality system.

Length of the umbilical cord is in most studies measured after birth. Using 2D ultrasound it is impossible to perform length measurements in second and third trimester. In our search for relevant studies concerning ultrasonic length measurements of the umbilical cord during the first trimester of the pregnancy we found only one study by Hill et al [9]. In this study, the umbilical cord was measured with 2D ultrasound in 53 normal embryos and 15 embryos with an intrauterine demise, ages ranging from 6.1 weeks to 11 weeks. They attempted to visualize the umbilical cord in its entirety and stated that the umbilical cord had to be taut and relatively straight. A close linear relationship was found between umbilical cord length and menstrual age in the normal group. 60% of 15 embryos with an intrauterine demise had cord lengths more than 2 SD below the expected value for menstrual age established in the normal embryos. Several studies have been published trying to unravel the mystery of why umbilical cord lengths can be of such different lengths at birth [10,11] and whether this has clinical implications [11-13]. It has been stated also that the umbilical cord of male infants is longer than the umbilical cord of female infants [11]. Although it would be very interesting to verify whether this difference in length is already present in the first trimester of pregnancy, the numbers in this study are too small for conclusions to be drawn based on the detection of these (small) differences. Sex was therefore not taken into account.

In all of the above studies mentioned, the length of the umbilical cord after birth was used for analyses. We did find one other study that tried to establish the length before birth. Durand et al [14] established the length of the umbilical cord during the third trimester of pregnancy using the propagation velocity of a pressure wave along the cord. The length was deduced by knowing the velocity of the pressure wave as well as the time interval between the systolic peak of two waves. The comparison between the calculated length and the actual length at birth measured showed a significant correlation. In general, most studies on the umbilical cord since the end of the 20th century focus on two different entities, being measurements of the diameter

and cross-sectional area of the umbilical cord and coiling of the umbilical cord, mostly expressed by the umbilical coiling index.

Measuring the diameter and cross-sectional area of the umbilical cord has been proposed as a tool to quantify the amount of Wharton's jelly [15-18]. The umbilical cord lacks adventitia and Wharton's jelly appears to serve that function, cushioning the umbilical blood vessels [19]. In our study, we found that the diameter of the cord was difficult to measure, in most cases because image resolution was not good enough to distinguish the exact boundaries of the circumference of the cord. We did find that the umbilical cord had the same diameter, both at the abdominal side as at the amniotic side.

The spiral twist of the umbilical cord was already described in 1954 by Edmonds [20]. Several studies have since been performed to evaluate the origin, direction and clinical significance of the umbilical cord twist [21-35]. Coiling of the umbilical cord is best visualized using Doppler or colour waves [36]. In this study a first trimester population was used and these diagnostic tools were not used. We were able to distinguish coiling and also the direction of the coiling, but only in a few cases and therefore, no conclusions could be drawn.

No previous studies were found about the length or diameter of the vitelline duct. Our results demonstrate that the growth patterns of the vitelline duct lengths of the individual cases show more variation than growth patterns of the umbilical cord lengths. Diameter remains the same both at yolk sac side as at the umbilical cord side. Because the length of the vitelline duct was difficult to measure at the advanced gestational ages, we were unable to identify whether a plateau or decline in growth would be present with advancing gestational ages. One would expect that since the yolk sac looses its function, the vitelline duct would stop growing.

The amniotic-placental (AP) distance was found to vary considerably between cases. It was a difficult measurement since the resolution and thus quality of the image determined the visibility of the boundaries. We did find it striking that in some embryos, the AP distance was very small or even not measurable, whereas in other embryos the AP distance was clearly visible and measured 4 or 5 mm in length.

We observed an interesting phenomenon, never mentioned before in literature. We tried to define the position of the yolk sac in relation to the embryo and whether this position changes over time. The embryo was placed in a neutral, 'upright' position, facing the operator. The position of the yolk sac was described as mentioned before in the method section. At 7 weeks of gestational age, the yolk sac could be on the right side, below the level of the shoulders, in front of the embryo. One week later, the yolk sac of that same embryo could be hovering above the head of the embryo. This indicates that something is turning or moving at ages where movements are believed to be only very subtle [37]. The length of the vitelline duct does not show significant shortening or elongation over such short time and it therefore does not seem logic that the yolk sac is moving. We think that it is most likely that the embryo is turning around, at a stage where it is not able to be really moving. We were unable to distinguish a certain pattern in which the embryo is turning.

All of the previous studies used conventional 2D ultrasound. The first study on the use of three-dimensional ultrasound in the assessment of the umbilical cord during 2nd and 3rd trimester was performed by Hata et al [38]. Their focus was on visualisation of the cord itself, coiling of the cord and the detection of any abnormalities. They concluded that 3D ultrasound technology has the potential to be a supplement to 2D ultrasound and might be useful in identifying abnormal umbilical cords in utero. Present 3D ultrasound techniques still do not offer real depth perception, and therefore length of the umbilical cord and vitelline duct cannot be measured using these techniques. The I-Space VR technique is unique in the ability to view and interact with the third dimension. The tracing function allows complex measurements of structures that are curved or looped. At present, the Erasmus MC is the only centre in the world that has use of such a system in combination with the necessary computer software to view medical images. However, the fully immersive I-Space version is also available as a desktop version, having the same benefits at a fraction of the cost. We foresee a future where 3D display technology is as common as 2D displays are today. Although the clinical relevance of the length of the umbilical cord and vitelline duct is not clear to us at this moment, further studies will reveal whether these parameters can be used in the detection of any anomalies in growth or development of the embryo.

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PART 5

EVALUATION OF THE APPLICABILITY OF THIS TECHNOLOGY IN CASES OF EMBRYONIC AND FOETAL MALFORMATIONS

CHAPTER 5.1

EMBRYONIC DELAY IN GROWTH AND DEVELOPMENT RELATED TO CONFINED PLACENTAL TRISOMY 16 MOSAICISM, DIAGNOSED BY VIRTUAL REALITY

Fertil Steril 2008; 90(5): 2017 e19-22

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ABSTRACT

Objective:

To demonstrate the use of a novel three dimensional (3D) virtual reality (VR) system in the visualisation of first trimester growth and development in a case of confined placental trisomy 16 mosaicsm (CPM+16).

Patient:

A 34-year old woman, gravida 1 para 0, was seen weekly in the first trimester for 3D ultrasound examinations. CVS was performed because of an enlarged NT measurement and low PAPP-A levels, followed by amniocentesis.

Results:

Amniocentesis revealed a confined placental trisomy 16 mosaicism. On 2D ultrasound and 3D ultrasound no structural anomalies were found, with normal foetal Dopplers. Growth remained below the *p*2.3. At 37 weeks a female child of 2010 grams (< *p*2.5) was born. After birth, growth climbed to the *p*50 in the first 2 months.

Conclusions:

The I-Space VR system provided information about phenotypes not obtainable by standard 2D ultrasound. In this case the delay in growth and development could be observed very early in pregnancy. Since first trimester screening programs are still improving and becoming even more important, systems as the I-Space open a new era for in vivo studies on physiologic and pathologic processes involved in embryogenesis.

CASE REPORT SUMMARY

A 34-year-old woman, gravida 1 para 0, participated weekly in a prospective study on first trimester embryonic growth and development using a 3D Virtual Reality system (I-Space). At the end of the first trimester an apparent delay in both growth and development became obvious. CVS was performed because of an enlarged NT measurement and low PAPP-A levels. Additional amniocentesis revealed a confined placental trisomy 16 mosaicism. At 37 weeks a female child of 2010 grams (< *p*2.5) was born. This is the first CPM+16 case illustrating that severe delay in embryonic growth and development already can be clearly apparent in the first trimester.

INTRODUCTION

In this paper, we describe a case of confined placental trisomy 16 mosaicism that was documented in detail in a prospective study on first trimester growth using three dimensional (3D) virtual reality (VR) [1]. The aim of this paper was to demonstrate that by using this imaging technique, delay in both growth and development can be depicted very early in the first trimester of the pregnancy.

It is expected that as many as 1% [2] to 1.5% [3] of all (clinically recognized) conceptions may have trisomy 16, which is the most frequent chromosome abnormality at conception [4]. In trisomy 16, an early embryonic arrest usually results in a miscarriage between 8 and 15 weeks of gestational age. Trisomy 16 miscarriages show empty sacs, disorganized embryos or minimal embryonic development [2]. Almost all cases of trisomy 16 surviving in the second trimester of pregnancy are found to be mosaic (meaning that the cell lines contain both euploid and trisomic cells) [5]. To survive, the mosaic trisomic 16 cell lines must be completely or at least predominantly confined to the placenta, and this phenomenon could be referred to as confined placental trisomy 16 mosaicism (CPM+16) [5]. Robinson et al [6] found that most cases of CPM+16 originate during maternal meiosis I. The 'rescue' means, that a chromosome 16 is lost in one of the cells of the trisomic conceptus, resulting in an euploid cell line. This can be either one of the two maternal chromosomes 16, resulting in biparental disomy 16 (BPD 16) or the paternal chromosome 16, resulting in uniparental disomy 16 (UPD 16) [7].

Besides an increased risk for (severe) foetal malformations, CPM+16 is associated with intrauterine growth restriction, which is described in both BPD and UPD 16 cases [8,9]. Early detection of placental confined trisomy 16 is important since patients are at increased risk for several maternal obstetrical complications, such as severe preeclampsia [10]. The aim of this paper is to present a case of mosaicsm trisomy 16 that caused an apparent delay in embryonic growth very early in pregnancy.

