First trimester size charts of embryonic brain structures

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Abstract

Study question: Can reliable size charts of human embryonic brain structures be created from three-dimensional ultrasound (3D US) visualisations?

Summary answer: Reliable size charts of human embryonic brain structures can be created from high-quality images.

What is known already: Previous studies on the visualisation of both the cavities and the walls of the brain compartments were performed using 2D-US, 3D US or invasive intrauterine sonography. However, the walls of the diencephalon, mesencephalon and telencephalon have not been measured non-invasively before. Last-decade improvements in transvaginal ultrasound techniques allow a better visualisation and offer the tools to measure these human embryonic brain structures with precision.

Study design, size, duration: This study is embedded in a prospective periconceptional cohort study. A total of 141 pregnancies were included before the sixth week of gestation and were monitored until delivery to assess complications and adverse outcomes.

Participants/materials, setting, methods: For the analysis of embryonic growth, 596 3D US scans encompassing the entire embryo were obtained from 106 singleton non-malformed live birth pregnancies between 7^{+0} and 12^{+6} weeks' gestational age (GA). Using 4D View (3D software) the measured embryonic brain structures comprised thickness of the diencephalon, mesencephalon and telencephalon, and the total diameter of the diencephalon and mesencephalon.

Main results and the role of chance: Of 596 3D scans, 161 (27%) high-quality scans of 79 pregnancies were eligible for analysis. The reliability of all embryonic brain structure measurements, based on the intra-class correlation coefficients (ICCs) (all above 0.98), was excellent. Bland–Altman plots showed moderate agreement for measurements of the telencephalon,

but for all other measurements the agreement was good. Size charts were constructed according to crown-rump length (CRL).

Limitations, reasons for caution: The percentage of high-quality scans suitable for analysis of these brain structures was low (27%).

Wider implications of the findings: The size charts of human embryonic brain structures can be used to study normal and abnormal development of brain development in future. Also, the effects of periconceptional maternal exposures, such as folic acid supplement use and smoking, on human embryonic brain development can be a topic of future research.

Introduction

The human brain is complex. During its rapid development it undergoes remarkable anatomical changes throughout pregnancy, even in the early first trimester of pregnancy. The detection of brain abnormalities by ultrasonography (US) during early pregnancy is therefore a challenge. In the first trimester the majority of structural anomalies detected by US comprise severe and lethal defects.[1] Recent studies suggest that periconceptional maternal exposures can affect the development of the human embryonic and foetal brain;[2, 3] however, the actual influence of these exposures remains unknown. Therefore, longitudinal studies investigating the growth of human embryonic brain structures might provide new insights.

In many countries, the structural anomaly scan is performed in the second trimester of pregnancy. However, first trimester detection of brain abnormalities has advantages compared with the detection in the second trimester of pregnancy, for example, it provides women more time for counselling and decision-making.[4] Early prenatal diagnosis requires, however, a profound knowledge of the anatomy of the developing human embryonic brain.

A first step is therefore to create size charts of human embryonic brain structures according to protocol, with validated non-invasive ultrasound techniques. During human embryonic brain development, the prosencephalon, mesencephalon and rhombencephalon can be distinguished at 5.5 weeks' gestational age (GA), as calculated from the first day of the last menstrual period (LMP). Between 6 and 8 weeks' GA, the prosencephalon divides into the telencephalon and diencephalon and the rhombencephalon divides into the metencephalon and myelencephalon.[5, 6]

Since the early 1990s, the availability of transvaginal high-velocity transducers with large apertures and/or annular array technology has offered the opportunity to perform studies on the visualisation of embryonic development with 2D-US and 3D US.[7-10] During the last decade, the human embryonic brain in particular has been a topic of interest in multiple studies.[1, 11, 12] Blaas et al. created growth trajectories from 7 weeks' GA onwards for the hemispheres of the telencephalon and for the fluid-filled vesicles of the diencephalon and mesencephalon.[13, 14] Tanaka et al. and Tanaka and Hata studied embryonic brain development using an intrauterine transducer for volume analysis of the fluid-filled brain cavities between 7 and 10 weeks' GA and for measurement of the embryonic brain mantle thickness between 6 and 11 weeks' GA.[11, 12]

The improvements in transvaginal ultrasound techniques over the last decade allow a better visualisation and offer the tools to measure these human embryonic brain structures with precision. Therefore, the aim of the present study was to create first trimester size charts of human embryonic brain structures in singleton non-malformed live birth pregnancies, using three-dimensional ultrasound (3D US) visualisation. We also determined the reliability of all measurements.

