Ultrasound Obstet Gynecol 2020; **56**: 187–195
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.21870.

Sex-specific reference ranges of cerebroplacental and umbilicocerebral ratios: longitudinal study

G. ACHARYA[1,2#], C. EBBING[3#], H. O. KARLSEN[3], T. KISERUD[3,4] and S. RASMUSSEN[4]

[1]*Women's Health and Perinatology Research Group, Department of Clinical Medicine, Faculty of Health Sciences, University of Tromsø and Department of Obstetrics and Gynecology, University Hospital of North Norway, Tromsø, Norway;* [2]*Department of Clinical Science, Intervention & Technology, Karolinska Institutet and Center for Fetal Medicine Karolinska, University Hospital, Stockholm, Sweden;* [3]*Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway;* [4]*Department of Clinical Science, University of Bergen, Bergen, Norway*

KEYWORDS: cerebroplacental ratio; Doppler; middle cerebral artery; sexual dimorphism; umbilical artery; umbilicocerebral ratio

CONTRIBUTION

What are the novel findings of this work?
Abnormal cerebroplacental ratio (CPR) or umbilicocerebral ratio (UCR) reflects fetal cardiac output redistribution in favor of the brain compared to the placenta. CPR and UCR have been shown to be useful in monitoring high-risk pregnancies. We found a small but significant difference in *Z*-scores of CPR and UCR in low-risk singleton pregnancies between male and female fetuses throughout the second half of pregnancy, and established sex-specific longitudinal reference ranges based on an adequate sample size.

What are the clinical implications of this work?
We established longitudinal reference intervals for CPR and UCR, which are more appropriate than the reference ranges based on cross-sectional data for serial monitoring of fetuses at risk of 'brain sparing' due to placental insufficiency. Considering sex-related differences in CPR and UCR may further refine the evaluation.

ABSTRACT

Objectives *Observational studies have shown that low cerebroplacental ratio (CPR) values predict an increased risk of adverse perinatal outcome. The inverse ratio, i.e. the umbilicocerebral ratio (UCR), has been suggested to be a better predictor as it rises with increasing degree of fetal compromise. However, longitudinal reference ranges for UCR have not been established, and whether gestational-age-dependent changes in CPR or UCR differ between male and female fetuses has not been studied. Thus, the aims of this study were to investigate sex-specific, gestational-age-associated serial changes in CPR and UCR during the second half of pregnancy and to establish longitudinal reference ranges.*

Methods *This was a secondary analysis of prospectively collected data from a dual-center longitudinal observational cohort study of low-risk singleton pregnancies. Doppler blood-flow velocity waveforms were obtained serially from the umbilical artery (UA) and fetal middle cerebral artery (MCA) from 19–41 weeks' gestation, and pulsatility indices (PIs) were determined. CPR and UCR were calculated as the ratios MCA-PI/UA-PI and UA-PI/MCA-PI, respectively. The course and outcome of pregnancies were recorded, and the sex of the fetus was determined after delivery. Reference intervals for CPR and UCR were constructed using multilevel modeling, and gestational-age-specific Z-scores in male and female fetuses were compared.*

Results *Of a total of 299 pregnancies enrolled, 284 (148 male and 136 female fetuses) were included in the final analysis, and 979 paired measurements of UA-PI and MCA-PI were used to construct sex-specific longitudinal reference intervals. The relationship of both CPR and UCR with gestational age was U-shaped, but in opposite directions. There was a small but significant difference in Z-scores of CPR and UCR between male and female fetuses throughout the second half of pregnancy (*$P = 0.007$*).*

Correspondence to: Prof. G. Acharya, Department of Clinical Science, Intervention & Technology, Karolinska Institutet, SE-141 86 Stockholm, Sweden (e-mail: ganesh.acharya@ki.se)

#G.A. and C.E. contributed equally to the study.

Accepted: 23 August 2019

Conclusions *We have established longitudinal reference ranges for CPR and UCR suitable for serial monitoring, with the possibility of refining assessment by using fetal sex-specific ranges and conditioning by a previous measurement. The clinical significance of such refinements needs further evaluation.* © *2019 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of the International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Fetal cerebral perfusion is stable over a range of blood pressures owing to autoregulation[1,2]. Brain blood flow is regulated mainly by pO_2 levels, and to a lesser extent by pCO_2 and blood glucose[3–5]. Hypoxemia assessed by cordocentesis in fetal growth restriction (FGR) is associated with high blood flow velocity and low pulsatility index (PI) in the middle cerebral artery (MCA)[6]. On the other hand, placental blood flow is low in FGR owing to increased vascular impedance[7,8]. As the flow and impedance in the cerebral and placental circulations tend to change in opposite directions during fetal hypoxemia, a ratio between the cerebral and placental blood flow or impedance could be a useful parameter for assessing fetal wellbeing.

Experimentally imposed fetal hypoxemia is associated with relative circulatory redistribution prioritizing the adrenals, heart and brain[9,10]. The cerebroplacental ratio (CPR), i.e. MCA-PI/umbilical artery (UA)-PI, has been suggested as a non-invasive measure of this 'brain-sparing' response, which might be able to predict perinatal outcome better than the individual parameters of MCA-PI and UA-PI separately[11]. CPR has been advocated as a measure of fetal adaptation to hypoxemia[12]. Several studies have reported its utility in predicting pregnancy outcome[13–17], although its benefit in identifying fetuses at risk and preventing perinatal death has yet to be confirmed in properly designed clinical trials[18–21].

Similarly, the umbilicocerebral ratio (UCR), i.e. UA-PI/MCA-PI, has also been reported to be of value in monitoring growth-restricted fetuses[22]. It has been found to be predictive of non-reactive computerized cardiotocography and intraventricular hemorrhage[23]. Recently it was suggested that UCR might be a better predictor of perinatal outcome than is CPR[19]. While several reference ranges for CPR, including those from one longitudinal study with an adequate sample size[24], have been published, longitudinal reference intervals for UCR are lacking. Furthermore, sex-specific differences are present in placental and cerebral circulations[25–27], but these have not been taken into account in any studies.

Thus, we aimed to explore differences in UCR and CPR between male and female fetuses, and to establish sex- and gestational-age-specific longitudinal reference ranges and their conditioning terms for serial measurements during the second half of pregnancy.

METHODS

This was a secondary analysis of prospectively collected data from a dual-center longitudinal observational cohort study conducted in Norway. Women attending antenatal clinics at Haukeland University Hospital, Bergen, and University Hospital of North Norway, Tromsø, for routine second-trimester ultrasound examination were recruited consecutively from 2004 to 2006 in Bergen and from 2009 to 2012 in Tromsø. The part of the study performed in Bergen has been described and CPR data have been published previously[24], but the UCR data and sex differences have not yet been analyzed and reported. Here, we present results based on analysis of the combined dataset. Another reason for combining the two datasets was to improve the reliability of clinically relevant extreme ranges.