CASE REPORT

A 34-year old woman, gravida 1 para 0, participated in a prospective study to determine the beneficiary aspects of a novel imaging technique for optimizing first trimester visualization. Women enrolled in this study early in pregnancy, and a 3D ultrasound scan was done weekly from about 5-6 weeks of gestation till 13-14 weeks. This patient had an accurately documented first day of last menstrual period and a positive pregnancy test on day 29 of her cycle. On the first ultrasound examination, gestational sac, yolk sac and an indication of embryonic structures were visualized. At 11 weeks an increased nuchal fold was seen, possibly foetal hydrops. A sonographer licensed by the Foetal Medicine Foundation (FMF; Certificate of Competence in the 11^{+0} – 13^{+6} – week scan) carried out a nuchal translucency measurement 6 days later. The nuchal fold was 2.9 mm. The free -HCG level was 70.70 IU/L (1.323MoM) and the pregnancy-associated plasma protein-A (PAPP-A) level was 0.013 IU/L (0,150 MoM) (AutoDELFIA™ analyzer and LifeCycle™ Elips software-package, PerkinsElmer®, Wallac, Turku, Finland). The crown-rump length (CRL) was only 41.3 mm (the expected range for 12 weeks of gestational age is between 46-63mm). The corrected risk for trisomy 21, 13 and 18 was 1 in 5. Following these results, a chorionic villus biopsy was performed. Five milligrams of chorionvilli were obtained, and in short term cultured villi an additional chromosome, most likely chromosome 16, was seen in all analysed cells. To discriminate between CPM+16 and true foetal mosaicism of trisomy 16, amniocentesis was performed. Using fluorescence in situ hybridisation with chromosome 16-specific probes, normal signal distributions were noted in 100 uncultured amniotic fluid cells, and a normal female karyotype was seen in 37 colonies of cultured amniotic fluid cells. UPD 16 was excluded. Since there still remained a residual risk

on foetal congenital anomalies due to somatic mosaicism, the pregnancy was carefully monitored with two dimensional (2D) and 3D ultrasound, which revealed no structural anomalies; foetal Doppler remained normal. The growth of the foetus remained below the *p*2.3 birth centile throughout her pregnancy. At 36 weeks the patient was admitted into hospital for pregnancy-induced hypertension. At 37 weeks, a Caesarean section was performed for failed induction of labour. A female infant was born with Apgar scores of 6 and 8 after 1 and 5 minutes. The infant had a birth weight of 2010 grams (< 2.5 percentile). The placenta weighed 735 grams after fixation. Besides localised chorangiomatosis in one slice of the placenta, no abnormalities were found. The infant was monitored on the paediatrics ward and was discharged 10 days after birth.

For the following year the girl developed normally and her growth climbed to the 50th percentile in the first 2 months and remained there. No congenital abnormalities were found.

MATERIALS AND METHODS

2D and 3D ultrasound scanning was performed on a GE Voluson 730 Expert system (GE Medical Systems, Zipf, Austria). The 3D volumes were transferred to a personal computer for offline evaluation using specialized 3D software (4DView, GE Medical Systems). These data were transferred to the BARCO I-Space at the department of Bioinformatics of the Erasmus MC. This 4-walled CAVE-like [11] virtual reality system is described in detail in several other studies [1,12-14].

Using this system, we measured standard biometry such as crown-rump length (CRL), biparietal diameter (BPD), and occipito-frontal diameter (OFD), and calculated the head circumference (HC). We also established the Carnegie Stage of the embryo. The embryo was staged according to the description of the external morphological features, mainly limb development, of the Carnegie Stages illustrated and described by O'Rahilly and Müller [15]. This method is described in detail in Verwoerd-Dikkeboom et al [1].

RESULTS

The results of the biometry measurement (mean of three measurements) in the I-Space and the assigned Carnegie stages are displayed in Table 5.1.1.
The Carnegie Staging system ends at day 57 post-conception, therefore when the patient was seen at gestational age 11 weeks + 1 day, assignment of Carnegie stages was no longer possible.

Table 5.1.1. *I-Space measurements.*

AGE	CARNEGIE STAGE (A)	CRL (B)	BPD (B)	HC (B)
7+1	14 (15-16)	5.2 (7-14)	3.1 (0-8)	16.4 (0-29)
8+1	16 (17-18)	9.6 (13-22)	6.5 (2-12)	28.1 (8-42)
9+4	19 (21-22)	19.2 (23-35)	8.0 (7-16)	32.0 (25-59)
11+1	--	32.9 (37-53)	11.5 (12-21)	38.0 (44-78)
12+0	--	41.3 (46-63)	13.5 (15-24)	47.0 (54-88)

Note: Age is gestational age in weeks.

a The expected Carnegie stage for that gestational age is given according to O'Rahilly an Muller [15], calculated as gestational age – 14 days to obtain the postovulatory age.

b The normal 5th-95th percentiles for that age are given for the different parameters. For CRL, the Robinson chart 23 was used; for BPD and HC, the Kusterman charts [24] were used.

DISCUSSION

Growth restriction

Most case reports on CPM+16 (or other chromosomes) start with the result of the chorionic villus sampling (CVS) or amniocentesis. Therefore, little is known about embryonic and/or placental phenotypes in the first trimester of these pregnancies. This patient demonstrated growth retardation already very early in pregnancy. Very early growth retardation can be mistaken for gestational age discrepancy. Adjusting the gestational age could then have serious consequences. We encountered another issue related to very early growth aberrations: problems with performing proper first trimester screening. For first trimester screening most research describes both a gestational age

period and CRL lengths. In first reports, this period was 10^{+0} - 14^{+0} weeks [16]. Nowadays, the FMF uses a 11^{+0} – 13^{+6} week period [17], corresponding with a CRL between 45 and 84mm. The First and Second Trimester Evaluation of Risk for Aneuploidy (FASTER) trial 18 used pregnancies with CRL's between 36 and 79mm, corresponding with 10^{+3} – 13^{+6} weeks. At 11 weeks of gestation, our patient had a CRL of only 33 mm; at 12 weeks this was 42 mm, indicating that in the desired period of first trimester screening (11-13^{+6} weeks) the minimal CRL requirement according to the FMF is still not met. This implies that the effect of any delay in embryonic growth and development on the reliability of the results of combined first trimester screening, is unclear.

PAPP-A

The combined first trimester screening for Down syndrome revealed that serum PAPP-A level in this patient was extremely low. Several studies have indicated the association between low levels of serum PAPP-A with a number of adverse pregnancy outcomes, such as an increased risk of pre-eclampsia [17,18], gestational hypertension [18] and intra-uterine growth restricion [17-19]. Smith et al [20] stated in their study that this predictive value of PAPP-A implies a fundamental role of this system in the development of the placenta in early pregnancy. This patient is a good example of the presumption that impaired placentation is reflected by low serum PAPP-A. To our knowledge, this is the first report that describes a low serum PAPP-A level in association with a placental confined trisomy 16. Groli et al [21] described five cases of trisomy 16 confined to the placenta that were found after high-risk results in a second trimester maternal serum screening program for Down syndrome. CVS and amniocentesis was performed. All five pregnancies displayed unusually high levels of human chorionic gonadotropin (hCG) and four out of five had raised alpha-fetoprotein values (AFP). All five pregnancies were complicated by foetal growth retardation. Other studies have also shown extremely high levels of hCG [2,22]. Our patient, however, displayed a hCG level within the normal range on first trimester screening; the AFP level was also within the normal range (26 IU/ml, measured at 16 weeks in amniotic fluid, normal range 15-30 IU/ml).

I-Space implementation

We analyzed the 3D volumes of this patient in the I-Space, and we were able to easily measure and calculate CRL, BPD, OFD and HC using this system. We also established the Carnegie Stage of the embryo. The Carnegie Stage we assigned to this embryo corresponded very well with the measured CRL compared with the original data of the Carnegie Collection described by O'Rahilly and Muller [15], indicating that growth and development were still in concordance. Delay in either growth or development would have meant that the assigned Carnegie Stages did not correspond with CRL measurements, for instance, a CRL measurement of a stage 21 embryo with morphological features of a stage 19 embryo or the exact opposite: morphological features of a stage 21 embryo with a CRL of a stage 19 embryo.

Age, however, did not correspond with CRL: the age discrepancy was more than 8 days in general. CRL parallels the Carnegie Stages in the discrepancy between CRL and gestational age. Since gestational age is not questioned in this patient the only conclusion can be that both growth and development were delayed very early in pregnancy. The question is whether this can all be attributed to the placental confined mosaicism. If it is, it implies that placentation is of vital importance already in the earliest stages of pregnancy.

We believe that it is essential to combine biometry measurements with evaluation of morphological features. The I-Space Virtual Reality system provides us with information about phenotypes not obtainable by standard 2D ultrasound. In this case, the delay in growth and development could be observed very early in pregnancy. Since first trimester screening programs are still improving and becoming even more important, we believe that systems such as the I-Space open a new era to study embryonic growth and development in vivo. This will eventually lead to better understanding of both physiologic and pathologic processes involved in embryogenesis.

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CHAPTER 5.2

USING VIRTUAL REALITY FOR EVALUATION OF FOETAL AMBIGUOUS GENITALIA

Ultrasound Obstet Gynecol 2008; 32: 510-514

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ABSTRACT

Objective:

The utility of a virtual reality system was examined in the visualization of three dimensional (3D) ultrasound images of foetal ambiguous genitalia.

Methods:

In 2005, foetal ambiguous genitalia were diagnosed in four patients referred to our department for prenatal ultrasound assessment. Patients were examined by two dimensional (2D) and 3D ultrasound and, subsequently, the volumes obtained with 3D ultrasound were visualized in the BARCO I-Space virtual reality system. This system projects stereoscopic images on three walls and the floor of a small 'room', allowing several viewers to see a 3D 'hologram' of the data being visualized. The results of 2D and 3D ultrasound examination and the virtual reality images of the I-Space were compared with diagnoses made post partum.

Results:

In all cases, prenatal diagnosis was unclear based on 2D ultrasound alone. Surface rendering of 3D data provided an impression of ambiguity, but diagnosis based on these data proved incorrect at birth in three cases. Conclusions based on the evaluation of 3D volumes in virtual reality best fitted the post partum diagnosis in all cases.

