International standards for fetal brain structures based on serial ultrasound measurements from the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project

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CONTRIBUTION

What are the novel findings of this work?

Previous charts of brain measures in current use are based on mostly small studies with poor methodology, and no follow up. In this international study we create standards for the size of five fetal brain structures, based on a prospective cohort of fetuses followed up into childhood demonstrating normal neuro-development.

What are the clinical implications of this work?

Clinical use of such objective fetal brain structure measurements may help to improve the screening and diagnostic performance of prenatal ultrasound; will allow a unified approach of fetal assessment by integrating with other standards from the same population, and will result in a common language when describing aberrations from expected norms.

ABSTRACT

Objective: To create prescriptive growth standards for five fetal brain structures, measured by ultrasound, from healthy, well-nourished women, at low risk of impaired fetal growth and poor perinatal outcomes, taking part in the Fetal Growth Longitudinal Study (FGLS) of the INTERGROWTH-21st Project.

Methods: This was a complementary analysis of a large, population-based, multicentre, longitudinal study. We measured, in planes reconstructed from 3-dimensional (3D) ultrasound volumes of the fetal head at different time points in pregnancy, the size of the parieto-occipital fissure (POF), Sylvian fissure (SF), anterior horn of the lateral ventricle (AV), atrium of the posterior ventricle (PV) and cisterna magna (CM). The sample analysed was randomly selected from the overall FGLS population, ensuring an equal distribution amongst the eight diverse participating sites and of 3D ultrasound volumes across pregnancy (range: 15 - 36 weeks' gestation). Fractional polynomials were used to the construct standards. Growth and development of the infants were assessed at 1 and 2 years of age to confirm their adequacy for constructing international standards.

Results: From the entire FGLS cohort of 4321 women, 451 (10.4%) were randomly selected. After exclusions, 3D ultrasound volumes from 442 fetuses born without congenital malformations were used to create the charts. The fetal brain structures of interest were identified in 90% of cases. All structures showed increasing size with gestation and increasing variability for the POF, SF, PV and CM. The 3rd, 5th, 50th, 95th and 97th smoothed centile are presented. The 5th centile of POF and SF were 2.8 and 4.3 at 22 weeks and 4.2 and 9.4mm at 32 weeks respectively. The 95th centile of PV and CM were 8.5 and 7.4 at 22 weeks and 8.5 and 9.4mm at 32 weeks respectively.

Conclusions: We have produced prescriptive size standards for fetal brain structures based on prospectively enrolled pregnancies at low risk of abnormal outcomes. We recommend these as international standards for the assessment of measurements obtained by ultrasound from fetal brain structures.

INTRODUCTION

In most settings, the anatomy of the fetal brain is routinely assessed as part of the mid-trimester anomaly scan at around 20 weeks' gestation, the main aims of which are to demonstrate anatomical integrity and diagnose abnormalities of the central nervous system (CNS). Measurement of intracranial structures forms part of the assessment, and includes the width of the atrium of the lateral ventricle measured posteriorly (PV) and cisterna magna (CM).[1,2] In more advanced neurosonography, undertaken due to indications such as a previous or suspected abnormality, other structures, e.g. the Sylvian fissure (SF), are examined either earlier in cases of a previous abnormality or late in pregnancy to assess gyration and sulcation patterns, which change with advancing gestational age.[3-8]

Fetal brain structures can be evaluated by assessing their appearance subjectively or measured quantitatively, which is recommended whenever possible as subjective assessment is associated with higher variability.[2] Currently, the normality of any measurements obtained is evaluated in relation to one of several reported reference charts for fetal brain structures.[2] However, many of studies reporting reference charts have important methodological limitations.[9] There can also be a lack of consistency in the interpretation of ultrasound images of the fetal CNS, leading to inconsistent clinical management, if the same measurement from a fetus is plotted on two different charts. These issues are generic to the measurement of all fetal anatomical structures, as reported in systematic reviews of studies aimed at creating charts for fetal biometry and pregnancy dating.[10,11]