Inclusion criteria were pregnant women of at least 18 years of age with a low-risk singleton pregnancy, gestational age > 17 and < 23 weeks at enrolment and absence of any major placental or fetal structural or chromosomal abnormality. Exclusion criteria were multifetal pregnancy, a history of pre-eclampsia, gestational diabetes, FGR, preterm birth before 34 weeks' gestation and pre-existing disease requiring regular medical treatment or that is known to have a significant effect on the outcome of pregnancy, such as chronic hypertension, diabetes or autoimmune disease. Gestational age was confirmed by fetal head biometry performed at 18–20 weeks' gestation[28]. Women were not included if there was a discrepancy of > 10 days between the last menstrual period-based and ultrasound biometry-based gestational age. Women were examined at approximately 4-week intervals (range, 3–5 weeks) from 19 to 41 weeks. A total of four physicians, each of whom had at least 3 years of training and experience in ultrasonography, performed the ultrasound measurements. At each visit, ultrasonography was performed using either a Vivid 7 Dimension machine equipped with a 4MS 1.5–4.3-MHz sector transducer (GE Vingmed Ultrasound AS, Horten, Norway) or a GE Voluson 730 Expert machine equipped with a 2–8-MHz curvilinear transabdominal transducer (GE Healthcare, Zipf, Austria). After confirmation of fetal viability, determination of placental location and assessment of amniotic fluid volume, fetal biometry was performed and fetal weight was estimated using the Hadlock III formula[29]. Fetal sex was not ascertained during the ultrasound examination.

Blood-flow velocity waveforms were obtained from the UA at a free-floating loop of the umbilical cord and from the proximal part of the fetal MCA at the point at which it emerges from the circle of Willis. Color Doppler and pulsed-wave Doppler were used in line with the techniques described previously, which have been shown to have acceptable reproducibility[24,30]. The Doppler gate (sample volume) was set liberally enough to ensure that the maximum velocity in the blood vessel was recorded, and the insonation was aligned with the vessel or as close to it as possible, always below 15°. The wall movement filter was set low (< 100 Hz). Acquisition of the Doppler blood-flow

velocity waveforms was performed during fetal quiescence. In the center in which more than one operator performed ultrasound measurements, all ultrasound images were stored, and randomly selected anonymized images were reviewed regularly to ensure quality.

Using the software available in the ultrasound systems, Doppler blood-flow velocity waveforms were traced automatically. PI was calculated as (peak systolic velocity – end-diastolic velocity)/time-averaged maximum velocity, and averaged for three or more consecutive cardiac cycles by the software and displayed on the screen. Sensitivity of the trace was adjusted if required and, when the angle was not 0°, angle correction was used to measure the velocities. UCR was calculated as the ratio of UA-PI to MCA-PI, and CPR was calculated as the ratio of MCA-PI to UA-PI.

To ensure the safety of the ultrasonography, the ALARA (as low as reasonably achievable) principle was used. Total scanning time never exceeded 60 min and mechanical indices and thermal for bone indices were always kept below 1.9 and 1.5, respectively; in the majority of the sessions, they were below 1.0.

The course and outcome of pregnancy were recorded prospectively. Gestational age at birth, mode of delivery, sex of the neonate, birth weight, placental weight and Apgar score were obtained from the electronic medical records. All neonates were examined once by a pediatrician during the first 3 postnatal days, and any abnormality was noted.

Sample size estimation

To construct gestational-age-specific reference ranges with adequate precision, 15 participants per gestational week has been suggested for cross-sectional studies of fetal biometry[31], which would result in a total of 330 observations covering a period of 19 to 41 weeks. The corresponding number of fetuses of either sex required for the purpose of establishing sex-specific longitudinal reference ranges can be calculated as 330/2.3 (i.e. 143 fetuses of each sex), where 2.3 is the design factor as suggested by Royston and Altman[32,33]. Thus, we estimated that a sample population of approximately 286 would be adequate to construct sex-specific reference ranges. The calculation of sample size required to construct sex-specific reference curves was performed *post hoc*.

Statistical analysis

Statistical analysis of data was performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA) and MLWin Version 3.01 (MLWin, Centre for Multilevel Modelling, University of Bristol, Bristol, UK). The distribution of data was checked for normality and power transformations were performed if required in order to best meet the criteria of a normal distribution. Fractional polynomials were used to achieve best-fitting curves in relation to gestational age for CPR and UCR. We used multilevel modeling to construct gestational-age-specific reference percentiles from each fitted model[33,34], which takes into account the fact that the repeated measurements within individuals are not independent. The 2.5th, 5th, 10th and 25th percentiles were calculated by subtracting 1.96 SD, 1.645 SD, 1.282 SD and 0.674 SD from the means, respectively, and the 97.5th, 95th, 90th and 75th percentiles were calculated by adding similar SD multiples, respectively. 95% CIs were calculated for the 5th, 50th and 95th percentiles. The conditional reference intervals were calculated from the conditional mean and variance and the level-2 (fetus) covariance; the level-1 (measurement) covariance is assumed to be zero[33]. Comparison of gestational-age-specific mean *Z*-scores of CPR and UCR between male and female fetuses was performed using the independent samples *t*-test for continuous variables. Comparison of these Doppler ratios between male and female fetuses was performed for each gestational week. To test homogeneity of variances for UCR and CPR across centers and fetal sexes, we compared medians of level-1 (measurement) and level-2 (fetus) variances using the independent samples Mann–Whitney *U*-test; statistical significance was set at a two-tailed P of < 0.05.

The study protocols were approved by the Regional Committees for Medical and Health Research Ethics – West and North Norway (REK Vest no. 203.03 and REK Nord 105/2008), and written informed consent was obtained from each participant.

RESULTS

In total, 299 women with a low-risk singleton pregnancy were recruited to the study. The baseline (at recruitment) demographic and clinical characteristics of the study population and outcome data of the pregnancies are presented in Table 1. Of the recruited women, 15 were excluded owing to missing data (lack of paired UA-PI and MCA-PI measurements), leaving 284 pregnancies in the final analysis. There were 148 male and 136 female fetuses. We were able to record Doppler velocity waveforms from both the MCA and UA in 979 out of 1218 (80.4%) observations.