Conclusions:

This study suggests that by evaluation of the genitals in the I-Space, a better impression of the ambiguity can be established. Binocular depth perception appeared particularly useful in distinguishing either a micropenis or enlarged clitoris from labia minora, since it helps in the estimation of size and position. Therefore, we see potential for the application of virtual reality not only for the evaluation of foetal ambiguous genitalia, but in all those cases where depth perception would improve the visualisation of anatomical structures.

INTRODUCTION

Determination of foetal sex is an important aspect in prenatal ultrasound assessment. Advances in two and three dimensional (2D and 3D) ultrasound technology have improved the visualization of foetal genitalia, allowing identification of foetal sex as early as the end of the first trimester [1,2]. Any uncertainty or doubt about a baby's sex is extremely worrying and unsettling for its parents and family, but the assessment and diagnosis of foetal genital anomalies remain challenging and difficult tasks for ultrasonographers.

The objective of this study was to asses the clinical potential of a virtual reality technique by visualizing foetal genitalia that were considered abnormal or ambiguous on routine ultrasound scan using an immersive virtual reality system, the BARCO I-Space (Barco, Kortrijk, Belgium). In the I-Space, binocular depth perception provides the investigator with a realistic 3D illusion that allows much better assessment of 3D images such as foetal genitalia as compared to conventional 3D rendering in surface mode on a workstation.

MATERIALS AND METHODS

I-Space: Volume visualization in a virtual environment

The BARCO I-Space installed at the department of Bioinformatics of the Erasmus MC is a four-walled CAVE™ -like virtual reality system [3], that surrounds investigators with computer generated stereoscopic images. These images are projected on three walls and the floor of a small 'room', by eight high-quality Digital Light Processing (DLP) projectors. The images need to be viewed through glasses with polarizing lenses to perceive depth. The CAVORE [4] volume rendering application is used to create a 'hologram' of the ultrasound volume that is being investigated, which can then be manipulated by means of a virtual pointer, controlled by a wireless joystick. Wireless tracking of the viewer's head allows the computer to provide the correct perspective and motion parallax, which in addition to the stereoscopic images helps in discerning fine details and understanding of 3D structures in the volumes. The CAVORE software was originally developed as a general-purpose volume rendering application for use

in immersive virtual environments such as the CAVE. It uses a combination of direct manipulation of the data set with a pointing device with six degrees-of-freedom and a simple graphical user interface with a drop-down menu and a 'widget' (a graphical object that can be manipulated by the user) to control the transfer function that assigns grey-scale and opacity values to the data.

Methods

Between January 2005 and May 2006 ambiguous genitalia were diagnosed as an isolated or an additional finding in the foetuses of four women referred to our department of obstetrics and prenatal medicine for prenatal assessment. 2D ultrasound examinations of all suspected foetal abnormalities were performed on a GE Voluson 730 Expert system (GE Medical Systems, Zipf, Austria). For the evaluation of the genital area we also performed 3D ultrasound examinations, but we used only 3D ultrasound rendering in surface mode (not the sectional plane mode). All foetal malformations in our department are discussed by a team of doctors and obstetricians, including ultrasonographers, but after reviewing the data from examination of the four cases under consideration analysis of the genital area was still unsatisfactory. To demonstrate the potential of the Barco I-Space in analyzing genital abnormalities, the 3D volumes were transferred to a personal computer for off-line evaluation using specialized 3D software (4DView, GE Medical Systems), without any image processing. These data were then saved as Cartesian (rectangular) volumes and transferred to the BARCO I-Space. In the I-Space, the volumes were screened for quality, completeness, and visibility of the genital area. Volumes were resized and turned around in space, and grey scale and opacity were adjusted. To obtain the best view of the genital area the hologram was directly manipulated by cutting away part of it using the virtual pointer.

RESULTS

Patient 1

A 27-year-old women, gravida 2 para 1, was referred at 20 weeks' amenorrhea because of a suspected foetal cardiac anomaly. Amniocentesis was performed at that time and karyotyping revealed 46 XY, although ultrasound examinations at 20 and 27 weeks'

gestational age had revealed normal female genitalia. Knowing the karyotype, the genitals were considered ambiguous on ultrasound examination at 34 weeks. On 3D surface rendering a bifid scrotum seemed most likely, with a small central structure, possibly a micro-penis. In the I-Space large labia majora were clearly recognizable with a prominent structure between them, which most resembled labia minora. An enlarged clitoris could neither be excluded nor confirmed (figure 5.2.1).

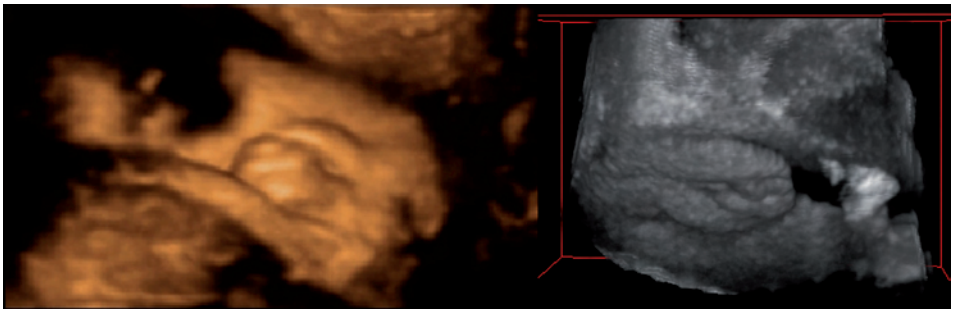

Figure 5.2.1. *Three dimensional (3D) image (a) and I-Space image (b) of the genital area of Patient 1. On the 3D image a bifid scrotum with a central micro-penis was diagnosed, whereas in the I-Space the labia minora are clearly recognizable. (A comparison of the two techniques from these pictures is not valid, since a picture of the I-Space is nothing more than a 3D picture on a two dimensional medium.)*

At 38 weeks the infant was delivered by Caesarean section. Examination of the external genitals revealed normally appearing female genitalia: the labia majora were normal, there were no palpable gonads, and the clitoris seemed enlarged (1.5cm) with a small palpable invagination. The introitus vaginae appeared normal. Ultrasound of the internal genitalia revealed normal aspects of the uterus and ovaries, with follicles visualized on both sides. The XY karyotype was confirmed and the infant was diagnosed as an XY-female [5].

Furthermore, the child was diagnosed with a hypertrophic cardiomyopathy, ventricular septal defect, atrial septal defect and hypoplasia of the right lung. Several cerebral malformations (hypomyelinisation) were found as well. The infant died at the age of eight months. Several syndromes and metabolic diseases were considered, but a definite diagnosis was not possible and parents refused autopsy.

Patient 2

A 23-year-old woman, gravida 3 para 1, was referred because of suspected foetal pyelectasia, polyhydramnios and ambiguous genitalia at 30 weeks' amenorrhea. Amniocentesis had already been performed and karyotyping revealed 46 XX. A series of ultrasound examinations confirmed polyhydramnios, a single-artery umbilical cord, two large cysts in the foetal pelvis and ambiguous genitalia; there was a prominent structure in between two skin creases. In the I-Space two small skin creases were visible in the genital area, with a prominent, protruding, blunt structure on top resembling a penis with hypospadia (figure 5.2.2).

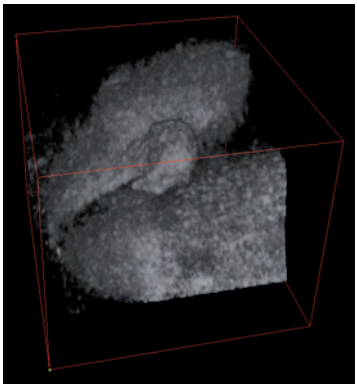

Figure 5.2.2. *Detailed I-Space image of the genital area of Patient 2, showing the blunt protruding structure.*

At 42 weeks the infant was born and several congenital anomalies were observed, including oesophageal atresia with a trachea-oesophageal fistula, anomalies of the S1-S2 vertebrae, urogenital sinus, hydronephrosis on both sides and ambiguous genitalia: labia majora, labia minora and a micropenis were observed. The anatomy of the urinary tract could not be distinguished as male or female and therefore it was concluded that it was probably an unusual variation of a cloacal malformation. The infant was diagnosed with VACTERL syndrome.

Patient 3

A 30-year-old woman, gravida 2 para 1, was referred because of a suspicion of hypospadias at 32 weeks' amenorrhea. 2D ultrasound examination revealed ambiguous genitalia. Severe hypospadia with micropenis and a bifid scrotum seemed most likely, but hypertrophy of the clitoris could not be excluded. Karyotyping following amniocentesis

revealed 46 XX. On 3D surface rendering mode two skin creases were visualized with a structure in between, the most likely diagnosis being an enlarged clitoris between the labia majora. In the I-Space, normal labia majora were found, with somewhat enlarged labia minora (figure 5.2.3).

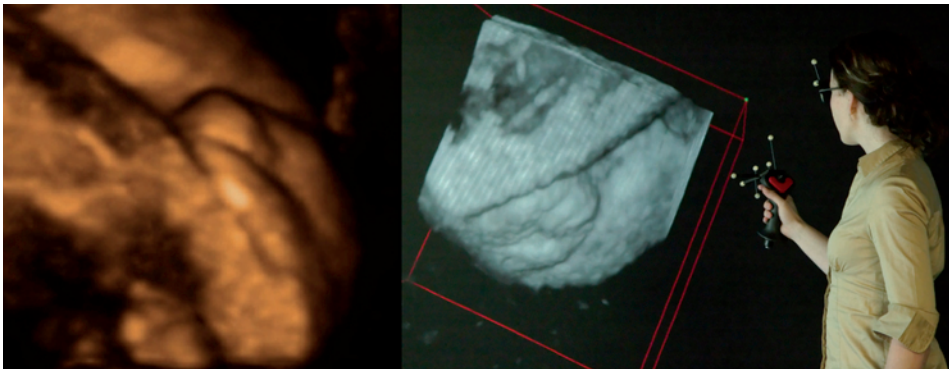

Figure 5.2.3. *(a) Three dimensional (3D) ultrasound surface-rendered image of the genital area of Patient 3. The skin creases were interpreted as labia majora and the clitoris was thought to be enlarged. (b) I-Space image in which the labia minora are quit obvious.*

At 41 weeks a healthy infant was delivered. Postpartum, the genitals were confirmed as female, with no evident abnormalities.

