The relationship between human placental morphometry and ultrasonic measurements of utero-placental blood flow and fetal growth.

N. Salavati [a,b,1]
Dr. U. Sovio[a,1]
R. Plitman Mayo[c,d]
Prof. D.S. Charnock-Jones[a,c]
Prof. G.C.S. Smith[a,c,2]

[a]Department of Obstetrics and Gynaecology, University of Cambridge; NIHR Cambridge Comprehensive Biomedical Research Centre, Cambridge, UK.
[b]Department of Obstetrics and Gynaecology, University Medical Centre of Groningen, University of Groningen, The Netherlands.
[c]Centre for Trophoblast Research (CTR), Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK.
[d]Department of Engineering, University of Cambridge, Cambridge, UK.

[1]N. Salavati and U. Sovio contributed equally to this work.

[2]Corresponding author:
Prof GCS Smith, Department of Obstetrics and Gynaecology, University of Cambridge, Box 223 The Rosie Hospital, Cambridge, CB2 0SW, UK.
Tel: +44 (0)1223 336871, Fax: +44 (0)1223 215327, E-mail: gcss2@cam.ac.uk

Email addresses: n.salavati@umcg.nl (NS), us253@medschl.cam.ac.uk (US), rp485@cam.ac.uk (RPM), dscj1@cam.ac.uk (DSCJ), gcss2@cam.ac.uk (GCS)

Abstract

Introduction: Ultrasonic fetal biometry and arterial Doppler flow velocimetry are widely used to assess the risk of pregnancy complications. There is an extensive literature on the relationship between pregnancy outcomes and the size and shape of the placenta. However, ultrasonic fetal biometry and arterial Doppler flow velocimetry have not previously been studied in relation to postnatal placental morphometry in detail.
Methods: We conducted a prospective cohort study of nulliparous women in The Rosie Hospital, Cambridge (UK). We studied a group of 2120 women who had complete data on uterine and umbilical Doppler velocimetry and fetal biometry at 20, 28 and 36 weeks' gestational age, digital images of the placenta available, and delivered a liveborn infant at term. Associations were expressed as the difference in the standard deviation (SD) score of the gestational age adjusted ultrasound measurement (z-score) comparing the lowest and highest decile of the given placental morphometric measurement.
Results: The lowest decile of placental surface area was associated with 0.87 SD higher uterine artery Doppler mean pulsatility index (PI) at 20 weeks (95% CI: 0.68 to 1.07, P<0.001). The lowest decile of placental weight was associated with 0.73 SD higher umbilical artery Doppler PI at 36 weeks (95% CI: 0.54 to 0.93, P<0.001). The lowest decile of both placental weight and placental area were associated with reduced growth velocity of the fetal abdominal circumference between 20 and 36 weeks (both P<0.001).
Conclusion: Placental area and weight are associated with uterine and umbilical blood flow, respectively, and both are associated with fetal growth rate.

Keywords: Placenta, morphometry, Doppler flow velocimetry, Fetal growth, Human

Abbreviations. AC, abdominal circumference; CCC, concordance correlation coefficient; CI, confidence interval; CV, coefficient of variation; FGR, fetal growth restriction; GV, growth velocity; LA, limits of agreement; PI, pulsatility index; POP, Pregnancy Outcome Prediction; SD, standard deviation; UmA, umbilical artery; UtA, uterine artery.

Introduction

Placental blood flow and gas and nutrient transport are major determinants of fetal growth [1]. The size, weight and shape of the placenta are all subject to wide variations [2] and placental size is related to its ability to transfer nutrients [2, 3]. Several studies have described the relationship between placental morphometry and adverse pregnancy outcome, including fetal growth restriction (FGR) [3]. Small placental size [4], decreased placental surface area [5] and small placental volume [6] have been associated with increased risk of FGR. Smaller surface area and a more oval shape are more common in pregnancies complicated by preeclampsia [6, 7]. Moreover, variations in size and placental thickness at birth are associated with increased rates of coronary disease and related disorders such as stroke, hypertension and type-2 diabetes in later life [8].