Reference charts for CPR and UCR for both sexes combined, with fitted means and 5th and 95th percentiles with 95% CIs for the same variables, are shown in Figure 1. Gestational age-specific reference values for both sexes combined, and for male and female fetuses separately, with corresponding 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97.5th percentiles, are presented in Tables 2–7.

Regression equations and terms for calculating means and variances as well as unconditional and conditional (expected mean and SD based on a previous measurement) reference ranges for CPR and UCR are presented in Appendices S1 and S2, respectively. A calculator for computing gestational-age-specific unconditional and conditional centiles as well as means and *Z*-scores is provided as a simple practical tool for routine clinical use (Appendix S3).

Mean CPR increased from 1.20 at 19 weeks' gestation, peaking at 2.31 at 33 weeks, and then decreased to 1.82 at 40 weeks. Mean UCR decreased from 0.83 at 19 weeks,

Table 1 Baseline characteristics and outcome in study population of 299 singleton pregnancies

Variable	*N*	*Value*
Maternal age (years)	299	30 (19–40)
Maternal weight (kg)*	293	65 (43–122)
Maternal height (cm)	296	167 (150–183)
Maternal BMI (kg/m^2)*	291	23.03 (18.01–40.76)
Nulliparous	299	143 (47.8)
Smoker	299	7 (2.3)
Gestational age at birth (days)	297	282 (234–298)
< 37 weeks		8 (2.7)
< 34 weeks		1 (0.3)
Pre-eclampsia	298	5 (1.7)
Mode of delivery	298	
Normal vaginal delivery		247 (82.9)
Operative vaginal delivery		14 (4.7)
Cesarean section		35 (11.7)
Breech vaginal delivery		2 (0.7)
Birth weight (g)	298	3630 (2251–4980)
Small-for-gestational age	298	15 (5.0)
Large-for-gestational age	298	17 (5.7)
Neonatal length (cm)	293	50 (44–55)
Ponderal index	293	28.1 (20.8–37.5)
Neonatal sex	298	
Male		156 (52.3)
Female		142 (47.7)
1-min Apgar score < 7	296	13 (4.4)
5-min Apgar score < 7	297	3 (1.0)
NICU admission	298	14 (4.7)
Placental weight (g)	287	650 (350–1200)
Umbilical cord length (cm)	253	60 (25–115)

Data are presented as median (range) or *n* (%). *N*-values differ between variables because of missing data. One participant failed to attend after the first examination; electronic medical records indicate that she died, but no other clinical details could be found. *Maternal weight and body mass index (BMI) at first examination. NICU, neonatal intensive care unit.

with the lowest value of 0.43 at 33 weeks, and then increased to 0.55 at 40 weeks.

Female fetuses had lower CPR and slightly higher UCR than did male fetuses. The mean difference in CPR *Z*-score between female and male fetuses was −0.17408 (95% CI, −0.29958 to −0.04858) and that of UCR *Z*-score was 0.17414 (95% CI, 0.04857–0.2992) ($P = 0.007$ for both). The differences were more pronounced earlier in gestation ($P = 0.006$ for both) and were not statistically significant after 33 weeks ($P = 0.42$ for both) (Figure 2). There was no association between MCA-PI, CPR or UCR and fetal head circumference or estimated fetal weight. Using the Mann–Whitney *U*-test, we found similar medians of level-1 (measurement) and level-2 (fetal) variances between the two centers and fetal sexes ($P > 0.05$ for all).

DISCUSSION

Principal findings

This study has established longitudinal reference ranges for CPR and UCR and has shown that these indices differ between male and female fetuses during the second half of pregnancy. We have provided sex-specific reference charts and tables of these Doppler indices based on an adequate sample size, and terms for calculating conditional reference ranges that will allow serial evaluation of the relationship between cerebral and placental blood flow impedances, helping to detect fetal brain-sparing.

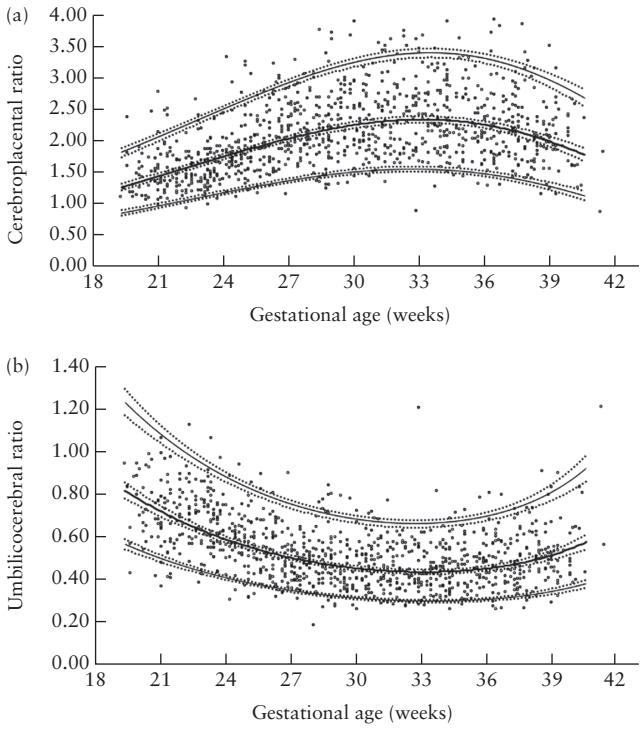

Figure 1 Longitudinal reference ranges of cerebroplacental ratio (a) and umbilicocerebral ratio (b) in second half of pregnancy for both sexes combined. ——, 5th, 50th and 95th percentiles; ·····, 95% CIs.

Strengths and limitations

This study had a prospective longitudinal design and a large enough sample size to allow construction of reference intervals with adequate precision for both sexes. Cross-sectional reference ranges are not optimal for serial assessment of fetal wellbeing. Longitudinal studies have more power and are more efficient than are cross-sectional studies[35]. However, only one longitudinal study with an adequate sample size has previously reported CPR[24], namely the Bergen part of the present study, but sex differences were not examined. In a recent systematic review of studies reporting reference ranges for CPR[36], this study[24] was rated among the top three with the highest methodological quality, which is reassuring. In our study, we applied similar methodology in both centers. We tested for homogeneity of variance of measurements, and found no evidence of systematic differences in distribution between centers or sexes. Three other studies with a sufficient number of observations per gestational week have been published previously, but all of them had a retrospective cross-sectional design[37–39].

By combining data from two centers, we were able to achieve a sufficient number of observations in the early part of mid-gestation and late term to construct reliable reference ranges for these gestational weeks.

A limitation of our study is that the pregnancies were not dated by first-trimester ultrasound, but gestational age was confirmed by second-trimester fetal biometry. This is because first-trimester dating ultrasound is not a routine practice in Norway and it is offered only to women with high-risk pregnancies.