Patient 4

A 25-year-old woman, gravida 2 para 0, was referred because of suspected genital abnormalities at 32 weeks' amenorrhea. 2D ultrasound examination revealed ambiguous genitalia. On 3D surface-rendering mode ultrasound labia majora with an enlarged clitoris seemed most likely. The adrenal glands were not enlarged. The parents refused amniocentesis for karyotyping. In the I-Space normal labia majora were visualized, with markedly enlarged labia minora. The clitoris did not seem enlarged (figure 5.2.4).

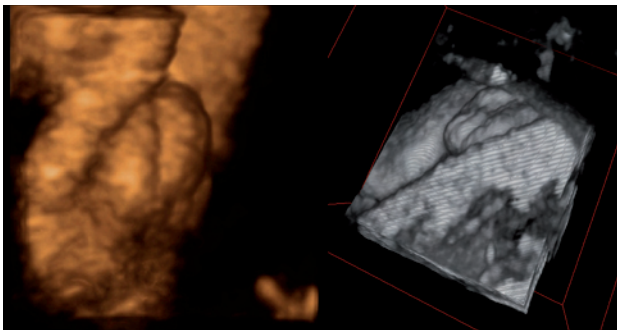

Figure 5.2.4. *(a) Three dimensional (3D) ultrasound image of the genital area of Patient 4, showing labia majora with an enlarged clitoris. (b) I-Space rendering image, showing the enlarged appearance of the labia minora; in virtual reality this was even more clearly recognizable than it is in this two dimensional representation.*

At 40 weeks the patient delivered a healthy infant. The genital area appeared female, with somewhat enlarged labia minora. The clitoris was not markedly enlarged. Ultrasound examination showed normal internal genital organs. A normal 46 XX karyotype was found.

Table 5.2.1. *Classification of the genitalia following the different examinations.*

		Classification on:				
Patient	**Referral Diagnosis**	**2D US**	**3D US**	**I Space**	**Karyotype**	**Postpartum diagnosis**
1	Female / Ambiguous	Female	Ambiguous	Female	XY	XY female, normal female genitalia
2	Ambiguous	Ambiguous	Ambiguous	Ambiguous	XX	VACTERL, ambiguous genitalia, cloacal malformation
3	Male hypospadias	Ambiguous	Clitoral hypertrophy	Normal female	XX	Normal female
4	Ambiguous	Ambiguous	Clitoral hypertrophy	Normal female	XX	Normal female

DISCUSSION

Prenatal diagnosis of foetal gender by ultrasound generally has a high rate of accuracy and is a well-established part of routine ultrasonography [6]. However, when foetal genitalia appear malformed or ambiguous, or gender assigned by ultrasound does not match gender by karyotype, a plethora of syndromes must be considered and the limitations of ultrasound imaging in observing the external contours of the genitals become obvious.

In this study, we tried to establish the clinical potential of a virtual reality technique in the assessment of foetal ambiguous genitalia after unsatisfying analysis of these malformations in four cases using 2D and 3D ultrasound. Although these scans were performed by senior ultrasonographers with more than 10 years of experience in the detection of foetal abnormalities, definite diagnosis of the genital malformations remained impossible. This study shows that by evaluation of the genitals in the I-Space, a better impression of ambiguity can be established. Depth perception appeared particularly useful in distinguishing either micropenis or enlarged clitoris from labia minora, since depth helps estimation of size and exact position. Although the numbers are small, we decided to present our results because the findings in the I-Space turned out to be extremely helpful in prenatal diagnosis. We learned that a protruding structure in the genital area can very well represent (normal) labia minora instead of malformations such as an enlarged clitoris or micropenis. This has already benefited the counselling of several patients seen after we conducted this study.

Using 3D ultrasound with conventional imaging systems still has the disadvantage that evaluation of the 3D ultrasound image is in fact performed in a 2D manner either on a print or on a computer screen. Therefore, the third dimension is not used optimally, since depth perception is not possible. In the I-Space the investigator views the equivalent of a hologram floating in space, thus benefiting from depth perception. When comparing the images of the 3D volumes with the images of the volumes in the I-Space it is important to remember the limitations of such comparisons, since the third dimension cannot be put on paper. Hence, the image of the volume in the I-Space shows neither all the information gathered from the volume in the I-Space itself nor all its beneficiary aspects.

Of course, this study has the limitation that we only used surface-mode rendering. Evaluating the 3D volumes in various modes, such as the sectional plane mode, enhances the information acquired from the volume. However, in this study we provided a valid comparison, since we evaluated only the surfaces of both 3D and I-Space volumes.

The CAVORE software can be used to visualize many different 3D imaging modalities, such as computed tomography (CT), magnetic resonance imaging (MRI),

single photon emission computed tomography (SPECT), positron emission tomography (PET) and, as in this study, 3D ultrasound. It can also animate time series of 3D data, also known as 4D data. This application has already been found effective and to have additional value in visualizing a foetal meningomyelocele [7] and dynamic 3D adult echo-cardiographic data [8,9]. In order to be integrated into daily clinical practice, there is a need to implement semi-immersive (such as a GeoWall http://geowall.geo.lsa.umich.edu]) or desktop (such as the Personal Space Station [http://www.ps-tech.com]) virtual reality systems in the hospital environment, for instance in consulting rooms and operating theatres. The user interface of CAVORE has already been adapted to allow it to be run on this type of virtual reality systems.

While a complete I-Space system at this time may cost in the region of $500,000, for this type of application a virtual reality system with a single projection surface would be sufficient. These can be put together for around $20,000 - $50,000, depending on projector and tracking choices. A potential side-effect of virtual reality might be that the systems using head-mounted-displays (virtual reality 'helmets') are associated with 'simulation sickness', i.e. nausea and/or dizziness similar to motion sickness. However, users of projection-based systems, and in particular the operator who views the correct perspective, suffer far less from this problem.

We conclude that there is potential for the use of the virtual reality technique in evaluating difficult anatomical structures where depth perception would improve visualization, as is the case with foetal ambiguous genitalia.

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GENERAL DISCUSSION

In this thesis the use of an innovative tool for the evaluation of developmental embryonic and early fetal morphology is demonstrated. The I-Space VR system is unique in its ability to view and measure the third dimension and its tracing function allows for complex measurements. In two studies we successfully established the reproducibility of I-Space measurements, for both biometric and volume measurements. We can therefore conclude that measurements in virtual reality are at least as reliable as routine 3D measurements with intra-observer and inter-observer variability within preferred limits. That reliability is an important factor, because during the last centuries, human growth has often been subject of study by investigating the relation between time (age) and size (biometric measurements) resulting in growth charts. Paediatricians had already made a serious break through in timely recognition of abnormal growth with the introduction of the use of growth charts during childhood. Birth weight charts already existed before the ultrasound period [1], partially constructed by using data on premature deliveries, which in fact were abnormal pregnancies. Intrauterine growth curves however were not available until after the introduction of ultrasound in the sixties [2-4].

Improvement of imaging techniques - like the development of high-resolution vaginal ultrasound, 3D ultrasound and virtual reality applications such as described in this thesis - all stress the discussion about what is normal embryonic growth. Growth is hard to define since two different parameters are required, the measurements of which having drawbacks. One is the determination of the age of the embryo. Menstrual history is established and/or when in doubt, measurements such as the crown-rump length (CRL) are used to determine the age of the embryo. However, the exact relation between the CRL and the duration of pregnancy against the background of individual growth rate and abnormal embryonic development is still not clear. Numerous articles have been published about CRL measurements and it's efficacy in estimating embryonic age and the duration of pregnancy [5-13]. Except for pregnancies from assisted reproduction programs, exact age is still hard to establish. The latter brings across even bigger dilemmas such as the question whether IVF/ICSI pregnancies can be regarded as normal pregnancies in this regard [10,14].

The other problem is, that biometry measurements are generally based on the comparison of measured values with predicted values derived from reference charts or equations from normal populations, which implicates that the embryonic period demonstrates uniform growth [5]. This is questioned in several studies [14-18]. One might state that measuring size alone does not adequately reflect embryonic growth and / or embryonic development.

Another way of looking at growth and development may therefore be to classify it into stages. The Carnegie Staging system is a well-established method that enables focusing on morphological features. Stages based on the morphological state of development have been devised for many species and the Carnegie Staging System has proven its value in the classification of human embryos for decennia [8,19-22]. Using the I-Space VR system, assigning Carnegie Stages to the embryos proved therefore relatively easy. Hence, combining length measurements with viewing developmental features using virtual reality techniques, will greatly improve knowledge of normal and abnormal embryonic growth, development and morphology.

The latter was demonstrated in the case report described in chapter 5.1 in which a case of placental confined trisomy 16 mosaicism is presented, that demonstrated growth retardation already very early in pregnancy. Biometric measurements were combined with the assignment of a Carnegie Stage. The CRL was found to parallel the Carnegie Stages in the discrepancy between CRL and gestational age and therefore both growth and development were shown to be delayed already very early in pregnancy. It does put emphasis on the fact that growth retardation is not necessarily the same as developmental delay and these two processes should be viewed at separately.