To overcome these issues with regard to ultrasound assessment of the fetal brain, we have again followed WHO recommendations and adopted a prescriptive approach to the construction of international size standards for five fetal brain structures, as a secondary analysis of data collected in the Fetal Growth Longitudinal Study (FGLS), one of the key components of the INTERGROWTH-21st Project (www.intergrowth21.org.uk).[12] Three of the brain structures relate to clinical evaluation of cerebrospinal fluid, namely the PV, CM and

anterior horn of the lateral ventricle (AV);[1] the two other structures are clinically relevant to the assessment of gyration and sulcation, namely the parieto-occipital fissure (POF) and SF. The international standards produced complement those previously published for early and late pregnancy dating,[13,14] fetal growth and estimated fetal weight,[15,16] symphysis-fundal height,[17] gestational weight gain,[18] newborn size at birth and body composition,[19,20] and postnatal growth of preterm infants.[21]

MATERIALS AND METHODS

Study population

INTERGROWTH-21st is an international, multicentre, population-based project, conducted between 2009 and 2016 in eight delimited geographical areas: Pelotas (Brazil), Turin (Italy), Muscat (Oman), Oxford (UK), Seattle (USA), Shunyi County in Beijing (China), the central area of Nagpur (India), and the Parklands suburb of Nairobi (Kenya). In FGLS, serial 2-dimensional (2D) and 3-dimensional (3D) fetal scans were performed every 5±1 weeks from 14+0 weeks' gestation to delivery.[15] Women participating in the study, who initiated antenatal care before 14 weeks' gestation, were selected based upon the WHO recommended criteria for optimal health, nutrition, education and socioeconomic status needed to construct international standards.[22,23] Hence, they had low-risk pregnancies that fulfilled well-defined and strict inclusion criteria at both population and individual levels.[23] Briefly, the individual inclusion criteria were maternal age between 18 and 35 years, body mass index (BMI) ≥18.5 and <30 kg/m^2, a naturally conceived singleton pregnancy, normal pregnancy history without relevant past medical history, no evidence of socioeconomic constraints likely to impede fetal growth, no use of tobacco or recreational drugs and no heavy alcohol consumption. Women also had to have a known date of the last menstrual period (LMP) with regular cycles without hormonal contraceptive use or breastfeeding in the 2 months before pregnancy. Gestational age was LMP-based provided that standardised ultrasound measurement of crown-rump length between 9+0 and 14+0 weeks was in agreement within 7 days.[24]

In FGLS, sonographers that were trained, standardised and regularly audited performed all ultrasound scans.[25,26] Identical, commercially available, ultrasound equipment (Philips HD-9, Philips Ultrasound, Bothell, WA, USA), with curvilinear abdominal 2D transducers (C5-2, C6-3) and curvilinear abdominal 3D transducer (V7-3), was used for all growth scans. For the purposes of the INTERGROWTH-21st Project, the manufacturer reprogrammed the

machine's software to ensure that the measurement values did not appear on screen during the scan in order to reduce operator "expected value" bias. A detailed description of the ultrasound methodology has been reported previously.[25]

Infants from sites that participated in the follow-up study (Brazil, India, Italy, Kenya and the UK) were assessed at age 1 and 2 years to obtain a detailed assessment of growth, nutrition, morbidity, and motor development. These data were collected by interviewing parents and assessment by a certified examiner. Achievement of milestones ('sitting without support', 'standing with assistance', 'hand-and-knees-crawling', 'walking with assistance', 'standing alone' and 'walking alone') were considered normal if the time of achievement was within the expected WHO windows (less than the 99th centile child age for each of the expected windows).[27]

The INTERGROWTH-21st Project was approved by the Oxfordshire Research Ethics Committee "C" (ref: 08/H0606/ 139), the research ethics committees of the individual institutions and the regional health authorities where the project was implemented; all the women involved gave informed written consent.

Structures measured and sample size considerations

The fetal brain structures were measured on ultrasound images extracted from 3D volumes of the fetal head, acquired in all eight participating sites. The decision regarding which structures to evaluate was based on a combination of factors: an extensive scoping exercise and review of the literature demonstrating their clinical utility[9]; structures that can be assessed in axial planes that are routinely acquired, and a pilot study involving 90 ultrasound volumes assessing feasibility and reproducibility.