Study population and methods

Study population

This study is embedded in the Rotterdam Predict Study, a prospective periconceptional cohort study investigating the influence of gene–environment interactions and epigenetic mechanisms on reproductive parameters and (extra) embryonic and pregnancy outcome at the Erasmus MC University Hospital in Rotterdam, the Netherlands. Enrolment of pregnant women over 18 years of age, from the outpatient clinic of the Department of Obstetrics and Gynaecology, took place before the sixth week of gestation. In the Rotterdam Predict study, 3D ultrasound examinations were performed weekly between 6^{+0} and 12^{+6} weeks' GA, during which a series of 3D sweeps was obtained, encompassing the whole embryo.

In total, 141 pregnancies were monitored until delivery to assess complications and adverse outcomes. Non-participating patients/dropouts (n = 3), miscarriages (n = 16), terminations of pregnancy (n = 2), multiple pregnancies (n = 3), intrauterine foetal deaths/neonatal deaths (n = 2), congenital anomalies (n = 3) and pregnancies dated on crown-rump length (CRL) (n = 6) were excluded, leaving a total of 106 pregnancies eligible for the study.

GA was calculated according to the LMP, and in cases of an irregular menstrual cycle, unknown LMP or a discrepancy of >3 days, GA was determined by the CRL measurements performed in the first trimester. In case of conception by means of IVF or ISCI, the conception date was used to calculate the GA.

All pregnant patients gave written informed consent before participation. Approval of the study was obtained from the Central Committee for Human Research in The Hague and the local Medical Ethical and Institutional Review Board of the Erasmus MC, University Medical Centre in Rotterdam.

Measurements

The measurements were performed using a 4.5–11.9-MHz transvaginal probe of the Voluson E8 system (GE Medical Systems, Zipf, Austria). The stored 3D volumes were displayed in the orthogonal multiplanar mode using specialised 3D software (4D View, version 5.0, GE Medical Systems). The quality of the 3D volumes was evaluated off-line and only volumes without motion artefacts and with a clear demarcation of the borders of the brain structures were accepted for further analysis.