A staging system such as the Carnegie Staging system takes morphological features such as development of arms and legs into account. Although we previously stated that measurements alone may not fully reflect or predict all developmental processes involved during embryogenesis, measuring is a well-known tool to quantify growth. We therefore established new embryonic growth charts, derived from longitudinal measurements. Of course growth charts for the standard biometric measurements such as CRL, BPD, HC and AC were created, but we also measured structures that needed depth perception for proper evaluation. New growth charts for

length of the arm, width of the shoulder and elbow and width of the hips and knees were established. The third dimension also offered the opportunity to measure for the first time the length of the umbilical cord, with all its loops as well as the length of the vitelline duct (chapter 4.2). Although the clinical applications of the latter measurements are not clear to us yet, these structures are vital for the development of the embryo and logically, one would expect that the length of these structures must also reflect embryonic wellbeing and developmental potential.

After birth, growth of an infant is monitored by following both length and weight gain. This provides a good estimation of the development of the child, but only when these parameters are combined. During pregnancy, several mathematical calculations have been developed to estimate foetal weight by combining several biometric measurements [23]. Although it does provide an impression of the weight of foetus, miscalculation of up to 20% of the real weight are no exceptions. It would be very interesting to know the weight of an embryo. One way of approximating the weight of an embryo might be the calculation of the total volume of the embryo. Since we demonstrated that in the I-Space volumes can be easily and reliably measured, volume measurements of total embryonic volumes is the next step in this ongoing study. Volumes of yolk sacs have already been measured (chapter 2.2). In the past, several studies have addressed the fact that shape, size and morphology of the yolk sac might have a relationship with embryonic wellbeing and developmental potential [24-27]. However, due to various reasons, amongst which image quality is an important one, an exact relationship has always been difficult to establish. Reliable measurements of the volume of the yolk sac in the I-Space may provide more insight as to whether such a relationship does exist. Charts for both normal pregnancies and abnormal pregnancies will need to be constructed.

As far as we know, the Erasmus MC is the only centre in the world to use such a system in combination with the necessary computer software to study medical images. As explained in the second part of this thesis, the fully immersive system can also be reduced to a desktop version, offering the same benefits at a fraction of the cost. Such a display system on the desk of the doctor will greatly benefit the counselling of patients by clearly illustrating embryonic / foetal anatomy. We foresee a future where

3D display technology is as common as 2D displays are today. Applying 'Virtual Embryoscopy' will enable us to study normal embryogenesis in vivo. The combination of biometric measurements with other methods of assessing embryonic growth and development such as the Carnegie Staging System will eventually offer an earlier and more accurate diagnosis of abnormal growth and developmental. We conclude that the I-Space offers an impressive new way of looking at growth and development during embryogenesis. Improved visualization of the first trimester of pregnancy may contribute to a shift of prenatal diagnosis of many congenital abnormalities from the second- towards the first trimester of pregnancy. Such 'Virtual Embryoscopy' may bring the era of 'Embryonic Medicine near by!'

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SUMMARY

The general aim of the studies presented in this thesis is to provide an accurate description of *in vivo* embryonic and early foetal growth and development using an innovative imaging technique, forming a basis for further studies on early pregnancy evaluation in high risk patients.

Part 1, chapter 1.1 is a brief introduction of the research described in this thesis and the main research objectives are presented. **Chapter 1.2** is an overview of the history of the development of virtual reality systems in general. Furthermore, the technical details and clinical applications of the I-Space Virtual Reality System are described. Future perspectives for clinical use of 3D ultrasound (3D) in virtual reality are addressed as well.

In **part 2, chapter 2.1** we established the reliability of routine 3D ultrasound measurements in early pregnancy using both specialized 3D imaging software and the I-Space. The application of virtual reality is a novel method of visualising 3D ultrasound data and the depth perception in the I-Space offers great possibilities of measuring non-planar structures. However, before reference charts can be created using this new imaging technique, reliability and reproducibility of this system needed to be established. This study demonstrates that measurements in virtual reality are at least as reliable as routine 3D measurements with intra-observer and inter-observer variability within preferred limits.

In **chapter 2.2** the reliability and reproducibility of the volume measuring application in the I-Space (implemented in V-Scope, the latest software application) is established. Four different techniques for volume estimation were compared: VOCAL, Inversion Mode, SonoAVC and V-Scope. For an *in vitro* study water filled balloons were used and the original volume was compared with the computer estimates. VOCAL and V-Scope turned out to be most reliable and intra- and interobserver variability were good in all four techniques. An *in vivo* study was also performed, in which yolk sac volumes were compared using the four techniques. Again, VOCAL and V-Scope turned out to be most reliable. Since VOCAL is a time-consuming technique, the semi-automatic volume tracing function of the I-Space offers great potential for the estimation of embryonic volumes and volumes of different organ systems, such as bladder, stomach and brain cavities.

In **part 3** we describe the use of the I-Space Virtual Reality system in the assignment of Carnegie Stages to embryos of IVF/ICSI pregnancies visualized with 3D ultrasound. This staging system is based on the apparent morphological state of development and therefore not directly dependent on chronological age or size. A number of features are taken into account, rendering individual differences less significant. We found that in the I-Space the Carnegie Stage of the embryo could be determined easily. Depth perception helped in estimating size and position. The presumed stages corresponded well with the measured CRL, although in general, stages seemed to have been reached earlier than previously described for the Carnegie Collection. We believe that the assignment of Carnegie Stages with 'Virtual Embryoscopy' provides a promising non-invasive tool for early pregnancy evaluation of embryogenesis. It also opens a new area to study the relationship between embryonic growth, development and morphology in relation to second and third trimester pregnancy complications.

In **part 4** normative data are presented relative to gestational age for both standard and non-standard biometric measurements. **Chapter 4.1** provides first trimester growth charts for standard biometric measurements of the crown-rump length (CRL), biparietal diameter (BPD), head circumference (HC) and abdominal circumference (AC). The depth perception in the I-Space also enabled us to construct new growth charts for other structures like length of the arm, width of the shoulder and elbow and width of the hips and knees.

In **chapter 4.2** for the first time ever, *in vivo* measurements are presented of the total length of the umbilical cord and vitelline duct during the first trimester of pregnancy. The change in position of the yolk sac in relation to the embryo in time is described as well. All these findings only can be observed with the use of the third dimension and virtual reality measurement tools.

In **part 5**, we evaluated the applicability of the virtual reality technology in cases of embryonic and foetal malformations. **Chapter 5.1** describes a case of trisomy 16 confined placental mosaicism. This patient was evaluated in the I-Space and both biometry measurements and morphological features were taken into account. Intra uterine growth restriction (IUGR) is common in patients with this type of mosaicism, but in

this patient delay in both growth and development was detected already very early in pregnancy. Serum PAPP-A levels were extremely low and this patient is a good example of the presumption that impaired placentation is reflected by low serum PAPP-A. We believe that systems such as the I-Space, open a new era to study embryonic growth and development *in vivo* clinically. This will eventually lead to better understanding of both physiologic and pathologic processes involved in embryogenesis.

In **chapter 5.2** we used the I-Space to visualize 3D ultrasound sets of foetal ambiguous genitalia. In all four patients evaluated, prenatal diagnosis was impossible based on 2D ultrasound alone. Surface renderings of 3D data provided an impression of ambiguity, however this proved incorrect at birth in three cases. Conclusions based on evaluation of 3D volumes in virtual reality best fitted post partum diagnosis in all cases. This study therefore suggests that by evaluation of the genitals in the I-Space, a better impression of the ambiguity can be established. Bi-ocular depth perception appeared particularly useful in distinguishing either a micropenis or enlarged clitoris from labia minora, since it helps in the estimation of size and position. We believe that the application of virtual reality could be useful in all cases where depth perception would improve the visualisation of anatomical structures.

In the **General Discussion:** In this thesis the use of this virtual reality technique in the study of embryogenesis and in daily clinical practice is discussed. In conclusion: the I-Space Virtual Reality system allows depth perception and therefore offers opportunities for visualizing structures not visible in two dimensions. Since any abnormalities in growth and/or development in the embryonic period will have major consequences for foetal and neonatal life, we believe it to be of vital importance to pay close attention to all developing structures as early as possible. Early diagnosis of delay in growth or development may eventually lead to new strategies for these patients and for patients at risk for other pregnancy associated complications. Preventative measures may be developed and 'Embryonic Medicine' thus awaits. It might be the case that 'Virtual Embryoscopy' develops as a powerful tool for first trimester pregnancy evaluation, stressing the necessity for various preventive treatment strategies and resulting in a new area of 'Embryonic Medicine.'

SAMENVATTING

INTRODUCTIE

De eerste 10 weken van de zwangerschap zijn essentieel voor het zich ontwikkelende embryo: abnormale groei en/of ontwikkeling zal naar alle waarschijnlijkheid gevolgen hebben voor de foetale groei in het 2e en 3e trimester en voor de gezondheid van de pasgeborene. Het is daarom van groot belang om de processen die plaatsvinden gedurende de humane embryogenese goed te definiëren en inzichtelijk te maken. Snelle detectie van problemen bij deze processen zijn belangrijk voor vroege detectie van afwijkingen, bijvoorbeeld in het geval van herhaalde miskramen en bij chromosoomafwijkingen.

Het is echter verbazend dat de klassieke beschrijving van de normale embryonale groei grotendeels gebaseerd is op informatie gebaseerd op abnormale zwangerschappen, zoals miskramen en ectopische zwangerschappen. Voor humane embryo's wordt gebruik gemaakt van het Carnegie Staging Systeem. Stageringssystemen zijn gebaseerd op morfologische ontwikkelingsstadia en hierdoor niet direct afhankelijk van bijvoorbeeld leeftijd of lengte. Het Carnegie Systeem is gebaseerd op zowel interne als externe fysieke karakteristieken van het embryo en is genummerd van 1 tot en met 23. Bij stadium 23 zijn alle interne orgaansystemen aangelegd en is daarom beschreven als het einde van de embryonale periode.