The sample size was based on pragmatic and statistical considerations. The main pragmatic consideration was the considerable length of time required for volume upload, manipulation, plane extraction and measurement (20mins per volume on average). As a

result, we decided to take a random sample from the entire FGLS cohort, bearing in mind the need for precision at the 5th and 95th centiles. A sample of 300 scans would obtain precision of 0.1 Standard Deviation (SD) at the 5th or the 95th centile.[28] Working on conservative estimates, we assumed a possible 5% exclusion rate due to loss to follow-up in pregnancy or at birth, withdrawal of consent, miscarriage, stillbirth, maternal death, fetal or neonatal structural abnormality or severely abnormal outcome at 2 year follow-up. This was defined as any of the following: meningitis, hearing loss, blindness or major visual problems, seizures, cerebral palsy, neurological disorders, malignancy, malaria, tuberculosis, hepatitis, HIV/AIDS, cystic fibrosis or haemolytic conditions. We also assumed that in up to 40% of the cases all five structures might not be measurable (based on a conservative estimate as the actual upper limit of the confidence interval from the pilot study was 20%, primarily due to movement artefact). Based on these assumptions, we estimated that 451 3D volumes would lead to a minimum of 300 measurements for each structure. Therefore, we selected 451 3D volumes from the overall FGLS population using computer randomisation, ensuring an equal distribution amongst the eight participating sites and of volumes across pregnancy (range: 15 - 36 weeks' gestation). The random selection was performed using SAS software (Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA).

The study was cross-sectional as only one volume per pregnancy was included.

Volume acquisition, offline analysis and quality control

Detailed descriptions of the volume acquisition methods are provided elsewhere.[25,29] Briefly, head volumes were acquired at the level of the axial transthalamic plane. Six predefined quality control criteria for the transthalamic plane had to be satisfied to acquire the volume (Table 1) (Figure 1).[26] Acquisition was undertaken with the volume data box and angle of sweep (usually 70°) adjusted to include the entire head during fetal quiescence, with the mother asked to hold her breath, and with the transducer held steady. The real-time image was observed during acquisition to confirm that the sweep included the entire

head with no maternal or fetal movement during the sweep, otherwise the process was repeated. All data were then sent to the Ultrasound Quality Coordinating Unit in Oxford.

Offline analysis was undertaken by four experienced sonographers at the Coordinating Unit. All were trained in neurosonography and specifically standardised for the purposes of this study in volume manipulation for plane reconstruction and measurement (Video S1, Figure 1). The volume manipulations and measurements were performed using the manufacturer software of the ultrasound machine or an open-source image analysis software program (MITK, Medical Imaging Interaction Toolkit MITK, version 0.12.2, German Cancer Research Center, Division of Medical and Biological Informatics, www.mitk.org).[30] This was done as the open source software was more user friendly. Comparability of measurements between the manufacturer software of the ultrasound machine and the open-source image analysis software program was confirmed (mean reproducibility was within 0.7 mm).

All sonographers were blinded to measures during the study. In addition, strict quality control was undertaken on the whole sample: for each extracted plane image quality criteria were used to ensure the maximum possible score for each extracted plane (Table 1) before measurement of the following five structures: the POF and SF in the transthalamic plane; AV and PV in the transventricular plane, and CM in the transcerebellar plane. The POF, SF, AV and PV were measured in the distal hemisphere of the respective plane (due to poorer visualisation in the proximal hemisphere). Further details of volume manipulation and caliper placement are given in the Appendix.

Reproducibility

Reproducibility was assessed in a subset of 90 volumes. The first sonographer uploaded the volume, manually extracted the three planes and measured the five structures twice (intra-observer reproducibility for plane reconstruction and measurement acquisition). A second sonographer re-uploaded the same volume and repeated this process (this second set of

data was used to assess interobserver reproducibility for plane reconstruction and measurement acquisition). To assess the contribution of caliper replacement, the second sonographer replaced the calipers on still images and repositioned them to measure all structures in each plane stored by the first sonographer (inter-observer reproducibility for caliper replacement on stored images). As in the main study, all sonographers were blinded to their own and the other sonographer's measurements during the reproducibility study.

Statistical analysis

We followed the modelling approach used previously by our group to construct fetal growth charts.[15] In summary, fractional polynomials that model the means and the SD were used to model biometric measurements of brain structures as a function of gestational age. Our overall aim was to produce centiles that change smoothly with age and maximise simplicity without compromising model fit. Goodness of fit was assessed by Q-Q plots and a scatter plot of Z-scores by gestational age. Mean differences between the observed and fitted centiles were also calculated.

For the reproducibility study, Bland-Altman plots were used to quantify the level of agreement and variability in the measurements. Differences between and within observers were expressed in absolute values (mm). All analyses were performed using STATA 11 (StataCorp, College Station, Texas, USA).