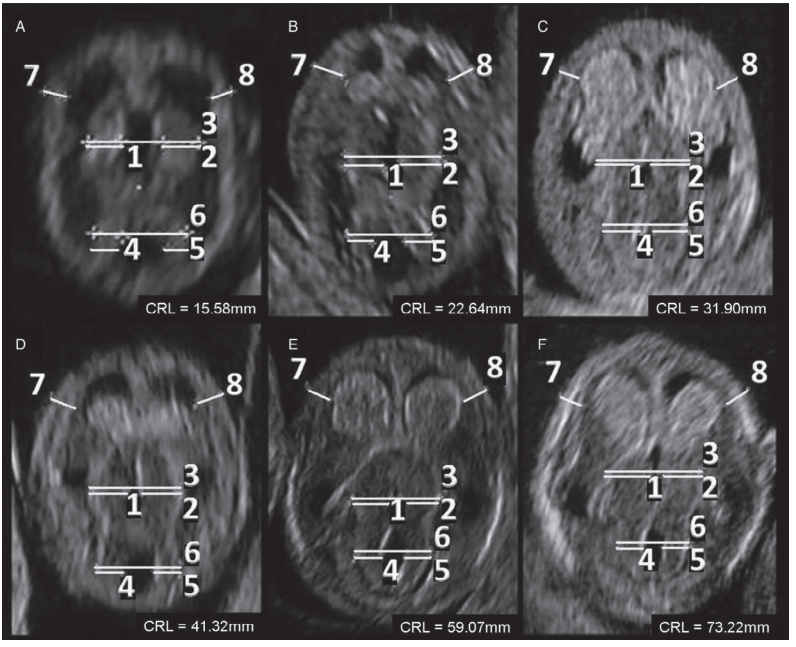

Figure 1: axial sections of the embryonic brain of embryos at 7–12 weeks' GA (A–F). The diencephalon thickness left (DTL; 1), diencephalon thickness right (DTR; 2), diencephalon total diameter (DTD; 3), mesencephalon thickness left (MTL; 4), mesencephalon thickness right (MTR; 5), mesencephalon total diameter (MTD; 6), telencephalon thickness left (TTL; 7) and telencephalon thickness right (TTR; 8) are measured using the 'distance two points' function. The respective CRL is shown in the image.

The following embryonic brain parameters were determined: left and right diencephalon thicknesses (DTL and DTR) and total diameter (DTD), left and right mesencephalon thicknesses (MTL and MTR) and total diameter (MTD) and left and right telencephalon thicknesses (TTL and TTR). According to a newly designed protocol, all measurements were performed in an axial or coronal section through the head of the embryo, with visualisation of the cavity of the diencephalon and mesencephalon (**Figure 1**). Measurements of the diencephalon and mesencephalon were performed by placing the callipers on the outer borders of these brain structures and the maximal thickness was measured. In the same axial plane also the cleavage of the telencephalic cavity, containing the choroid plexus, was visualised. In a line 45° from the longitudinal axis of the axial plane of the embryonic head, the diameters of the left and right lateral wall of the hemispheres of the telencephalon were measured.

All measurements in the same volume were independently repeated three times and the mean values were used for analysis. To assess intra- and inter-observer reproducibility, a randomly selected subset of 30 volumes from 30 randomly selected pregnancies was measured a second time by the same examiner (M.G.) and independently by another examiner (H.B.). For this purpose, five volumes were selected of each gestational week. Both examiners were blinded to the results of each other's measurements, each volume was unadjusted (raw data) and each measurement required manual adjustment of the volume to obtain the right image.

Statistical analysis

Using SPSS (Release 17.0 for Windows, IBM, USA), intra-class correlation coefficients (ICCs) were calculated to quantify the inter- and intra-observer reliability of the measurements. To assess the agreement between and within the two examiners Bland–Altman plots were created. The mean difference and mean percentage difference with corresponding 95% limits of agreement [mean (percentage) difference ± 1.96 SD] were calculated.[15]

We analysed the data cross-sectionally. We used the GAMLSS method[16] as implemented in R (version 2.15) to estimate percentile curves (P5, P50 and P95). We used a model that uses a spline to describe the trend, as a function of CRL, by P-splines. In all cases the logarithm (to base 10) of the measurements is taken as the dependent variable and a normal distribution of the variations around the trend is assumed. The logarithm of the standard deviation is allowed to change linearly with the CRL. The GAMLSS model specification formula is 'gamlss(*y* ~ps(crl), sigma.formula = ~ crl, family = 'NO')'. Here '*y*' is the logarithm of the size of the brain structure to be modelled.

Results

The mean age of the 106 pregnant patients was 32.4 years (SD: 4.91 years) and the mean BMI was 24.6 kg/m^2 (range: 19.1–38.3 kg/m^2). Fifty-five healthy girls and 51 healthy boys were born at a median GA of 39^{+4} weeks' GA (range: 26^{+5}–42^{+0} weeks' GA) with a median birthweight of 3378 g (range: 450–4700 g). A non-response analysis, comparing the characteristics between the pregnancies with (*n* = 79) and without measurable images (*n* = 27), did not show any significant differences. In gestational weeks 8

	Diencephalon	Mesencephalon	Telencephalon
Number of images	596	596	596
Number of measurements (%)	161 (27)	152 (26)	133 (22)
Number of patients with ≥1 measurement (%)	79 (75)	77 (73)	75 (71)
1 measurement (%)	40.5	41.6	52.0
2 measurements (%)	29.1	29.9	24.0
3 measurements (%)	16.5	18.2	10.6
4 measurements (%)	11.4	10.4	13.4
5 measurements (%)	2.5	0	0

Table 1: ultrasound data per brain structure for all included patients in the growth analysis (n = 106)

and 11, the embryonic brain structures of smaller embryos, according to CRL, were measured more often compared with bigger embryos ($P < 0.05$).