Om echter embryogenese *in vivo* te bestuderen zijn andere technieken nodig. In 1957 werd voor het eerst een foetus zichtbaar gemaakt met echoscopie door Ian Donald. Embryonale groei werd in een curve geplaatst door Robinson in 1975, waarbij hij gebruik maakte van de kop-stuit lengte (CRL). Nog steeds is het meten van de CRL een standaard onderdeel van echoscopisch onderzoek in het eerste trimester. De driedimensionale (3D) echoscopie werd geïntroduceerd in het begin van de jaren '80. Tegenwoordig is de toegevoegde waarde van deze techniek voor het beoordelen van foetale afwijkingen, vooral aan gezicht, ledematen, thorax en wervelkolom onomstreden. Het embryo kan door deze techniek ook zeer goed in beeld worden gebracht. Echter, hoewel 3D echoscopie in naam drie dimensies heeft, worden de beelden bekeken vanaf tweedimensionale (2D) media, namelijk papier of een computerscherm. De derde dimensie, diepte, gaat dus feitelijk weer verloren.

DE I-SPACE VIRTUAL REALITY SYSTEEM

Dit diepteverlies kan ondervangen worden door de toevoeging van een stereoscopisch beeldtechniek. Op 24 maart 2005 werd op de afdeling Bioinformatica van het Erasmus MC in Rotterdam de BARCO I-Space officieel geopend. De I-Space is gebaseerd op een 3D projectietechniek, waarbij verschillende beelden voor het linker- en rechteroog geprojecteerd worden. De hersenen zijn in staat om uit deze twee 'platte' beelden een beeld met diepte te vormen. De I-Space bestaat uit 8 projectoren, geplaatst achter 4 projectiewanden. Deze projectiewanden zijn de drie muren en vloer van een kleine kamer, die aan de voorkant open is. Op iedere wand wordt door twee projectoren twee verschillende beelden geprojecteerd. Een speciaal voor dit systeem ontwikkelde volume rendering applicatie (CAVORE / V-Scope) wordt gebruikt om een 'hologram' te creëren van het te onderzoeken volume. Dit kunnen 3D echobeelden zijn, maar ook CT, MRI of PET scans. Bij binnenkomst krijgt de toeschouwer een bril met gepolariseerde glazen. Het linker- en rechteroog van de gebruiker zien niet hetzelfde beeld op de wanden en vloer, maar beelden die precies zo van elkaar verschillen dat het lijkt of het object driedimensionaal is en als hologram in de ruimte zweeft. Het hologram kan worden gemanipuleerd door een virtuele 'pointer', gekoppeld aan een draadloze joystick en kan qua grootte, oriëntatie, helderheid en transparantie worden aangepast. Ook kunnen willekeurige delen 'weggesneden' worden, zodat in het volume gekeken kan worden. Het grote voordeel voor de onderzoeker is, dat deze precies ziet wat hij of zij doet, in het juiste perspectief. Het bewerken van het beeld gaat dus op een heel natuurlijke manier. Ook kunnen meerdere mensen tegelijk de beelden bekijken in de I-Space, waardoor het ook voor onderwijsdoeleinden zeer geschikt is.

In dit proefschrift wordt het gebruik van de I-Space geëvalueerd bij het beoordelen van humane embryonale groei en ontwikkeling. De volgende onderzoeksdoelen werden gedefinieerd:

1. De reproduceerbaarheid van embryonale biometrie metingen en volume-metingen vaststellen gebruik makend van deze nieuwe techniek.
2. Kennis van embryogenese *in vivo* verbeteren door gebruik te maken van een stageringssysteem waarbij zowel gelet wordt op morfologie als op biometrie.

3. Het opstellen van normatieve data gerelateerd aan zwangerschapsduur voor zowel standaard embryonale biometrie metingen als voor nieuwe, niet-standaard biometrie metingen.
4. De toepasbaarheid van deze techniek beoordelen bij embryonale en foetale afwijkingen.

De reproduceerbaarheid van metingen verricht in de I-Space (**part 2**) werd vastgesteld in 2 verschillende studies.
De eerste studie (**chapter 2.1**) beschrijft de betrouwbaarheid van diverse 3D metingen vroeg in de zwangerschap. Hiervoor werden 28 zwangerschappen met zwangerschapsduren tussen de 6 en 14 weken geincludeerd. Er werden 3D volumes gemaakt en de volgende metingen werden bepaald: de dooierzakdiameter (YS), de kop-stuit lengte (CRL), de biparietale diameter (BPD), de hoofdomtrek (HC) en de buikomtrek (AC). De 3D datasets werden vervolgens naar de I-Space getransporteerd.. Hier werden dezelfde metingen verricht. Alle metingen werden driemaal verricht, waarbij het gemiddelde werd gebruikt om te vergelijken. Om de intra-onderzoeker variabiliteit te bepalen werden alle metingen 2x verricht door 1 onderzoeker. Voor de inter-onderzoeker variabiliteit werden alle metingen ook nog verricht door een andere onderzoeker. Vervolgens werden intraclass correlatie coëfficiënten (ICC) bepaald, waarbij een ICC waarde > 0,90 betekent dat er sprake is van een goede overeenstemming. Wanneer de 3D metingen vergeleken werden met de I-Space metingen bleek voor alle parameters de ICC groter te zijn dan 0,97. De intra- en interonderzoeker variabiliteit waren > 0,96 voor de 3D metingen en > 0,98 voor de I-Space metingen. Hieruit kan geconcludeerd worden metingen met de I-Space Virtual Reality techniek in de vroege zwangerschap betrouwbaar is en minimaal even goed is als de bestaande 2D en 3D meettechnieken.

De tweede studie (**chapter 2.2**) onderzoekt de betrouwbaarheid en nauwkeurigheid van 4 verschillende volume meetmethoden. Hedendaagse echoscopische apparatuur biedt diverse mogelijkheden om volumes te bepalen. In deze studie wordt gekeken naar de volgende technieken:

- VOCAL: een volume meet algoritme wat gebruik maakt van 2D segmentatie rondom een centrale roterende as. Het aantal stappen waarmee om de as gedraaid

wordt kan door de onderzoeker bepaald worden. De computer software berekend het volume op 2 manieren ofwel automatisch ofwel nadat de onderzoeker handmatig in de verschillende vlakken het volume heeft aangegeven.

- Inversion Mode: een segmentatie algoritme waarbij enkel volumes bepaald kunnen worden van hypo-echoische structuren. De grijswaarde van de verschillende voxels bepaald het uiteindelijke volume, waarbij de drempelwaarde voor de grijswaarde door de onderzoeker kan worden ingesteld.
- SonoAVC: dit algoritme identificeert en kwantificeert hypo-echoische regionen binnen een 3D dataset en geeft een automatische schatting van het volume.
- V-Scope: volume rendering applicatie binnen de I-Space. Het segmentatie algoritme groeit vanuit een punt binnen het te onderzoeken volume tot het een vooraf bepaalde drempelwaarde bereikt.

Bij deze studie werd gekozen voor een *in vitro* opzet en een *in vivo* opzet. Voor het *in vitro* gedeelte werden met water gevulde ballonnen in een testreservoir geplaatst, waarna 3D volumes bepaald werden. Vervolgens werd het vooraf vastgestelde volume vergeleken met de volumes die berekend werden door de verschillende technieken. Hierbij bleek SonoAVC standaard te kleine volumes te meten, terwijl de andere methoden ongeveer gelijkwaardig waren. Intra- en inter-onderzoeker variabiliteit waren goed voor alle technieken. Het in vivo gedeelte maakte gebruik van de dooierzakken van 24 zwangerschappen met een gemiddelde zwangerschapsduur van 9 weken (spreiding 6-11 weken). Hierbij bleek de ICC waarde voor alle technieken > 0,91 te zijn. V-Scope en VOCAL hadden de beste ICC met het kleinste betrouwbaarheidsinterval. Hieruit kunnen we concluderen dat van de 4 technieken alleen SonoAVC niet in alle gevallen nauwkeurige en betrouwbare volumes kon meten. De nieuw geïntroduceerde V-Scope applicatie bleek accuraat en betrouwbaar.

In **part 3** van dit proefschrift beschrijven we hoe kennis van embryogenese in vivo toe kan nemen door gebruik te maken van een stageringsysteem. In deze studie werd van embryo's gevisualiseerd in de I-Space, het Carnegie Stadium bepaald. Hierbij werd gebruik gemaakt van 19 IVF/ICSI zwangerschappen met zwangerschapsduren tussen de 7 en 10 weken. In totaal werden 48 3D volumes beoordeeld. De embryo's werden gestagieerd op basis van enkel externe morfologische kenmerken waarbij de nadruk lag op de ledemaatontwikkeling. Na het stageren werd de kop-stuitlengte

(CRL) gemeten. Het stadium en de lengte werd vervolgens vergeleken met de leeftijd gebaseerd op de punctiedatum en met de klassieke data uit de embryologie literatuur over de Carnegie collectie. De resultaten lieten zien dat het relatief eenvoudig was om de embryo's te stageren: door de dieptеperceptie in de I-Space gaat het bepalen van de positie en locatie van bijvoorbeeld de armen zeer natuurlijk. De bepaalde stadia bleken goed te corresponderen met de gemeten lengte. Echter, wanneer de leeftijd gebaseerd op de punctiedatum vergeleken werd met de leeftijd in de embryologieliteratuur voor de verschillende stadia bleek in 28 van de 48 gevallen het embryo voor te lopen in leeftijd. Het stadium werd dus eerder bereikt dan voorheen beschreven. Hier zijn meerdere verklaringen voor te bedenken. De studiepopulatie bestond uit IVF/ICSI zwangerschappen, waarbij eraan kan worden getwijfeld of deze zwangerschappen in alle opzichten als normaal kunnen worden beschouwd. Ook wordt in deze studie enkel naar externe morfologie gekeken. Echter, een andere verklaring zou kunnen zijn dat de Carnegie collectie bestaat uit embryo's na miskramen en ectopische zwangerschappen, welke dus niet volledig normaal hoeven te zijn. Tevens was de zwangerschapsduur (leeftijd) gebaseerd was op menstruatiedata, hetgeen ook niet volledig betrouwbaar is. Concluderend kan gesteld worden dat met behulp van de I-Space groei en ontwikkeling gedurende de embryogenese goed kan worden gevolgd. Door lengtemetingen te combineren met een stageringssysteem als het Carnegie systeem wordt een beter en completer beeld gekregen van het zich ontwikkelende embryo.