RESULTS

After exclusions, 442/451 (98.0%) volumes were used to reconstruct planes and create the charts (Figure 2). No congenital malformations were detected antenatally or postnatally in the selected fetuses, and no infants met the exclusion criteria set for the 2-year follow-up. As expected, given the random selection, maternal demographics and pregnancy outcomes were similar to the overall FGLS population, confirming a low risk of perinatal complications (Table S1).

Of the 442 infants, 297 (67.2%) were assessed by their parent(s) at age 1; of these, 289 (97.3%) were also assessed by a certified examiner at mean 12.3 (range 10.9 - 19.4) months. As reported by the parent(s), 99% of the infants had entirely normal motor development; only three infants (1%) did not achieve the milestones sitting 'without support' and 'standing with assistance', centiles of brain structures for these children were within the 5th and the 95th centile. There was overall good agreement between the achievement of milestones, as reported by the parent(s), and that found on examination (average agreement 96%, range 92 to 100%). Reassuringly, in almost all cases where disagreement was present, the examiner reported more precocious milestone achievement than the parent(s), confirming the low risk for abnormal long-term outcome in our cohort. Follow-up at the age of 2 years was available in 304 children; the findings of this detailed assessment demonstrate comparability to the morbidity reported in children from the overall cohort who underwent motor and neurodevelopment assessment (Table S2, Figure 3).[31] The mean and SD of the children's weight, length and head circumference at 2 years of age were 12.3 kg (1.7), 87.4 cm (3.7), 47.7 cm (1.6) respectively, and z-scores were within the expected values of the WHO Child Growth Standards. Motor development for the two milestones not reached by age 1 ('standing alone' and 'walking alone') was confirmed normal in 99% and 98%, respectively.

In total, 2439 measurements of fetal brain structures were acquired.

On average, structures were measurable in a high quality extracted plane in 90% of cases, with the CM being the structure least frequently measurable. After removal of outliers, measurements were available to create centiles for POF, SF, AV, PV and CM in 420 (95%), 404 (91%), 378 (85%), 422 (95%) and 352 (80%) cases, respectively. The mean (SD) values in mm were POF=5.47 (1.91), SF=9.45 (4.22), AV=7.61 (1.54), PV=6 (1.59) and CM=5.27 (1.66). All fetal brain measurements were normally distributed conditional on gestational age.

The best fitting powers were provided by second-degree fractional polynomials and further modelled in a multilevel framework to account for the cross-sectional design of the study. The gestational age-specific 3rd, 5th, 50th, 95th and 97th smoothed centiles for POF, SF, AV, PV and CM are presented in Figure 4 and reported in Table 2. One infant had PV > 10mm and 1 infant a CM > 10mm. They both had a normal perinatal outcome.

Both visual assessment of scatter plots of z-scores by gestational age and goodness of fit tests, assessed by gestational age-specific comparisons of empirical centiles to smoothed centile curves, showed good agreement.

The equations for the mean and SD from the fractional polynomial regression models for each structure measured are presented in Table 3, allowing for calculations of any desired centiles according to gestational age in exact weeks.

Results of the reproducibility study are shown in Table 4. All measurements were reproducible within less than 3mm or 12% (all mean differences were less than 0.1mm and 0.5%). The greatest proportion of variability was due to caliper replacement accounting for more than 50% of the intra- and inter-observer variability for all structures, as previously observed in fetal biometry (Supplementary Figure 1).[32]

DISCUSSION

We have produced international size standards for ultrasound measurements of clinically relevant brain structures. The population consisted of women at low risk of adverse pregnancy and perinatal outcomes.[15] Uniquely, we followed up the infants and demonstrated satisfactory growth and development at 1 and 2 years of age, confirming that our initial selection criteria met the WHO requirements for constructing international growth standards.[12,31] The sequence and timing of attainment of neurodevelopmental milestones and associated behaviours in early childhood were strikingly similar to those previously reported by our group, i.e. we have demonstrated similarities across diverse geographical regions as long as nutritional and health needs are met.[31]