A total number of 596 3D US scans between 7^{+0} and 12^{+6} were performed in 106 patients, with an average number of 5.6 scans per patient. Measurements could be performed in 161 scans (27%) in 79 patients (75%) (**Table 1**). As an example, **Figure 3** shows all measurements.

Table 2 depicts the embryonic brain structure measurements per week GA with the corresponding mean value and SD value. We were able to measure the mesencephalon, diencephalon and telencephalon as early as 7^{+1} weeks' GA.

Ultrasound characteristics	7 weeks' GA Mean (SD)	8 weeks' GA Mean (SD)	9 weeks' GA Mean (SD)	10 weeks' GA Mean (SD)	11 weeks' GA Mean (SD)	12 weeks' GA Mean (SD)
CRL (mm)	12.25 (3.27)	18.42 (3.46)	25.93 (4.30)	36.17 (5.90)	48.46 (7.13)	61.54 (7.49)
N	15/95	36/97	34/100	34/104	25/103	17/97
DTL (mm)	0.85 (0.19)	1.21 (0.33)	1.69 (0.28)	2.34 (0.42)	3.24 (0.48)	3.90 (0.57)
DTR (mm)	0.83 (0.18)	1.23 (0.33)	1.68 (0.26)	2.29 (0.39)	3.19 (0.45)	3.88 (0.57)
DTD (mm)	2.85 (0.39)	3.41 (0.76)	4.37 (0.50)	5.64 (0.75)	7.20 (0.93)	8.61 (1.08)
MTL (mm)	0.74 (0.19)	0.92 (0.22)	1.24 (0.26)	1.66 (0.31)	2.07 (0.20)	2.40 (0.29)
MTR (mm)	0.77 (0.14)	0.95 (0.21)	1.23 (0.29)	1.71 (0.29)	2.08 (0.23)	2.34 (0.26)
MTD (mm)	2.52 (0.47)	3.21 (0.60)	4.08 (0.52)	5.16 (0.56)	6.00 (0.39)	6.42 (0.37)
TTL (mm)	0.58 (0.12)	0.76 (0.22)	1.15 (0.26)	1.38 (0.21)	1.76 (0.32)	1.88 (0.31)
TTR (mm)	0.58 (0.07)	0.80 (0.22)	1.17 (0.27)	1.49 (0.28)	1.82 (0.29)	2.05 (0.35)

Table 2: mean estimations with the corresponding SD and number (N) of images per complete gestational week
CRL, crown-rump length; DTL, diencephalon thickness left; DTR, diencephalon thickness right; DTD, diencephalon total diameter; MTL, mesencephalon thickness left; MTR, mesencephalon thickness right; MTD, mesencephalon total diameter; TTL, telencephalon thickness left; TTR, telencephalon thickness right; SD, standard deviation

The mean (percentage) difference, the limits of agreement and the ICC values for the intra- and inter-observer reproducibility of the 3D measurements are displayed in **Table 3**. All ICC values were >0.98, representing very good reliability between the measurements. The Bland–Altman statistics showed good agreement between the measurements for all parameters except