Interpretation of results

CPR increased during gestational weeks 19–33, then decreased gradually until 41 weeks. UCR had the opposite trend. A similar trend in CPR has been reported previously in a longitudinal study and some cross-sectional studies[24,39,40]. However, our longitudinal reference percentiles are different from those based on cross-sectional data[37,38,40], and we found significant sex-related differences.

Table 2 Longitudinal reference percentiles of cerebroplacental ratio in male fetuses

Gestational age (weeks)	*2.5th percentile*	*5th percentile*	*10th percentile*	*25th percentile*	*50th percentile*	*75th percentile*	*90th percentile*	*95th percentile*	*97.5th percentile*
19	0.74	0.81	0.90	1.06	1.27	1.50	1.74	1.89	2.03
20	0.80	0.88	0.97	1.15	1.36	1.61	1.86	2.02	2.17
21	0.87	0.95	1.05	1.23	1.46	1.72	1.98	2.15	2.31
22	0.94	1.02	1.13	1.32	1.56	1.83	2.11	2.29	2.45
23	1.00	1.09	1.20	1.41	1.66	1.95	2.24	2.43	2.60
24	1.07	1.16	1.28	1.49	1.76	2.06	2.37	2.56	2.74
25	1.13	1.23	1.36	1.58	1.86	2.17	2.49	2.69	2.88
26	1.20	1.30	1.43	1.66	1.95	2.28	2.61	2.82	3.02
27	1.25	1.36	1.49	1.74	2.04	2.38	2.72	2.94	3.14
28	1.30	1.41	1.55	1.80	2.12	2.47	2.82	3.05	3.26
29	1.35	1.46	1.60	1.86	2.19	2.55	2.91	3.15	3.36
30	1.38	1.50	1.64	1.91	2.24	2.62	2.99	3.23	3.45
31	1.41	1.53	1.67	1.95	2.29	2.67	3.05	3.30	3.53
32	1.42	1.54	1.69	1.97	2.32	2.70	3.09	3.35	3.58
33	1.42	1.54	1.70	1.98	2.33	2.72	3.12	3.37	3.61
34	1.41	1.53	1.69	1.97	2.32	2.72	3.12	3.38	3.61
35	1.38	1.51	1.66	1.94	2.29	2.69	3.10	3.36	3.60
36	1.34	1.47	1.62	1.90	2.25	2.65	3.05	3.31	3.55
37	1.29	1.41	1.56	1.84	2.18	2.58	2.98	3.24	3.48
38	1.22	1.34	1.49	1.76	2.10	2.49	2.88	3.14	3.38
39	1.14	1.26	1.40	1.66	2.00	2.38	2.77	3.02	3.25
40	1.05	1.16	1.30	1.55	1.88	2.25	2.62	2.87	3.10

Table 3 Longitudinal reference percentiles of cerebroplacental ratio in female fetuses

Gestational age (weeks)	*2.5th percentile*	*5th percentile*	*10th percentile*	*25th percentile*	*50th percentile*	*75th percentile*	*90th percentile*	*95th percentile*	*97.5th percentile*
19	0.75	0.80	0.87	0.99	1.14	1.30	1.46	1.56	1.65
20	0.81	0.87	0.94	1.07	1.24	1.42	1.60	1.71	1.82
21	0.86	0.93	1.01	1.16	1.34	1.54	1.75	1.87	1.99
22	0.92	1.00	1.09	1.25	1.45	1.67	1.90	2.04	2.17
23	0.98	1.06	1.16	1.34	1.56	1.80	2.05	2.20	2.35
24	1.04	1.13	1.23	1.43	1.67	1.93	2.20	2.37	2.53
25	1.10	1.19	1.31	1.51	1.77	2.06	2.35	2.53	2.70
26	1.16	1.26	1.38	1.60	1.87	2.18	2.49	2.69	2.87
27	1.21	1.31	1.44	1.67	1.97	2.30	2.63	2.84	3.04
28	1.25	1.36	1.50	1.75	2.05	2.40	2.75	2.98	3.18
29	1.29	1.41	1.55	1.81	2.13	2.49	2.86	3.10	3.31
30	1.33	1.44	1.59	1.86	2.19	2.57	2.95	3.20	3.42
31	1.35	1.47	1.62	1.90	2.24	2.63	3.02	3.27	3.51
32	1.36	1.49	1.64	1.92	2.27	2.67	3.07	3.33	3.56
33	1.37	1.49	1.64	1.93	2.28	2.68	3.09	3.35	3.59
34	1.36	1.48	1.63	1.92	2.27	2.67	3.08	3.34	3.58
35	1.34	1.46	1.61	1.89	2.24	2.64	3.04	3.30	3.54
36	1.30	1.42	1.57	1.84	2.19	2.57	2.97	3.22	3.46
37	1.26	1.37	1.52	1.78	2.11	2.49	2.87	3.11	3.34
38	1.20	1.31	1.45	1.70	2.01	2.37	2.73	2.97	3.18
39	1.13	1.24	1.37	1.60	1.90	2.23	2.57	2.79	2.99
40	1.06	1.15	1.27	1.49	1.76	2.07	2.38	2.59	2.77

Placental weight[41], umbilical cord length[42] and UA-PI[25,26] are known to be affected by the sex of the fetus, with female fetuses having a smaller placenta, shorter cord and higher UA-PI, although the magnitude of these differences is small and may be modulated by parity and gestational age[26,41]. Male fetuses have a larger head circumference than do female fetuses[28]. Prior *et al.*[27] examined 388 term fetuses before the onset of active labor (cervical dilation < 3 cm) and reported CPR to be slightly higher in female than in male fetuses (1.81 *vs* 1.74), although the difference was not significant. In our study, we found mean CPR to be slightly lower in female than in male fetuses. We have reported similar findings in a cross-sectional study previously[25]. The discrepancy in findings between our study and those of the study of Prior *et al.*[27] could be related to differences in study design (longitudinal *vs* cross-sectional) and timing of examination (antenatal *vs* in early latent

Table 4 Longitudinal reference percentiles for cerebroplacental ratio in male and female fetuses combined