In **part 4** worden normatieve data in relatie tot zwangerschapsduur gepresenteerd voor verschillende biometrie metingen.
In de studie in **chapter 4.1** werden in een longitudinale studieopzet 3D metingen verricht bij 32 zwangerschappen met zwangerschapsduren tussen de 6 en 14 weken. In totaal werden 125 3D volumes geanalyseerd in de I-Space. Er werden groeicurven gemaakt van de volgende parameters: kop-stuitlengte (CRL), biparietale diameter (BPD), hoofdomtrek (HC), buikomtrek (AC), lengte van de arm, breedte van de schouder, breedte van de elleboog, breedte van de heupen en breedte van de knieën. De CRL, BPD en HC kon in meer dan 96% van alle patiënten gemeten worden. De AC in 78%. De breedte van schouder, elleboog, heup en knie in meer dan 95% en de lengte van de arm in 82%. De CRL curve werd vergeleken met de als eerste ter wereld gepubliceerde curve van Robinson en Fleming uit 1975 en deze bleek vrijwel identiek

te zijn. Geconcludeerd kan worden dat, met behulp van een innovatieve techniek als de I-Space, het opstellen van groeicurven van zowel bestaande parameters als nog niet eerder gemeten structuren goed haalbaar is. Door de dieptеperceptie is het mogelijk om metingen te verrichten in drie dimensies, waardoor een beter totaalbeeld ontstaat van de groei en ontwikkeling van het embryo in het eerste trimester.

In **chapter 4.2** is dezelfde studiepopulatie gebruikt als in chapter 4.1. Nu zijn echter metingen verricht van de lengte van de navelstreng, de lengte van de ductus vitellinus en de breedte van deze structuren bij de verschillende aanhechtingspunten. Ook is gekeken hoe de positie veranderd van de dooierzak ten opzichte van het embryo in het verloop van de tijd. Lengtemetingen van de navelstreng en ductus vitellinus kunnen alleen verricht worden wanneer van 3 dimensies gebruik gemaakt kan worden. In de I-Space kan gebruik gemaakt worden van een 'tracing-functie', waardoor ook structuren die erg gekronkeld zijn gemeten kunnen worden. De lengte van de navelstreng bleek in 55% te meten en bleek een nauwe relatie te vertonen met de zwangerschapsduur. De lengte van de ductus vitellinus kon gemeten worden in 42%. Er bleek geen relatie te zijn tussen de lengte van deze structuur en de zwangerschapsduur. Wanneer naar de positie van de dooierzak gekeken werd ten opzichte van het embryo bleek dat de dooierzak zich steeds verplaatst. Er leek geen sprake te zijn van een vaste richting. Wel kan hieruit geconcludeerd worden dat er 'iets' moet bewegen, bij een zwangerschapsduur waarbij in principe slechts zeer geringe bewegingen beschreven zijn. De navelstreng is vaak bestudeerd, maar doordat lengte voorheen niet meetbaar was gedurende de zwangerschap is ook nog niet vastgesteld of er een relatie is tussen de lengte van de navelstreng en bijvoorbeeld zwangerschapsuitkomst. Hetzelfde geldt voor de ductus vitellinus. Echter, aangezien deze structuren het embryo verbinden met de structuren waaruit zij voeding ontvangen (eerst de dooierzak, dan de placenta) lijkt het niet meer dan logisch dat ook deze structuren essentieel zijn voor de groei en ontwikkeling van het embryo.

In **part 5** wordt geëvalueerd wat de toepasbaarheid van deze nieuwe techniek is bij embryonale en foetale afwijkingen.
Chapter 5.1 beschrijft een casus van een tot de placenta beperkte trisomie 16 mozaïek. De patiënte werd reeds vanaf de 5^{e} week van de zwangerschap echoscopisch gezien

en ondanks een zekere termijn bleek de groei en ontwikkeling al zeer vroeg achter te lopen. Omdat bij de 10e week sprake bleek van een verdikte nekplooi (2.9mm) en de PAPP-A waarde extreem laag was, werd een vlokkentest en vervolgens een vruchtwaterpunctie verricht. De uitslagen toonden een normaal vrouwelijk karyogram, maar in de placenta bleek een mozaïek trisomie 16 aanwezig. De zwangerschap werd nauwkeurig vervolgd. Hoewel de groei steeds onder de p2.5 bleef waren verder geen aanwijzingen voor congenitale afwijkingen. De bevalling werd ingeleid op basis van een door de zwangerschap geïnduceerde hypertensie. Bij 37 weken zwangerschapsduur werd een meisje geboren met een geboortegewicht van 2010 gram (< *p*2.5). In het volgende jaar klom haar groei naar het 50e percentiel in de eerste 2 maanden en was er sprake van een normale ontwikkeling. Deze casus is bijzonder omdat niet eerder werd beschreven dat de groei al zo vroeg vertraagd kan zijn bij een trisomie 16 mozaïek in de placenta. Ook was niet eerder een relatie gelegd tussen een lage PAPP-A waarde en deze diagnose. Tevens werd duidelijk dat het enkel meten van de CRL er mogelijk toe kan leiden dat een eventuele groeivertraging niet ontdekt wordt doordat op basis van de CRL de zwangerschapstermijn bijgesteld wordt. Het belang van het bestuderen van embryogenese in verband met de gevolgen van eventuele afwijkingen gedurende deze periode voor het verdere verloop van de zwangerschap wordt door deze casus geïllustreerd. De I-Space heeft toegevoegde waarde doordat het deze embryogenese al vanaf vroeg in de zwangerschap duidelijk zichtbaar maakt.

In **chapter 5.2** wordt de I-Space gebruikt bij het evalueren van 4 patiënten waarbij echoscopisch werd vastgesteld dat er mogelijk sprake was van een ambigu genitaal. Deze diagnose was met 2D en 3D echoscopie gesteld, maar in alle gevallen bleef er onduidelijkheid over de precieze afwijking. De 3D echobeelden werden beoordeeld in de I-Space en gekeken werd of in de I-Space bepaald kon worden of het genitaal mannelijk, vrouwelijk dan wel ambigu was. De I-Space diagnose werd vergeleken met de 2D, 3D en post-partum diagnose. In alle 4 casus bleek de I-Space diagnose correct. In 3 casus bleek sprake van een vrouwelijk genitaal, waarbij 1 patiënt met een normaal vrouwelijk genitaal bij een XY karyogram. In 1 casus bleek sprake van een ambigu genitaal. In de I-Space was niet vast te stellen wat de afwijking precies inhield, maar dit bleek ook post-partum zeer moeizaam. Het meest opvallend was, dat in 2 casus op 2D echoscopie de diagnose ambigu genitaal was gesteld, met 3D

echoscopie sprake leek van hypertrofie van de clitoris, terwijl zowel in de I-Space als post-partum een normaal vrouwelijk genitaal werd gezien. Wel werd in de I-Space vastgesteld dat de labia minora wat vergroot leken, echter normaal passend bij de zwangerschapsduur. De I-Space heeft dus toegevoegde waarde bij het beoordelen van congelitale afwijkingen waarbij diepteperceptie helpt bij het inschatten van de positie en grootte van de verschillende structuren.

CONCLUSIES

In dit proefschrift wordt gebruik gemaakt van een innovatieve beeldvormende techniek bij het bestuderen van normale humane embryonale groei en ontwikkeling. De I-Space Virtual Reality systeem is uniek in zijn vermogen de derde dimensie zichtbaar te maken en om metingen te kunnen verrichten in deze derde dimensie. Aangezien de metingen betrouwbaar uitgevoerd blijken te kunnen worden konden nieuwe biometrie groeicurven werden opgesteld van zowel al bekende parameters als van metingen aan structuren die voorheen niet meetbaar waren met gangbare echoscopische technieken. Ook kunnen in de I-Space morfologische kenmerken als ledemaatontwikkeling duidelijk zichtbaar gemaakt worden, waardoor een stageringsysteem als het Carnegie systeem ook *in vivo* kan worden toegepast. Al deze mogelijkheden leiden ertoe dat er een completer beeld ontstaat van de groei en ontwikkeling van een embryo in het eerste trimester. Dit kan als basis dienen voor het vaststellen wat normaal is en wat abnormale groei is, waardoor de implicaties hiervan voor het verdere verloop van de zwangerschap eerder duidelijk zullen worden. Een volgende stap zal zijn om volumes te bepalen van het totale embryo, waarbij wederom vastgesteld dient te worden wat normaal is en wat niet.

Het Erasmus MC is voor zover wij weten het enige centrum ter wereld wat gebruik maakt van een dergelijk systeem voor het bestuderen van medisch beeldmateriaal. De versie zoals gebruikt in deze studie, waarbij een volledige kamer gebruikt wordt, kan echter gereduceerd worden tot een desktop applicatie, waardoor in principe elke dokter medisch 3D beeldmateriaal op zijn eigen bureau in alle drie de dimensies kan beoordelen. Dit zal het counselen van de patiënt een stap verder brengen, omdat

bijvoorbeeld embryonale en foetale anatomie ook voor niet-medici dan zeer duidelijk zichtbaar is.