We performed a systematic review of the literature that analysed the methodology used to create fetal brain structure charts.[9] This showed that some studies did not strictly adhere plane standardization. Using different planes in fetal head biometry can lead to significant measurement differences.[33] In some studies, landmarks for plane acquisition are not specified,[34-44] while in others, various oblique planes with numerous landmarks are proposed.[45,46] One of the strengths of our study is the use of standardised axial planes recommended in routine clinical practice for biometry assessment (Table 1).. We believe that this approach of using standardised planes improves reproducibility, a view that is supported by previous studies.[46,47] In our case, this led to a high proportion of structures that could be measured on stored volumes (90% on average) and resulted in reproducible measurements, with 95% limits of agreement within <3mm (or <6%) (Table 4). Studies involving experts in neurosonography report similar results in visualizing structures from volume analysis.[48] This is in contrast to previous studies on subjective assessment of brain fissures, which report variable results in terms of reproducibility (Kappa coefficients varying from 0.56 to 0.95).[45,49] Improving reproducibility was also one of the motivations of our study: to move to quantitative assessment of fetal brain development.[45,46,50]

To achieve our objectives, we used international guidelines to obtain measurements of PV and CM,[1,2] and we provide detailed methods for AV, POF and SF measurements, based on existing publications (Appendix), as we were unable to find generally accepted guidelines.

Our study overcomes many of the methodological limitations of previous studies.[9] These include a high risk of bias in the selection of the population, ultrasound protocol and data analysis. For example, less than 10% of previous studies reported on maternal and fetal inclusion/exclusion criteria, pregnancy outcomes or ultrasound quality control. Goodness of fit of the model to create the charts was reported in only than 35% of the studies. Most importantly, no studies reported long-term infant outcomes, most probably due to their retrospective descriptive design (30%); thus, data were often not collected specifically for the purpose of the study. Not surprisingly, these are some of the same challenges seen in previous studies to construct fetal biometry charts.[10,11] Nevertheless, some previous studies did have a relatively low risk of methodological bias and the range of our observed measurements did not differ substantially from their findings.[34,51-54]

Limitations and strengths

We used a large number of sonographers; however, this reflects more accurately clinical practice.[55] In addition, the quality of the images obtained in the study was of a high standard and in accordance to a predefined protocol.[25] We set near-optimal conditions for scanning to minimise the potential contribution of confounding factors, which could also be seen as a strength. It is possible that measurements acquired on planes extracted from 3D volumes are not equivalent to measurements made from 2D image acquisition. Although volumetry is associated with a high degree or variability if not standardised,[50] once rigorous methodology is adopted, 2D measurements from reconstructed planes can be as reproducible as measurements obtained in real-time.[29,37]

A key strength of our study is that we adopted a prescriptive design as recommended by WHO. We identified urban regions where women were at low-risk of perinatal complications; participants were then enrolled within these regions based on their individual characteristics. All ultrasound measurements were taken specifically for the purpose of constructing international standards with standardisation of all study sites, using centrally trained staff and specially adapted ultrasound equipment to allow masking of measurements. For the offline analysis, we developed a novel quality control strategy. Finally, the most appropriate statistical methods were used to analyse the dataset.

It can be argued that only longitudinal data should be preferred to assess fetal growth. However, given the design of FGLS where women had mostly equal number of visits during pregnancy and these visits were according to what was pre-specified in the protocol, cross-sectional data were acquired in order to ensure a representative number of brain structure measurements per gestational week. The model fitted took this in account.

The INTERGROWTH-21st Project and WHO Multicentre Group Reference Study have previously demonstrated the generalisability across geographically diverse international populations of anthropometric standards produced using the prescriptive approach.[12,31,56] Follow-up of infants in the FGLS cohort has also been reported, and demonstrates striking similarities across sites when assessed by variance components analysis and standardised site differences, showing that the sequence and timing of attainment of neurodevelopmental milestones and associated behaviours in early childhood are likely innate and universal.[31]

Conclusion

We report international standards for the size of five fetal brain structures throughout gestation. These standards use reproducible and highly controlled ultrasound measurements, and have been created using a prospective cohort of fetuses that was

followed up into childhood. Clinical use of such objective measurements may help to improve the screening and diagnostic performance of prenatal ultrasound; will allow a unified approach of fetal assessment by integrating with other standards from the same population, and will result in a common language when describing aberrations from expected norms.[57,58] The standards proposed should not replace currently accepted cut-off values for triggering referral or further investigation; for example, we do not propose that we should redefine the diagnosis of antenatally diagnosed ventriculomegaly. This is because previous studies relating infant outcomes to antenatally detected congenital brain abnormalities cannot simply be replicated.[57-59]

Conflict of interest: ATP and JAN are Senior Advisors of Intelligent Ultrasound. All other authors declare no competing interests.