Agreement	Mean difference (mm)	95% CI mean difference (mm)	95% limits of agreement (mm)	Mean difference (%)	95% limits of agreement (%)	ICC	95% CI ICC
Intra-observer							
DTL	−0.01	−0.03 to 0.00	−0.09 to 0.07	−0.52	−6.48 to 5.45	0.999	0.999–1.000
DTR	−0.02	−0.06 to 0.01	−0.21 to 0.17	−0.72	−12.74 to 11.31	0.996	0.993–0.998
DTD	−0.02	−0.05 to 0.01	−0.17 to 0.13	0.34	−3.33 to 2.65	0.999	0.999–1.000
MTL	0.02	−0.01 to 0.05	−0.14 to 0.18	1.75	−9.90 to 13.41	0.993	0.986–0.997
MTR	0.00	−0.03 to 0.33	−0.18 to 0.18	−0.31	−11.23 to 10.61	0.991	0.982–0.996
MTD	−0.01	−0.05 to 0.03	−0.22 to 0.21	0.00	−4.45 to 4.46	0.997	0.995–0.999
TTL	−0.02	−0.06 to 0.01	−0.22 to 0.17	−1.82	−18.45 to 14.82	0.986	0.971–0.993
TTR	−0.01	−0.04 to 0.02	−0.19 to 0.17	0.29	−17.51 to 18.09	0.990	0.980–0.995
Inter-observer							
DTL	−0.01	−0.03 to 0.01	−0.12 to 0.11	−0.62	−7.46 to 6.22	0.999	0.998–0.999
DTR	−0.03	−0.06 to 0.00	−0.19 to 0.14	−0.95	−13.00 to 11.09	0.997	0.994–0.999
DTD	0.04	−0.00 to 0.08	−0.19 to 0.27	−0.74	−3.60 to 5.07	0.998	0.996–0.999
MTL	−0.03	−0.06 to 0.00	−0.20 to 0.14	−2.74	−18.39 to 12.91	0.992	0.982–0.996
MTR	0.00	−0.04 to 0.04	−0.21 to 0.21	−1.15	−18.50 to 16.20	0.988	0.974–0.994
MTD	0.05	−0.01 to 0.11	−0.27 to 0.37	1.66	−7.18 to 10.50	0.994	0.987–0.997
TTL	−0.05	−0.09 to −0.01	−0.26 to 0.16	−6.87	−31.39 to 17.64	0.980	0.950–0.991
TTR	0.03	−0.01 to 0.07	−0.20 to 0.26	0.45	−20.28 to 21.19	0.982	0.963–0.992

Table 3: mean difference with the corresponding CI, the limits of agreement and the ICCs with the corresponding 95% CI
DTL, diencephalon thickness left; DTR, diencephalon thickness right; DTD, diencephalon total diameter; MTL, mesencephalon thickness left; MTR, mesencephalon thickness right; MTD, mesencephalon total diameter; TTL, telencephalon thickness left; TTR, telencephalon thickness right; CI, confidence interval; ICC, intra-class correlation coefficient

the telencephalon thickness. Measurements of the total diameters of the diencephalon and mesencephalon showed better agreement compared with those of the individual left and right thicknesses.

There was no significant difference between the left and right thicknesses of the mesencephalon (mean difference = −0.02 mm, range −0.04–0.00 mm; P = 0.068). However, there was a small statistically significant difference between the left and right thicknesses of the diencephalon (mean difference = 0.02 mm, range 0.00–0.04 mm; P = 0.013) and between the left and right thicknesses of the telencephalon (mean difference = −0.07 mm, range −0.10 to −0.05 mm; P < 0.0001). Since we considered these differences too small to be clinically significant, we averaged left and right data to create the size charts of the embryonic brain structures.

The size charts of the embryonic brain structures are shown in **Figure 2**.