Gestational age (weeks)	*2.5th percentile*	*5th percentile*	*10th percentile*	*25th percentile*	*50th percentile*	*75th percentile*	*90th percentile*	*95th percentile*	*97.5th percentile*
19	0.73	0.80	0.88	1.02	1.20	1.40	1.61	1.74	1.86
20	0.79	0.86	0.95	1.10	1.30	1.52	1.74	1.88	2.01
21	0.86	0.93	1.02	1.19	1.40	1.64	1.87	2.03	2.17
22	0.92	1.00	1.10	1.28	1.50	1.76	2.01	2.17	2.32
23	0.99	1.07	1.18	1.37	1.61	1.88	2.15	2.32	2.48
24	1.05	1.14	1.25	1.46	1.71	2.00	2.29	2.47	2.64
25	1.12	1.21	1.33	1.55	1.81	2.12	2.42	2.62	2.80
26	1.17	1.28	1.40	1.63	1.91	2.23	2.55	2.76	2.95
27	1.23	1.34	1.47	1.71	2.00	2.34	2.67	2.89	3.09
28	1.28	1.39	1.53	1.78	2.09	2.44	2.79	3.01	3.22
29	1.32	1.44	1.58	1.84	2.16	2.52	2.89	3.12	3.34
30	1.36	1.47	1.62	1.89	2.22	2.59	2.97	3.21	3.44
31	1.38	1.50	1.65	1.92	2.26	2.65	3.04	3.29	3.52
32	1.39	1.52	1.67	1.95	2.29	2.69	3.08	3.34	3.57
33	1.39	1.52	1.67	1.95	2.31	2.70	3.10	3.36	3.60
34	1.38	1.51	1.66	1.94	2.30	2.70	3.10	3.36	3.60
35	1.36	1.48	1.63	1.92	2.27	2.67	3.07	3.33	3.57
36	1.32	1.44	1.59	1.87	2.22	2.62	3.02	3.28	3.51
37	1.27	1.39	1.54	1.81	2.15	2.54	2.93	3.19	3.42
38	1.21	1.32	1.46	1.73	2.06	2.44	2.82	3.07	3.30
39	1.13	1.24	1.38	1.63	1.95	2.31	2.68	2.92	3.15
40	1.04	1.15	1.28	1.52	1.82	2.17	2.52	2.75	2.96

Table 5 Longitudinal reference percentiles for umbilicocerebral ratio in male fetuses

Gestational age (weeks)	*2.5th percentile*	*5th percentile*	*10th percentile*	*25th percentile*	*50th percentile*	*75th percentile*	*90th percentile*	*95th percentile*	*97.5th percentile*
19	0.49	0.53	0.58	0.67	0.79	0.94	1.11	1.23	1.35
20	0.46	0.50	0.54	0.62	0.73	0.87	1.03	1.14	1.24
21	0.43	0.46	0.50	0.58	0.68	0.81	0.95	1.05	1.15
22	0.41	0.44	0.47	0.55	0.64	0.76	0.89	0.98	1.07
23	0.39	0.41	0.45	0.51	0.60	0.71	0.83	0.91	1.00
24	0.36	0.39	0.42	0.48	0.57	0.67	0.78	0.86	0.93
25	0.35	0.37	0.40	0.46	0.54	0.63	0.74	0.81	0.88
26	0.33	0.35	0.38	0.44	0.51	0.60	0.70	0.77	0.84
27	0.32	0.34	0.37	0.42	0.49	0.58	0.67	0.74	0.80
28	0.31	0.33	0.35	0.41	0.47	0.55	0.64	0.71	0.77
29	0.30	0.32	0.34	0.39	0.46	0.54	0.62	0.68	0.74
30	0.29	0.31	0.33	0.38	0.45	0.52	0.61	0.67	0.72
31	0.28	0.30	0.33	0.37	0.44	0.51	0.60	0.66	0.71
32	0.28	0.30	0.32	0.37	0.43	0.51	0.59	0.65	0.71
33	0.28	0.30	0.32	0.37	0.43	0.51	0.59	0.65	0.70
34	0.28	0.30	0.32	0.37	0.43	0.51	0.59	0.65	0.71
35	0.28	0.30	0.32	0.37	0.44	0.52	0.60	0.66	0.72
36	0.28	0.30	0.33	0.38	0.44	0.53	0.62	0.68	0.75
37	0.29	0.31	0.34	0.39	0.46	0.54	0.64	0.71	0.78
38	0.30	0.32	0.35	0.40	0.48	0.57	0.67	0.75	0.82
39	0.31	0.33	0.36	0.42	0.50	0.60	0.71	0.80	0.87
40	0.32	0.35	0.38	0.45	0.53	0.64	0.77	0.86	0.95

phase of labor) and gestational age (19–41 weeks *vs* 37–42 weeks).

Clinical implications

Relatively low pO_2 and higher pCO_2 of the fetal blood facilitate cerebral blood flow *in utero* by reducing cerebrovascular impedance[43]. This fetal brain-sparing response to hypoxemia is well known and has been experimentally validated[44]. However, direct invasive measurement of blood-flow volume to the human fetal brain and placenta cannot, of course, be performed for ethical reasons, and non-invasive measurements using Doppler are perceived as difficult and error prone, although technically possible[7,45]. Therefore, the ratio between the surrogate indices of cerebral and placental

Table 6 Longitudinal reference percentiles for umbilicocerebral ratio in female fetuses

Gestational age (weeks)	*2.5th percentile*	*5th percentile*	*10th percentile*	*25th percentile*	*50th percentile*	*75th percentile*	*90th percentile*	*95th percentile*	*97.5th percentile*
19	0.60	0.64	0.69	0.77	0.88	1.01	1.15	1.24	1.34
20	0.55	0.58	0.63	0.70	0.81	0.93	1.06	1.15	1.24
21	0.50	0.53	0.57	0.65	0.75	0.86	0.99	1.07	1.16
22	0.46	0.49	0.53	0.60	0.69	0.80	0.92	1.00	1.08
23	0.43	0.45	0.49	0.55	0.64	0.75	0.86	0.94	1.02
24	0.40	0.42	0.45	0.52	0.60	0.70	0.81	0.89	0.96
25	0.37	0.39	0.43	0.49	0.56	0.66	0.77	0.84	0.91
26	0.35	0.37	0.40	0.46	0.53	0.63	0.73	0.80	0.86
27	0.33	0.35	0.38	0.44	0.51	0.60	0.69	0.76	0.83
28	0.31	0.34	0.36	0.42	0.49	0.57	0.67	0.73	0.80
29	0.30	0.32	0.35	0.40	0.47	0.55	0.65	0.71	0.77
30	0.29	0.31	0.34	0.39	0.46	0.54	0.63	0.69	0.75
31	0.29	0.31	0.33	0.38	0.45	0.53	0.62	0.68	0.74
32	0.28	0.30	0.33	0.38	0.44	0.52	0.61	0.67	0.73
33	0.28	0.30	0.32	0.37	0.44	0.52	0.61	0.67	0.73
34	0.28	0.30	0.33	0.37	0.44	0.52	0.61	0.68	0.74
35	0.28	0.30	0.33	0.38	0.45	0.53	0.62	0.69	0.75
36	0.29	0.31	0.34	0.39	0.46	0.54	0.64	0.70	0.77
37	0.30	0.32	0.35	0.40	0.47	0.56	0.66	0.73	0.80
38	0.31	0.34	0.37	0.42	0.50	0.59	0.69	0.76	0.83
39	0.33	0.36	0.39	0.45	0.53	0.62	0.73	0.81	0.88
40	0.36	0.39	0.42	0.48	0.57	0.67	0.79	0.87	0.95