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FIGURE LEGENDS

Figure 1: Planes reconstructed and caliper placement for brain structure acquisition at different weeks' gestation.

W: completed weeks' gestation, TT: transthalamic plane, TV: transventricular plane, TC: transcerebellar plane, POF: parieto-occipital fissure, SF: Sylvian fissure, AV: anterior horn of the lateral ventricle, PV: atrium of the posterior ventricle, TCD: transcerebellar diameter, CM: cisterna magna.

Figure 2: Study flow chart

Figure 3: Median age of achievement (3^{rd} and 97^{th} centiles) of four gross motor development milestones for children that were included in the INTERGROWTH-21^{st} Fetal Growth Standards (purple) and children included in the current study (green). For comparison, the median, 3^{rd} and 97^{th} centiles of the WHO windows of achievement for the same milestones are presented as grey bars.

Figure 4: Fitted 5^{th}, 50^{th}, and 95^{th} smoothed centile curves of fetal brain structure measurements.

A: parieto-occipital fissure (POF), B: Sylvian fissure (SF), C: anterior horn of the lateral ventricle (AV), D: atrium of the posterior ventricle (PV), E: cisterna magna (CM).

Table 1: Quality criteria for acquisition of the three planes.

TRANSTHALAMIC PLANE	TRANSVENTRICULAR PLANE	TRANSCEREBELLAR PLANE
Symmetrical hemispheres	Symmetrical hemispheres	Symmetrical hemispheres
Cavum of the septum pellucidum present	Cavum of the septum pellucidum present	Cavum of the septum pellucidum present
Thalami visible	Lateral ventricles visible	Thalami visible
No cerebellum visible	No cerebellum visible	Cerebellum present at the maximum diameter
Magnification of 30% image	Magnification of 30% image	Magnification of 30% image

Table 2A: Smoothed centiles for parieto-occipital fissure (in mm) according to exact gestational age (in weeks).

GA	Sample	C3	C5	C50	C95	C97
15w+0d	18	1.29	1.39	2.14	2.88	2.99
16w+0d	18	1.55	1.69	2.66	3.63	3.77
17w+0d	18	1.79	1.96	3.12	4.28	4.45
18w+0d	19	2.02	2.21	3.53	4.85	5.04
19w+0d	19	2.24	2.45	3.90	5.35	5.56
20w+0d	21	2.44	2.67	4.24	5.80	6.03
21w+0d	16	2.64	2.87	4.54	6.21	6.44
22w+0d	18	2.82	3.07	4.82	6.57	6.82
23w+0d	21	2.99	3.25	5.08	6.90	7.17
24w+0d	18	3.16	3.43	5.32	7.21	7.48
25w+0d	20	3.32	3.59	5.54	7.49	7.77
26w+0d	19	3.47	3.75	5.75	7.75	8.04
27w+0d	19	3.61	3.90	5.95	7.99	8.29
28w+0d	19	3.75	4.05	6.13	8.22	8.52
29w+0d	22	3.89	4.19	6.31	8.43	8.74
30w+0d	21	4.02	4.32	6.48	8.63	8.94
31w+0d	20	4.14	4.46	6.64	8.83	9.14
32w+0d	17	4.27	4.58	6.80	9.01	9.32
33w+0d	22	4.39	4.71	6.94	9.18	9.50
34w+0d	21	4.51	4.83	7.09	9.35	9.67
35w+0d	19	4.62	4.95	7.23	9.51	9.84
36w+0d	15	4.74	5.07	7.37	9.67	9.99
Total Measurements	420					

GA: gestational age in weeks (w) and days (d); C3: 3rd centile; C5: 5th centile; C50: 50th centile; C95: 95th centile; C97: 97th centile.

Table 2B: Smoothed centiles for Sylvian fissure (in mm) according to exact gestational age (in weeks).