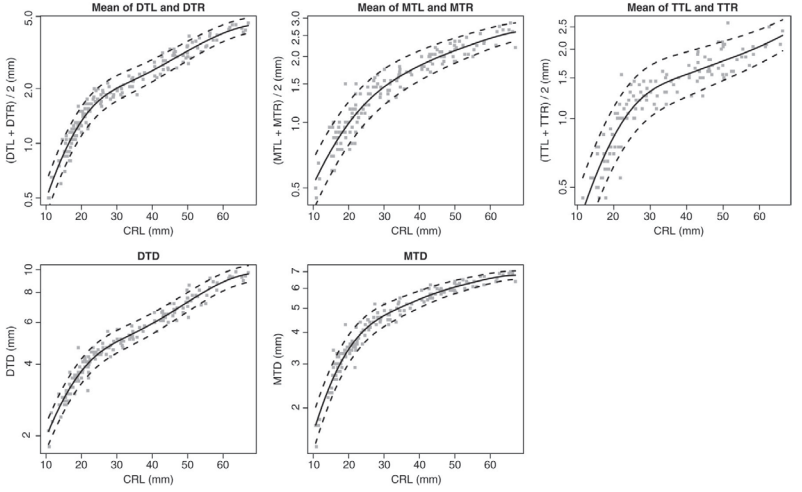

Figure 2: the embryonic brain structures against the CRL. The lines represent the p5, p50 and p95 reference lines. DTL, diencephalon thickness left; DTR, diencephalon thickness right; DTD, diencephalon total diameter; MTL, mesencephalon thickness left; MTR, mesencephalon thickness right; MTD, mesencephalon total diameter; TTL, telencephalon thickness left; TTR, telencephalon thickness right.

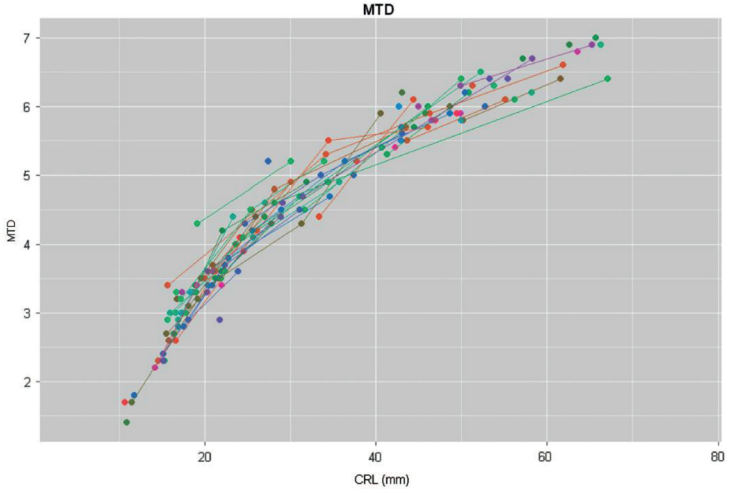

Figure 3: the raw longitudinal representation of all measurements of the mesencephalon total diameter (MTD) against the crown-rump length (CRL, with lines connecting pregnancies with multiple observations.

Discussion

This explorative study shows that the use of a high-frequency transvaginal transducer allows for accurate and reproducible measurements of embryonic brain structures on 3D data sets from 7 weeks' GA onwards, namely the diencephalon, mesencephalon and telencephalon, to create size charts.

A good reliability was found for intra-observer and inter-observer measurements of all brain parameters. However, measurements of the total diameters of the diencephalon and mesencephalon showed a better agreement compared with those of the individual left and right thicknesses. This may be due to a better visualisation of the outer borders compared with the inner borders of the diencephalon and mesencephalon. The wider limits of agreement found for the telencephalon thickness can be explained by the poor

demarcation of the borders of the telencephalon and the large variability in size with minimal adjustment of the scan. In addition, because of the low success rate of the measurements of the telencephalon (22%), the measurement errors reduce the precision and reliability of these measurements. We point out that our assessment of reproducibility is limited. It is based on the same stored ultrasound images, evaluated by different observers. For a full assessment, it would be necessary to collect and measure repeated (suitable) images of the same embryos. Such data are not available. In addition, in this study each volume required manual adjustment to obtain the right image for measurement, which implies that also image reading was part of the reproducibility that has been tested.

Blaas and Eik-Nes described human brain development in the first trimester of pregnancy.[1] 3D US reconstructions of the embryo and the embryonic brain (cavities) in particular have been available in the literature since the early 1990s.[7, 9, 10, 17] Due to improvements in ultrasound equipment and their increasingly widespread availability, the embryonic and foetal brain can be investigated in much larger populations. Measurements of developing embryonic brain structures allow us to create size charts, which may enable differentiation between anatomically normal and abnormal human brain development in early pregnancy.