Table 7 Longitudinal reference percentiles for umbilicocerebral ratio in male and female fetuses combined

Gestational age (weeks)	*2.5th percentile*	*5th percentile*	*10th percentile*	*25th percentile*	*50th percentile*	*75th percentile*	*90th percentile*	*95th percentile*	*97.5th percentile*
19	0.54	0.58	0.62	0.71	0.83	0.98	1.14	1.26	1.37
20	0.50	0.53	0.58	0.66	0.77	0.91	1.05	1.16	1.26
21	0.46	0.49	0.53	0.61	0.71	0.84	0.98	1.07	1.17
22	0.43	0.46	0.50	0.57	0.66	0.78	0.91	1.00	1.08
23	0.40	0.43	0.47	0.53	0.62	0.73	0.85	0.93	1.01
24	0.38	0.40	0.44	0.50	0.58	0.69	0.80	0.87	0.95
25	0.36	0.38	0.41	0.47	0.55	0.65	0.75	0.83	0.90
26	0.34	0.36	0.39	0.45	0.52	0.61	0.71	0.78	0.85
27	0.32	0.35	0.37	0.43	0.50	0.59	0.68	0.75	0.81
28	0.31	0.33	0.36	0.41	0.48	0.56	0.66	0.72	0.78
29	0.30	0.32	0.35	0.40	0.46	0.54	0.63	0.70	0.76
30	0.29	0.31	0.34	0.39	0.45	0.53	0.62	0.68	0.74
31	0.28	0.30	0.33	0.38	0.44	0.52	0.61	0.67	0.72
32	0.28	0.30	0.32	0.37	0.44	0.51	0.60	0.66	0.72
33	0.28	0.30	0.32	0.37	0.43	0.51	0.60	0.66	0.72
34	0.28	0.30	0.32	0.37	0.44	0.51	0.60	0.66	0.72
35	0.28	0.30	0.33	0.37	0.44	0.52	0.61	0.67	0.74
36	0.28	0.31	0.33	0.38	0.45	0.53	0.63	0.69	0.76
37	0.29	0.31	0.34	0.39	0.47	0.55	0.65	0.72	0.79
38	0.30	0.33	0.35	0.41	0.49	0.58	0.68	0.76	0.83
39	0.32	0.34	0.37	0.43	0.51	0.61	0.73	0.81	0.89
40	0.34	0.36	0.40	0.46	0.55	0.66	0.78	0.87	0.96

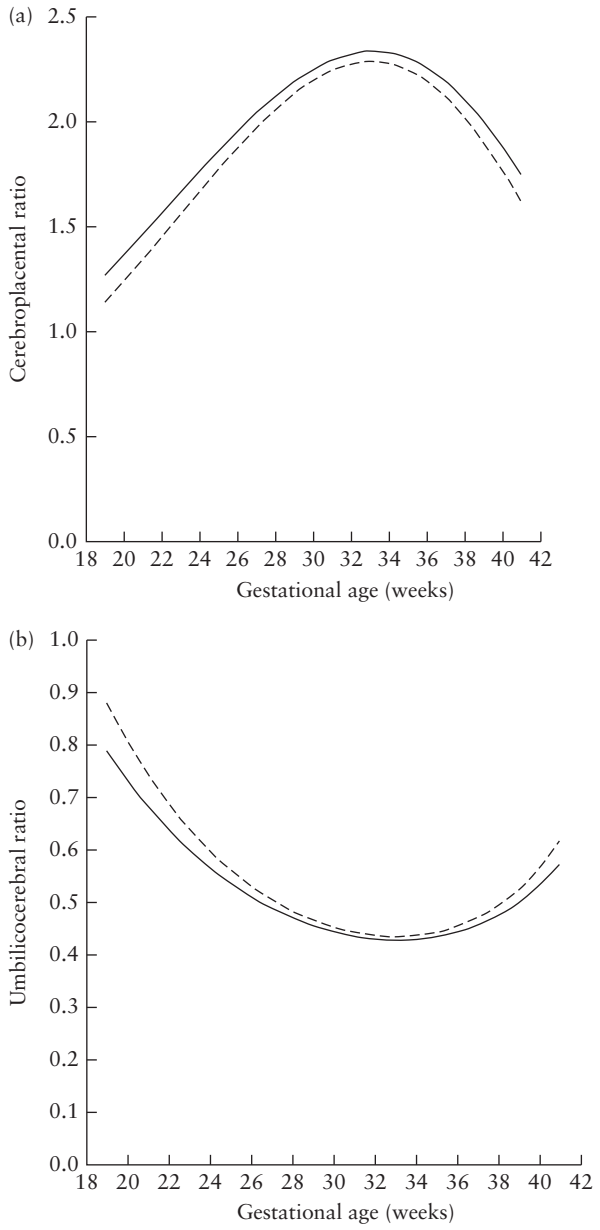

Figure 2 Cerebroplacental ratio (a) and umbilicocerebral ratio (b) in second half of pregnancy according to gestational age in male (——) and female (– – -) fetuses.

vascular impedances has been used in clinical practice to reflect the degree of brain-sparing.

UA Doppler alone is a poor discriminator of FGR in late gestation, and abnormal CPR is suggested as the most reliable Doppler measure of late-onset FGR[12,46]. CPR has been validated experimentally in sheep fetuses as a sensitive marker of acute reduction in pO_2[44]. However, abnormal CPR can result from increased placental, or reduced cerebrovascular, impedance, or both. The ratio may be abnormal even when both UA-PI and MCA-PI are within the normal ranges[13]. Thus, abnormal CPR has to be defined. A fixed CPR cut-off value ranging from 1.0 to 1.08 has been suggested by some[13,47,48], while others recommend the use of gestational-age-specific reference percentiles to define abnormality[40]. In our study, serial evaluation of CPR and UCR showed their dependence on gestational age; therefore, we recommend the use of longitudinal reference ranges rather than a fixed cut-off.