Placental function can be assessed *in vivo* by utero-placental Doppler flow velocimetry and fetal growth can be assessed by serial ultrasonic biometry. Prior to pregnancy, flow velocity waveforms in the uterine artery (UtA) tend to be high resistance, and the development of a low resistance pattern of flow in the first half of pregnancy is thought to be due to invasion of the maternal resistance vessels by the trophoblast [9, 10]. Persistence of high resistance patterns of flow in the UtA in mid-gestation has been associated with an increased risk of obstetric complications [11, 12]. The flow velocity waveform in the umbilical artery (UmA) is normally low resistance in the last trimester of pregnancy, and this is thought to reflect the development of the villous vascular tree [2]. A high resistance pattern of flow in the umbilical artery is widely used as an indicator of placental dysfunction, where the structural correlate of high resistance flow is maldevelopment of the tertiary villi [1].

Most studies on the inter-relationships between antenatal utero-placental Doppler blood flow velocimetry and the post-natal findings have focused on the microscopic and ultrastructural characteristics of the placenta and placental bed. However, utero-placental blood flow could also be related to the gross morphology of the placenta. We are unaware of any study employing an appropriate design, methodology and a sufficient sample size that aims to determine these relationships. In the present study, we analysed data from 2120 unselected women having first singleton

pregnancies who were recruited to a prospective cohort study. All women delivered at term and there were data from both serial blinded ultrasound scans, and a standardised series of digital images of each placenta obtained after the delivery. The aim of the study was to determine the inter-relationships between the size and shape of the placenta (assessed following birth), utero-placental Doppler flow velocimetry and the rate of growth of the fetal abdomen between 20 and 36 weeks' gestational age (GA).

Methods

Ethical Approval

Ethical approval for the study was given by the Cambridgeshire 2 Research Ethics Committee (reference number 07/H0308/163). All participants gave an informed written consent.

Overview and recruitment

The Pregnancy Outcome Prediction (POP) study was conducted at the Rosie Hospital, Cambridge (UK) and has been previously described in detail [13, 14]. In brief, the study design was a prospective cohort study. Nulliparous women with a viable singleton pregnancy who attended for their dating ultrasound scan at the hospital's ultrasound department between 14/08/2008 and 31/07/2012 were eligible. Women recruited to the study had follow-up research ultrasound scans at 20, 28 and 36 weeks of gestation. Following delivery, the fetal and maternal sides of the placenta were photographed and the placenta cut to 1cm thick strips which were also photographed. Subsequently, the results of the research ultrasound scans were un-blinded, and their associations with placental measurements assessed.

Study group

Among the participants of the POP study, the inclusion criteria for the present analysis were delivery of a liveborn baby at term (≥ 37 weeks' GA) and the availability of digital images of the placenta taken after delivery. Photographs of placentas were available for this study only from 01/10/2010. We excluded women who withdrew or were lost to follow up and women who had ultrasonic measurements missing from the 20 or 36 week scans.

Research ultrasonography

Methods of ultrasonic measurements have previously been described in detail [13, 14]. In brief, all study participants had UtA Doppler measurements at 20 weeks, UmA Doppler measurement at 36 weeks, and measurement of abdominal circumference (AC) at 20 weeks and 36 weeks for the purposes of research. The outcome of the research scans was not revealed to the women or the clinician, unless there was a clinically important finding at 36 weeks [14]. This occurred in 108 [5%] women in the

study group, 94 [87%] of them being previously undiagnosed breech presentation. GA was defined on the basis of early ultrasonographic examination, as recommended [15].