GA	Sample	C3	C5	C50	C95	C97
15w+0d	18	0.40	0.57	1.77	2.98	3.15
16w+0d	15	0.91	1.13	2.65	4.17	4.38
17w+0d	18	1.46	1.72	3.49	5.27	5.52
18w+0d	18	2.03	2.31	4.31	6.30	6.59
19w+0d	17	2.60	2.91	5.09	7.27	7.58
20w+0d	20	3.18	3.51	5.85	8.18	8.51
21w+0d	15	3.75	4.10	6.57	9.04	9.40
22w+0d	18	4.32	4.69	7.27	9.86	10.23
23w+0d	20	4.87	5.26	7.95	10.64	11.02
24w+0d	17	5.42	5.82	8.60	11.38	11.78
25w+0d	20	5.96	6.37	9.23	12.09	12.50
26w+0d	18	6.49	6.91	9.84	12.77	13.19
27w+0d	16	7.01	7.44	10.43	13.42	13.85
28w+0d	19	7.52	7.95	11.00	14.05	14.48
29w+0d	22	8.01	8.45	11.55	14.65	15.09
30w+0d	20	8.49	8.94	12.09	15.23	15.68
31w+0d	20	8.97	9.42	12.61	15.79	16.25
32w+0d	17	9.43	9.89	13.11	16.33	16.79
33w+0d	22	9.88	10.34	13.60	16.86	17.32
34w+0d	22	10.32	10.79	14.07	17.36	17.83
35w+0d	18	10.75	11.22	14.54	17.85	18.33
36w+0d	14	11.17	11.64	14.99	18.33	18.80
Total Measurements	404					

GA: gestational age in weeks (w) and days (d); C3: 3rd centile; C5: 5th centile; C50: 50th centile; C95: 95th centile; C97: 97th centile.

Table 2C: Smoothed centiles for anterior horn of the lateral ventricle (in mm) according to exact gestational age (in weeks).

GA	Sample	C3	C5	C50	C95	C97
15w+0d	17	4.34	4.62	6.61	8.59	8.87
16w+0d	15	4.39	4.67	6.65	8.63	8.91
17w+0d	17	4.44	4.72	6.70	8.68	8.97
18w+0d	18	4.49	4.78	6.76	8.74	9.02
19w+0d	19	4.56	4.84	6.82	8.80	9.09
20w+0d	20	4.63	4.91	6.89	8.87	9.16
21w+0d	15	4.71	4.99	6.97	8.95	9.24
22w+0d	18	4.79	5.08	7.06	9.04	9.32
23w+0d	21	4.89	5.17	7.15	9.13	9.42
24w+0d	15	4.99	5.27	7.25	9.24	9.52
25w+0d	19	5.10	5.38	7.37	9.35	9.63
26w+0d	18	5.22	5.51	7.49	9.47	9.75
27w+0d	17	5.35	5.64	7.62	9.60	9.88
28w+0d	19	5.49	5.78	7.76	9.74	10.02
29w+0d	22	5.65	5.93	7.91	9.89	10.17
30w+0d	19	5.81	6.09	8.07	10.05	10.34
31w+0d	17	5.98	6.26	8.24	10.23	10.51
32w+0d	13	6.17	6.45	8.43	10.41	10.69
33w+0d	18	6.36	6.65	8.63	10.61	10.89
34w+0d	17	6.57	6.85	8.84	10.82	11.10
35w+0d	15	6.79	7.08	9.06	11.04	11.32
36w+0d	9	7.03	7.31	9.29	11.27	11.56
Total Measurements	378					

GA: gestational age in weeks (w) and days (d); C3: 3rd centile; C5: 5th centile; C50: 50th centile; C95: 95th centile; C97: 97th centile.

Table 2D: Smoothed centiles for atrium of the posterior ventricle (in mm) according to exact gestational age (in weeks).

GA	Sample	C3	C5	C50	C95	C97
15w+0d	18	4.71	4.99	6.94	8.88	9.16
16w+0d	19	4.49	4.77	6.78	8.78	9.07
17w+0d	18	4.28	4.58	6.64	8.70	9.00
18w+0d	19	4.10	4.40	6.52	8.64	8.94
19w+0d	19	3.92	4.23	6.41	8.58	8.89
20w+0d	22	3.76	4.08	6.31	8.54	8.86
21w+0d	16	3.61	3.94	6.22	8.51	8.84
22w+0d	18	3.46	3.80	6.14	8.49	8.82
23w+0d	21	3.33	3.67	6.07	8.47	8.81
24w+0d	18	3.20	3.55	6.00	8.46	8.81
25w+0d	20	3.07	3.43	5.94	8.46	8.82
26w+0d	19	2.95	3.32	5.89	8.46	8.83
27w+0d	19	2.84	3.22	5.84	8.46	8.84
28w+0d	19	2.73	3.11	5.79	8.48	8.86
29w+0d	22	2.62	3.01	5.75	8.49	8.88
30w+0d	21	2.52	2.92	5.71	8.51	8.91
31w+0d	20	2.42	2.83	5.68	8.53	8.94
32w+0d	17	2.32	2.74	5.65	8.55	8.97
33w+0d	22	2.23	2.65	5.62	8.58	9.00
34w+0d	22	2.14	2.57	5.59	8.61	9.04
35w+0d	19	2.05	2.49	5.56	8.64	9.08
36w+0d	14	1.96	2.41	5.54	8.67	9.12
Total Measurements	422					