Validity of findings

All ultrasound examinations were performed by qualified physicians with appropriate training and adequate experience, and no participant was lost to follow-up. Once included, women were not excluded for any complication occurring during the course of pregnancy in order to avoid a 'supernormal' population. Only 5% of neonates had a birth weight > 90th percentile and 5.7% had a birth weight < 10th percentile, based on Norwegian birth-weight centiles[49], and, postnatally, none was diagnosed with a congenital abnormality. As our study was performed in two centers over two different time periods, study site could be considered as a third level in the multilevel model. We analyzed our data including this third level in the regression model, in addition to the measurement occasion (level 1) and fetus (level 2). However, it did not add significantly to the variance; therefore, it was not included in the final model. The baseline characteristics and pregnancy outcome data confirm that the population studied was representative of the Scandinavian population. One could argue that the findings from a relatively homogeneous Nordic population cannot be generalizable to other multi-ethnic populations, but Doppler blood-flow studies in normal pregnancies are less likely to be affected by ethnic differences[50].

Conclusions

We have established longitudinal reference ranges for CPR and UCR that are suitable for serial monitoring of the fetal brain-sparing phenomenon, with the possibility of refining the assessment by using fetal sex-specific ranges and conditioning by a previous measurement. Significant sex-related differences were present in CPR and UCR during the second half of normal pregnancies. Therefore, the use of sex-specific reference ranges might provide more precise physiological information. However, as the magnitude of the differences was small, further studies are needed to ascertain the clinical value of using sex-specific reference ranges.

ACKNOWLEDGMENTS

We would like to thank Åse Vårtun, Bodil Hvingel and Norbert Szunyogh for their help with the recruitment and examination of pregnant women. This research was supported by the Northern Norway Regional Health Authority (Grant No. SFP873/ID1646) and Western Norway Regional Health Authority (Grant No. 911160). The funders had no role in designing the study, collection, analysis and interpretation of data, writing of the report, or in the decision to submit the article for publication.