One will not expect that measurements of the brain mantle are helpful in the diagnosis of all congenital malformations that occur in the embryonic period, such as holoprosencephaly and neural tube defects. In these pathological conditions, diagnosis is made by identification of gross anatomical derangements. However, in cases of ventriculomegaly, one could argue that measurements of the total diameters of the mesencephalon and diencephalon are more easily performed than measurements of the cavities due to better demarcation of the outer borders in comparison with the inner borders of the brain mantle. Our standardised cross sections of the embryonic brain allow reliable measurements of total mesencephalon and diencephalon diameters.

Several studies have been performed measuring embryonic brain cavities.[11, 13, 18] When subtracting the diameters of the walls from the total diameters, the widths of the brain cavities are obtained. Both the widths of the cavities of the diencephalon and the mesencephalon in this study show virtually identical values from 7 to 12 weeks compared with those measured by Blaas et al.[13]

Tanaka and Hata performed measurements of the walls of the brain vesicles, diencephalon, mesencephalon, metencephalon and telencephalon using 2D intrauterine sonography. We have measured slightly greater thicknesses of the diencephalon, mesencephalon and telencephalon, which may be explained by a somewhat different measurement method. In their study, 71 scans were used when compared with 161 scans in our study. The relatively lower feasibility can be explained by the use of a 20 MHz intrauterine transducer, a technique that cannot be implemented in daily practice. In comparison with the use of our transvaginal transducer, transducer movement is less limited, allowing a better visualisation of the embryonic structures. Other differences are that their patients were about to undergo therapeutic termination of pregnancy, foetal abnormalities could not be ruled out, and the measurements were performed one sided which suggests a less strict selection of scans.

In our study, we not only measured both right and left thicknesses of the diencephalon and mesencephalon but also measured the right and left thicknesses of the telencephalon. We found a slight asymmetry in the thicknesses of the diencephalon and the telencephalon. An explanation could be the 3D acquisition and measurement biases. Another explanation is that brain asymmetry (like asymmetry of the lateral ventricles in normal foetuses) is already present in the embryo as a normal physiological variation. For now, we pooled the data of the left and right thicknesses of the brain structures, because of the lack of clinical significance of these small differences. This reduced the measurement noise appreciably, increasing precision and validity of the size charts of the embryonic brain structures.

There are several explanations for the low percentage (27%) of scans suitable for analysis of these embryonic brain structures. Although high-frequency transvaginal transducers result in a better resolution and allow for more magnification, the quality of the scans declines rapidly with increasing depth. Movement artefacts due to embryonic or maternal movements were another important reason for exclusion of 3D volumes. Finally, the present study was embedded in a prospective cohort study. 3D US sweeps encompassing the whole embryo, to measure the embryonic volume for example, were obtained weekly without specific brain-targeted imaging; 3D US examinations dedicated to the embryonic brain (structures) would have undoubtedly leaded to a higher success rate. However, when accounting for the number of weekly measurements per pregnancy, the average percentage of successful measurements per number of scans per pregnancy was >33% for all measurements. The non-response analysis comparing the characteristics between the pregnancies that did and did not provide measurable scans did not show any significant differences. Therefore, selection bias is not very likely. Surprisingly, the embryonic brain structures of smaller embryos could be measured more often. Since neither the size of the embryo nor the characteristics of the pregnancies can explain the cause of data loss, motion artefacts or other reasons for low-quality images become more likely.

The average number of successive measurements is only 2. This implies that the current study contains both longitudinal and cross-sectional data, and is therefore a mixed study. Since in 41–52% of pregnancies only one measurement could be performed and we are not interested in confidence intervals in our size charts at this moment, we chose to carry out a cross-sectional analysis in which we described the trends and spread in the study population of this observational study. A limitation of this type of analysis is that we handled the data as being cross-sectional, although for a (small) fraction of the embryos multiple measurements were included. Hence, not all data points were independent and our charts should be interpreted carefully.

In all graphs we see a 'bump', being more prominent in the graphs of the diencephalon and telencephalon. This corresponds to a GA between 9 and 10 weeks and can be explained by a relatively increased growth in this period due to the transfer of histiotrophic to haemotrophic nutrition of the embryo.[19, 20] In our periconception cohort study, more research onto this phenomenon is being carried out.