GA: gestational age in weeks (w) and days (d); C3: 3rd centile; C5: 5th centile; C50: 50th centile; C95: 95th centile; C97: 97th centile.

Table 2E: Smoothed centiles for cisterna magna (in mm) according to exact gestational age (in weeks).

GA	Sample	C3	C5	C50	C95	C97
15w+0d	19	1.71	1.82	2.82	4.36	4.64
16w+0d	17	1.96	2.08	3.20	4.92	5.24
17w+0d	17	2.19	2.33	3.56	5.44	5.79
18w+0d	18	2.41	2.56	3.89	5.92	6.29
19w+0d	19	2.61	2.77	4.20	6.36	6.75
20w+0d	21	2.80	2.97	4.48	6.76	7.17
21w+0d	15	2.97	3.15	4.73	7.12	7.55
22w+0d	18	3.12	3.31	4.97	7.45	7.90
23w+0d	21	3.26	3.46	5.18	7.75	8.21
24w+0d	16	3.39	3.60	5.37	8.02	8.50
25w+0d	17	3.51	3.72	5.55	8.27	8.76
26w+0d	19	3.62	3.83	5.71	8.50	8.99
27w+0d	15	3.72	3.94	5.85	8.70	9.21
28w+0d	16	3.81	4.03	5.99	8.89	9.41
29w+0d	20	3.90	4.12	6.11	9.06	9.59
30w+0d	16	3.97	4.20	6.22	9.22	9.75
31w+0d	13	4.04	4.27	6.33	9.36	9.90
32w+0d	14	4.11	4.34	6.42	9.49	10.04
33w+0d	12	4.17	4.40	6.51	9.62	10.17
34w+0d	13	4.22	4.46	6.59	9.73	10.28
35w+0d	11	4.27	4.51	6.66	9.83	10.39
36w+0d	5	4.32	4.56	6.73	9.92	10.49
Total Measurements	352					

GA: gestational age in weeks (w) and days (d); C3: 3rd centile; C5: 5th centile; C50: 50th centile; C95: 95th centile; C97: 97th centile.

Table 3: Equations for the estimation of the mean and SD (in mm) of each fetal brain structure measure according to exact gestational age (in weeks).

Parieto-occipital fissure	
Mean	10.29428 - 122.8447*GA^-1 + 0.00001038*GA^3
SD	1.596042 - 257.2297*GA^-2
Sylvian fissure	
Mean	80.27012 - 32.7877*GA^-0.5 - 100.1593*GA^-0.5*LN(GA)
SD	2.304501 - 353.814*GA^-2
Anterior horn of the lateral ventricle	
Mean	6.396214 + 0.00006205*GA^3
SD	1.204454
Atrium of the posterior ventricle	
Mean	4.389214 + 38.10015*GA^-1 + 0.0000020063*GA^3
SD	0.6707227 + 0.034258*GA
Cisterna magna	
Mean	EXP (2.098095 -239.0659*GA^-2 -0.0000001547*GA^3)
SD	0.2297936 + 8.1872*GA^-2

LN: natural logarithm, GA=exact gestational age.

Table 4: Reproducibility study.

	Intra-observer reproducibility mean (95% LOA)	Inter-observer reproducibility mean (95% LOA)	Caliper replacement reproducibility mean (95% LOA)
Parieto-occipital fissure (mm)	-0.02 (1.6)	0 (0.19)	-0.01 (0.19)
Sylvian fissure (mm)	-0.01 (2.1)	0 (0.22)	0 (0.28)
Anterior horn of the lateral ventricle (mm)	-0.01 (0.18)	-0.02 (0.2)	0 (0.1)
Atrium of the posterior ventricle (mm)	0 (0.11)	0 (0.18)	0.01 (1.7)
Cisterna magna (mm)	0 (1.6)	-0.02 (0.19)	0.01 (1.85)

LOA; limits of agreement.