REFERENCES

1. Papile LA, Rudolph AM, Heymann MA. Autoregulation of cerebral blood flow in the preterm fetal lamb. *Pediatr Res* 1985; **19**: 159–161.
2. Purves MJ, James IM. Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb. *Circ Res* 1969; **25**: 651–667.
3. Pearce W. Hypoxic regulation of the fetal cerebral circulation. *J Appl Physiol* 2006; **100**: 731–738.
4. Ashwal S, Dale PS, Longo LD. Regional cerebral blood flow: studies in the fetal lamb during hypoxia, hypercapnia, acidosis, and hypotension. *Pediatr Res* 1984; **18**: 1309–1316.
5. Peeters LL, Sheldon RE, Jones MD Jr, Makowski EL, Meschia G. Blood flow to fetal organs as a function of arterial oxygen content. *Am J Obstet Gynecol* 1979; **135**: 637–646.
6. Vyas S, Nicolaides KH, Bower S, Campbell S. Middle cerebral artery flow velocity waveforms in fetal hypoxaemia. *Br J Obstet Gynaecol* 1990; **97**: 797–803.
7. Kiserud T, Ebbing C, Kessler J, Rasmussen S. Fetal cardiac output, distribution to the placenta and impact of placental compromise. *Ultrasound Obstet Gynecol* 2006; **28**: 126–136.
8. Trudinger BJ, Stevens D, Connelly A, Hales JR, Alexander G, Bradley L, Fawcett A, Thompson RS. Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation. *Am J Obstet Gynecol* 1987; **157**: 1443–1448.
9. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. *Am J Obstet Gynecol* 1974; **120**: 817–824.
10. Jensen A, Berger R. Fetal circulatory responses to oxygen lack. *J Dev Physiol* 1991; **16**: 181–207.
11. Arbeille P, Roncin A, Berson M, Patat F, Pourcelot L. Exploration of the fetal cerebral blood flow by duplex Doppler–linear array system in normal and pathological pregnancies. *Ultrasound Med Biol* 1987; **13**: 329–337.
12. DeVore GR. The importance of the cerebroplacental ratio in the evaluation of fetal well-being in SGA and AGA fetuses. *Am J Obstet Gynecol* 2015; **213**: 5–15.
13. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral–umbilical Doppler ratio as a predictor of adverse perinatal outcome. *Obstet Gynecol* 1992; **79**: 416–420.
14. Bahado-Singh RO, Kovanci E, Jeffres A, Oz U, Deren O, Copel J, Mari G. The Doppler cerebroplacental ratio and perinatal outcome in intrauterine growth restriction. *Am J Obstet Gynecol* 1999; **180**: 750–756.
15. Prior T, Mullins E, Bennett P, Kumar S. Prediction of intrapartum fetal compromise using the cerebroumbilical ratio: a prospective observational study. *Am J Obstet Gynecol* 2013; **208**: 124.e1–6.
16. Khalil AA, Morales-Rosello J, Morlando M, Hannan H, Bhide A, Papageorghiou A, Thilaganathan B. Is fetal cerebroplacental ratio an independent predictor of intrapartum fetal compromise and neonatal unit admission? *Am J Obstet Gynecol* 2015; **213**: 54.e1–54.e10.
17. Sirico A, Diemert A, Glosemeyer P, Hecher K. Prediction of adverse perinatal outcome by cerebroplacental ratio adjusted for estimated fetal weight. *Ultrasound Obstet Gynecol* 2018; **51**: 381–386.
18. Vollgraff Heidweiller-Schreurs CA, De Boer MA, Heymans MW, Schoonmade LJ, Bossuyt PMM, Mol BWJ, De Groot CJM, Bax CJ. Prognostic accuracy of cerebroplacental ratio and middle cerebral artery Doppler for adverse perinatal outcome: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018; **51**: 313–322.
19. Stampalija T, Arabin B, Wolf H, Bilardo CM, Lees C; TRUFFLE investigators. Is middle cerebral artery Doppler related to neonatal and 2-year infant outcome in early fetal growth restriction? *Am J Obstet Gynecol* 2017; **216**: 521.e1–521.e13.
20. Dunn L, Sherrell H, Kumar S. Systematic review of the utility of the fetal cerebroplacental ratio measured at term for the prediction of adverse perinatal outcome. *Placenta* 2017; **54**: 68–75.
21. Conde-Agudelo A, Villar J, Kennedy SH, Papageorghiou AT. Predictive accuracy of cerebroplacental ratio for adverse perinatal and neurodevelopmental outcomes in suspected fetal growth restriction: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018; **52**: 430–441.
22. Hecher K, Spernol R, Stettner H, Szalay S. Potential for diagnosing imminent risk to appropriate- and small-for-gestational-age fetuses by Doppler sonographic examination of umbilical and cerebral arterial blood flow. *Ultrasound Obstet Gynecol* 1992; **2**: 266–271.
23. Piazze J, Padula F, Cerekja A, Cosmi EV, Anceschi MM. Prognostic value of umbilical–middle cerebral artery pulsatility index ratio in fetuses with growth restriction. *Int J Gynaecol Obstet* 2005; **91**: 233–237.
24. Ebbing C, Rasmussen S, Kiserud T. Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: longitudinal reference ranges and terms for serial measurements. *Ultrasound Obstet Gynecol* 2007; **30**: 287–296.
25. Widnes C, Flo K, Acharya G. Exploring sexual dimorphism in placental circulation at 22–24 weeks of gestation: A cross-sectional observational study. *Placenta* 2017; **49**: 16–22.
26. Widnes C, Flo K, Wilsgaard T, Kiserud T, Acharya G. Sex differences in umbilical artery Doppler indices: a longitudinal study. *Biol Sex Differ* 2018; **9**: 16.
27. Prior T, Wild M, Mullins E, Bennett P, Kumar S. Sex specific differences in fetal middle cerebral artery and umbilical venous Doppler. *PLoS One* 2013; **8**: e56933.
28. Johnsen SL, Rasmussen S, Sollien R, Kiserud T. Fetal age assessment based on ultrasound head biometry and the effect of maternal and fetal factors. *Acta Obstet Gynecol Scand* 2004; **83**: 716–723.
29. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of Fetal Weight with the Use of Head, Body and Femur Measurements – A Prospective Study. *Am J Obstet Gynecol* 1985; **151**: 333–337.
30. Acharya G, Wilsgaard T, Berntsen GK, Maltau JM, Kiserud T. Reference ranges for serial measurements of umbilical artery Doppler indices in the second half of pregnancy. *Am J Obstet Gynecol* 2005; **192**: 937–944.
31. Altman DG, Chitty LS. Charts of Fetal Size. 1. Methodology. *Br J Obstet Gynaecol* 1994; **101**: 29–34.
32. Royston P, Altman DG. Design and analysis of longitudinal studies of fetal size. *Ultrasound Obstet Gynecol* 1995; **6**: 307–312.
33. Royston P. Calculation of unconditional and conditional reference intervals for foetal size and growth from longitudinal measurements. *Stat Med* 1995; **14**: 1417–1436.
34. Royston P, Altman DG. Regression Using Fractional Polynomials of Continuous Covariates: Parsimonious Parametric Modelling. *Appl Statist* 1994; **43**: 429–467.
35. Ohuma EO, Altman DG; International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st Project). Design and other methodological considerations for the construction of human fetal and neonatal size and growth charts. *Stat Med* 2019; **38**: 3527–3539.
36. Oros D, Ruiz-Martinez S, Staines-Urias E, Conde-Agudelo A, Villar J, Fabre E, Papageorghiou AT. Reference ranges for Doppler indices of umbilical and fetal middle cerebral arteries and cerebroplacental ratio: systematic review. *Ultrasound Obstet Gynecol* 2019; **53**: 454–464.
37. Arduini D, Rizzo G. Normal values of pulsatility index from fetal vessels: a cross–sectional study on 1556 healthy fetuses. *J Perinat Med* 1990; **18**: 165–172.
38. Kurmanavicius J, Florio I, Wisser J, Hebisch G, Zimmermann R, Müller R, Huch R, Huch A. Reference resistance indices of the umbilical, fetal middle cerebral and uterine arteries at 24–42 weeks of gestation. *Ultrasound Obstet Gynecol* 1997; **10**: 112–120.
39. Ciobanu A, Wright A, Syngelaki A, Wright D, Akolekar R, Nicolaides KH. Fetal Medicine Foundation reference ranges for umbilical artery and middle cerebral artery pulsatility index and cerebroplacental ratio. *Ultrasound Obstet Gynecol* 2019; **53**: 465–472.
40. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. *Ultrasound Obstet Gynecol* 2003; **21**: 124–127.
41. Wallace JM, Bhattacharya S, Horgan GW. Gestational age, gender and parity specific centile charts for placental weight for singleton deliveries in Aberdeen, UK. *Placenta* 2013; **34**: 269–274.
42. Linde LE, Rasmussen S, Kessler J, Ebbing C. Extreme umbilical cord lengths, cord knot and entanglement: risk factors and risk of adverse outcomes, a population-based study. *PLoS One* 2018; **13**: e0194814.
43. Giussani DA. The fetal brain sparing response to hypoxia: physiological mechanisms. *J Physiol* 2016; **594**: 1215–1230.
44. Arbeille P, Maulik D, Fignon A, Stale H, Berson M, Bodard S, Locatelli A. Assessment of the fetal PO2 changes by cerebral and umbilical Doppler on lamb fetuses during acute hypoxia. *Ultrasound Med Biol* 1995; **21**: 861–870.
45. Vimpeli T, Huhtala H, Wilsgaard T, Acharya G. Fetal aortic isthmus blood flow and the fraction of cardiac output distributed to the upper body and brain at 11–20 weeks of gestation. *Ultrasound Obstet Gynecol* 2009; **33**: 538–544.
46. Nassr AA, Abdelmagied AM, Shazly SA. Fetal cerebro-placental ratio and adverse perinatal outcome: systematic review and meta-analysis of the association and diagnostic performance. *J Perinat Med* 2016; **44**: 249–256.
47. Devine PA, Bracero LA, Lysikiewicz A, Evans R, Womack S, Byrne DW. Middle cerebral to umbilical artery Doppler ratio in post-date pregnancies. *Obstet Gynecol* 1994; **84**: 856–860.
48. Arias F. Accuracy of the middle-cerebral-to-umbilical-artery resistance index ratio in the prediction of neonatal outcome in patients at high risk for fetal and neonatal complications. *Am J Obstet Gynecol* 1994; **171**: 1541–1545.
49. Skærven R, Gjessing HK, Bakketeig LS. Birthweight by gestational age in Norway. *Acta Obstet Gynecol Scand* 2000; **79**: 440–449.
50. Jacquemyn Y, Verdonk P. Doppler ultrasound of the fetomaternal circulation: a preliminary study on differences between ethnic groups. *Clin Exp Obstet Gynecol* 2001; **28**: 277–279.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Appendix S1 Regression equations for cerebroplacental ratio and terms for calculating means and variances and unconditional and conditional reference ranges

Appendix S2 Regression equations for umbilicocerebral ratio and terms for calculating means and variances and unconditional and conditional reference ranges

Appendix S3 Calculators for centiles of cerebroplacental ratio and umbilicocerebral ratio and conditional centiles