De I-Space biedt in zijn huidige vorm een opzienbarende, nieuwe manier om de groei en ontwikkeling van embryogenese te bestuderen. Dit zal uiteindelijk leiden tot een verschuiving van prenatale diagnostiek in vooral het 2e en 3e trimester van de zwangerschap naar ook het 1e trimester. Hierdoor kan deze 'Virtual Embryoscopy' ertoe bijdragen dat embryonale geneeskunde een stap dichterbij gekomen is.

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- Bridge meetings on Bio Informatics. Erasmus MC Rotterdam. Molecular Medicine Research School. '3D Ultrasound and visual representation of early pregnancies'. (12 juni 2007)

Oral poster presentations:

17th World Congress on Ultrasound in Obstetrics and Gynaecology (ISUOG). Florence, 2007.

- Assigning Carnegie Stages to embryos visualized with 3D ultrasound using a virtual reality system.
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DANKWOORD

Het meest gelezen deel van een proefschrift...
Aan de totstandkoming van een proefschrift werken veel verschillende mensen mee, op zeer uiteenlopende manieren. De patiënten die de moeite hebben genomen om wekelijks een echo te laten maken vroeg in hun zwangerschap ben ik zeer erkentelijk voor hun medewerking.
Daarnaast wil ik in het bijzonder de volgende mensen bedanken voor hun hulp, moeite en inzet:

Allereerst de promotoren. Prof.dr. Steegers. Beste Eric, in 2002 ben ik als 4e-jaars student eens bij je binnengelopen om te vragen of er naast de 'bekende' afstudeeronderzoeken niet ook nog andere projecten waren op de afdeling Verloskunde en Vrouwenziekten. Je adviseerde me toen om vooral een onderzoek te kiezen waar eventueel een publicatie uit kon voortkomen. Dit advies heb ik ter harte genomen en heeft uiteindelijk geleid tot de aanzet voor dit proefschrift. Het geeft maar weer aan hoe belangrijk een keuzeonderzoek kan zijn voor je verdere carrière! Ik ben nog steeds erg blij met de door jou geboden kans om aan dit onderzoek te beginnen. De vraagstelling was in het begin wat breed (wat kunnen we überhaupt met dit apparaat) en ook technische problemen (waterschade) bleven ons niet bespaard. De volgende promovendus is echter al bezig, dus er is een goede lijn ingezet. Ik heb bewondering voor de inspirerende manier waarop jij met wetenschap bezig bent.
Prof.dr. van de Spek. Beste Peter, hoewel wij elkaar niet zo frequent zagen en spraken was je altijd op de achtergrond aanwezig. En jij bent natuurlijk een van de drijvende krachten geweest achter het naar Rotterdam halen van de I-Space! Bedankt voor alle mogelijkheden die de afdeling Bioinformatica geboden heeft om dit onderzoek tot stand te brengen.

De copromotoren. Laat ik beginnen met Anton Koning. Vanaf het begin was je betrokken bij dit project. Wat zul je af en toe hebben moeten zuchten om mijn ongeduld en onbegrip als het om technische problemen ging. Heel veel dank voor je luisterend oor, je adviezen en tips bij het uitwerken van de verschillende projecten en je snelle

correcties van mijn manuscripten.
Niek Exalto, hartelijk dank voor de goede ideeën en de begeleiding. Ik weet dat je je af en toe blauw gelachen hebt in de trein om mijn soms bijzondere woordspelingen. Het is toch nog redelijk goed gekomen!

Ook een woord van dank aan Christoph Brezinka. Beste Christoph, ik ben blij dat je je draai weer gevonden hebt in Oostenrijk. Je was altijd erg enthousiast over de I-Space en alle mogelijkheden die het bood. Bedankt voor je hulp en begeleiding in het eerste deel van mijn promotietraject.

Wim Hop, bedankt voor je hulp met de statistische analyses. Je hebt de meest bijzondere vorm van papiermanagement die ik ooit heb meegemaakt!
Jolanda Claessens, bedankt voor alle hand en span diensten die je in de afgelopen tijd voor me hebt verricht, je hebt me er enorm mee geholpen!

De afdeling Prenatale Geneeskunde wil ik van harte danken voor hun medewerking aan (de voorbereidingen voor) dit onderzoek. Vooral Irene Groenenberg ben ik zeer erkentelijk voor alles wat ze voor dit onderzoek betekend heeft. Een bijzonder woord van dank aan Els Grijseels, het hart van de afdeling! Lieve Els, dank voor je luisterend oor, je adviezen en vooral je oprechte aandacht en belangstelling! Peter van Heesch, kamergenoot op de meest druk bezochte kamer van het He-gebouw: ik vond het erg gezellig om een kamer met je te delen. Heel veel succes met je eigen onderzoek!

De afdeling Bioinformatica: allereerst natuurlijk Lennard Goedknegt. Vooropgesteld dat het altijd erg gezellig was, wil ik je enorm bedanken voor je hulp en moeite bij het maken van de voorkant van dit proefschrift en voor het realiseren van de anaglyf plaatjes! Ronald Nanninga, bedankt voor de mogelijkheid om dit proefschrift met brilletjes een derde dimensie te geven. Mirjam van den Hout - van Vroonhoven: bedankt voor je hulp met al die ingewikkelde formules.

En dan de sectie onderzoekers.... Natuurlijk wil ik jullie allemaal bedanken voor jullie gezelligheid, aanmoediging en bereidheid om naar mijn toch-wat-ver-van-jullie-bed-sores te luisteren. Na een wat eenzame start van mijn onderzoek was het een verademing

om gezamenlijk te gaan lunchen en buiten ijsjes te nuttigen. Jullie belangstelling tijdens mijn wat gecompliceerde zwangerschap heeft me erg veel goed gedaan! Heel veel succes allemaal met jullie eigen onderzoek en carrière!
In het bijzonder aandacht voor Melek Rousian, mijn 'opvolgster'. Erg gezellig dat je het laatste jaar het team bent komen versterken en veel succes met de voortzetting van dit project!

De afdeling huisartsgeneeskunde. Natuurlijk alle groepsgenoten en begeleiders, maar vooral mijn huidige huisartsopleider Marco Neeteson en Yvonne, Gabrielle, Mariëtte en Pascale. Jullie weten gelukkig al dat ik het erg naar mijn zin heb bij jullie in de praktijk. Bedankt voor de flexibiliteit om ook onderzoeksactiviteiten te kunnen verrichten.

Mijn paranimfen wil ik bedanken dat ze het aandurven om naast me te komen staan op dit toch wel spannende moment. Lieve Annemieke, we hebben al veel met elkaar beleefd. Vanaf dag 2 van de studie geneeskunde delen we lief en leed met elkaar en ik hoop dat we dit nog lang vol gaan houden. Lieve Elly, ik heb bewondering voor alles wat jij in je leven bereikt hebt. Ik voel me gesteund met jou als sectie van de 'koude kant'!

Lieve Marjon, uitgerekend nu ben je natuurlijk uitgerekend. Bijzonder dat we dezelfde richting ingegaan zijn met zo'n verschillend voortraject. Erg leuk dat het contact zo goed blijft!

Alle andere familie en vrienden: bedankt voor jullie aanwezigheid, steun en begrip voor dit voor jullie toch vaak onbegrijpelijke onderzoek. Helma, bedankt voor de gezelligheid tijdens de carpoolritjes en alle andere momenten natuurlijk, jammer dat je er niet bij kan zijn! Marina en Peter, zonder goede oppas was er van dit onderzoek niets meer terecht gekomen. Bedankt voor het warme thuis dat jullie Lydia bieden als wij aan het werk zijn. Oma Dikkeboom, u had vast niet gedacht dat u dit nog mee zou maken, maar in 99 jaar kan veel gebeuren zoals u ziet! Pa Verwoerd, erg leuk dat u al eens in de I-Space naar onze toen nog ongeboren Lydia heeft gekeken. Ma Dikkeboom, wat jammer dat pa er niet meer is om dit mee te maken, hij zou zo trots

geweest zijn! Ik heb heel veel bewondering voor hoe jij het leven weer opgepakt hebt. Bedankt voor je steun en alle mogelijkheden die jij en pa mij geboden hebben.

Als laatste natuurlijk mijn grote liefde Gijsbert. Lieve Gijs, wie had ooit kunnen denken dat een bijbaantje in een ziekenhuis zou uitlopen op een geëmancipeerde doktersroman? Dat hebben we samen toch maar mooi van de grond getild! Dat ik blij met je ben en ontzettend veel van je houd moge duidelijk zijn. Bijzonder hoe Lydia al in menig internationaal tijdschrift staat en nu ook ons tweede kindje op zo'n mooie manier geportretteerd is. Ik voel me bevoorrecht om moeder en echtgenote te mogen zijn in dit gezin!

ABOUT THE AUTHOR

Christine Verwoerd-Dikkeboom was born in Papendrecht on September 11th 1980. She passed secondary school at 'De Lage Waard' secondary school in Papendrecht, where she graduated in 1998. That year she started to study medicine at the Erasmus MC, University Medical Centre in Rotterdam. During the third year of her study she did an internship at Dormaa Presbyterian Hospital in Dormaa Ahenkro, Ghana. In the fourth year she performed a scientific research project at the Department of Obstetrics and Gynaecology, division of Prenatal Medicine under the supervision of Professor Wladimiroff. She graduated cum laude from Medical School in 2004. Subsequently, she worked as a resident at the department of Obstetrics and Gynaecology at the IJsselland Ziekenhuis in Capelle a/d IJssel. In 2005 she started working at the Erasmus MC, University Medical Centre in Rotterdam. First, she worked as a junior resident at the Department of Obstetrics and Gynaecology. In September 2005 she started with the PhD research described in this thesis under the supervision of Professors Steegers and Van der Spek. She was awarded the Prof. Dr. Juriy Wladimiroff Research award in 2008. In September 2008 she started her training as a general health practitioner at the Erasmus MC, University Medical Centre in Rotterdam.
She is married to Gijsbert Verwoerd. They are the proud parents of daughter Lydia and are expecting their second child in August 2009.