In conclusion, it was feasible to create reliable size charts of human embryonic brain structures, even with the limited eligibility of scans. In high-quality images, the reliability of embryonic brain structure measurements is excellent. The size charts of human embryonic brain structures can be used to study normal and abnormal brain development in future. Also, the effects of periconceptional maternal exposures, such as folic acid supplement use and smoking on human embryonic brain development can be a topic of future research. Multivariate analyses will allow us to come to a better understanding of very early human brain development.

References

1. Blaas HG, Eik-Nes SH. Sonoembryology and early prenatal diagnosis of neural anomalies. Prenat Diagn. 2009;29(4):312-25.

2. Thompson BL, Levitt P, Stanwood GD. Prenatal exposure to drugs: effects on brain development and implications for policy and education. Nat Rev Neurosci. 2009;10(4):303-12.

3. Willford J, Day R, Aizenstein H, Day N. Caudate asymmetry: a neurobiological marker of moderate prenatal alcohol exposure in young adults. Neurotoxicol Teratol. 2010;32(6):589-94.

4. Fong KW, Toi A, Salem S, Hornberger LK, Chitayat D, Keating SJ, et al. Detection of fetal structural abnormalities with US during early pregnancy. Radiographics. 2004;24(1):157-74.

5. O'Rahilly R, Muller F. Significant features in the early prenatal development of the human brain. Ann Anat. 2008;190(2):105-18.

6. O'Rahilly R, Muller F. Developmental stages in human embryos: revised and new measurements. Cells Tissues Organs. 2010;192(2):73-84.

7. Timor-Tritsch IE, Monteagudo A. Transvaginal sonographic evaluation of the fetal central nervous system. Obstet Gynecol Clin North Am. 1991;18(4):713-48.

8. Timor-Tritsch IE, Monteagudo A, Warren WB. Transvaginal ultrasonographic definition of the central nervous system in the first and early second trimesters. Am J Obstet Gynecol. 1991;164(2):497-503.

9. Blaas HG, Eik-Nes SH, Berg S, Torp H. In-vivo three-dimensional ultrasound reconstructions of embryos and early fetuses. Lancet. 1998;352(9135):1182-6.

10. Berg S, Torp H, Blaas HG. Accuracy of in-vitro volume estimation of small structures using three-dimensional ultrasound. Ultrasound Med Biol. 2000;26(3):425-32.

11. Tanaka H, Senoh D, Yanagihara T, Hata T. Intrauterine sonographic measurement of embryonic brain vesicle. Hum Reprod. 2000;15(6):1407-12.

12. Tanaka H, Hata T. Intrauterine sonographic measurement of the embryonic brain mantle. Ultrasound Obstet Gynecol. 2009;34(1):47-51.

13. Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR. Early development of the forebrain and midbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. Ultrasound Obstet Gynecol. 1994;4(3):183-92.

14. Blaas HG, Eik-Nes SH, Kiserud T, Berg S, Angelsen B, Olstad B. Three-dimensional imaging of the brain cavities in human embryos. Ultrasound Obstet Gynecol. 1995;5(4):228-32.

15. Bland JM, Altman DG. Applying the right statistics: analyses of measurement studies. Ultrasound Obstet Gynecol. 2003;22(1):85-93.

16. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape. J Roy Stat Soc C-App. 2005;54:507-44.

17. Timor-Tritsch IE, Monteagudo A, Santos R. Three-dimensional inversion rendering in the first- and early second-trimester fetal brain: its use in holoprosencephaly. Ultrasound Obstet Gynecol. 2008;32(6):744-50.

18. Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR. Early development of the hindbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. Ultrasound Obstet Gynecol. 1995;5(3):151-60.

19. Burton GJ, Hempstock J, Jauniaux E. Nutrition of the human fetus during the first trimester—a review. Placenta. 2001;22 Suppl A:S70-7.

20. van Uitert EM, Exalto N, Burton GJ, Willemsen SP, Koning AH, Eilers PH, et al. Human embryonic growth trajectories and associations with fetal growth and birthweight. Hum Reprod. 2013;28(7):1753-61.