Ultrasonic measurements

Quantification of the UtA and UmA Doppler flow velocity waveforms was by the pulsatility index (PI). UtA PI was quantified as the mean PI of the left and right uterine arteries. Measurements were converted into GA-adjusted z-scores defined within the POP study [13], to adjust for minor variation in the exact GA at the scan. Abdominal circumference growth velocity (ACGV) was obtained by calculating the difference in AC z-score between the 20 week and 36 week scans [13].

Maternal and fetal characteristics

Several maternal characteristics were examined in relation to both placental and ultrasonic measurements to assess potential confounding. Maternal age was defined as age at recruitment. Body mass index (BMI) at the 12 week scan was used as a proxy measurement for pre-pregnancy BMI. Maternal ethnicity, age at leaving full-time education (FTE), smoking and alcohol consumption were defined by self-report at the 20 week questionnaire. Birth weight was measured directly after delivery.

Collection of placentas

After delivery, the placenta was given as soon as possible to the research technicians when there was no reason to send the placenta to pathology for further investigation. The median collection time in the study cohort was 4 hours (interquartile range was 20 minutes to 10 hours). Placental biopsies were obtained as soon after birth as possible [14]. The placentas were then stored for 24 hours before dissection. After the membranes and cord were removed, both sides of the placenta were photographed with a ruler and patient identification number (ID) on a clean surface (Canon Powershot A480 camera, Supplementary Figure 1). The placenta was then cut along the longest diameter in 1cm thick strips using a disposable brain knife. The placental strips were tipped to the left so that the right hand face of each strip was uppermost, after which a picture was taken with the ruler and patient ID besides the placenta.

Image analysis in Matlab

Placental measurements (the first seven parameters in Supplementary Table 1) were calculated using Matlab (version r2014a, The Mathworks, Natick, MA, USA). Custom code (available on request from the authors) was written to analyse all the pictures in an objective and reproducible manner. The pixel size in each image was calculated by determining the number of pixels within 20mm on the ruler in the photograph (Supplementary Figure 1). To correct for the wedge biopsy taken at the placental edge (Supplementary Figure 1, red arrow) the outer boundary of the placenta was manually adjusted. Afterwards the umbilical cord insertion was noted if visible (black asterisk). In the photographs of the placental strips, infarcts, defined as white/pale regions in the placenta, were identified and manually drawn by the observer. All measurements were performed by a single individual, blinded to all clinical and research data available for the given pregnancy. Images were excluded from the analysis if the fetal side of the placenta was not photographed, if the placenta was photographed without a ruler, if there was an accessory lobe, holes in the placenta, or if the placenta was in parts.

Intra-observer repeatability

After 40% of the pictures had been analysed in Matlab, a note was made of the next 51 image IDs, which were reanalysed after 60% of all the images had been processed (both sets of measurements performed by NS). The first measurements were used for the main analysis. The results of the blinded, repeated measurements from the same placenta were used to calculate coefficients of variation (CV), the proportion of variance explained (R^2), concordance correlation coefficients (CCC), the difference between the two measurements with the 95% limits of agreement (LA) and the Kappa statistic (as appropriate) to assess repeatability and reliability of placental morphometry.

Statistics

In preliminary analyses, continuous variables were compared using Spearman correlation and categorical variables were compared using the Pearson Chi-square test. The Breusch-pagan/Cook-Weisberg test for heteroscedasticity was used to assess the change of variation in placental measurements by GA. Placental measurements were converted into GA-adjusted z-scores where the measurement

varied by GA at term. The association between deciles of placental measurements and continuous ultrasonic measurements was first tested using the Kruskal-Wallis rank test to allow for any nonlinearity in the association. The associations between placental measurements (exposure) and continuous ultrasonic measurements (outcome) were then estimated by linear regression in three categories, combining deciles 2 to 9 into a single category. We report the coefficient for the lowest decile of the continuous placental measurement referent to the highest decile and for the presence of any infarcts referent to absence of visible infarcts. Confounding by maternal characteristics was investigated by comparing the effect sizes on ultrasonic measurements between adjusted and unadjusted linear regression models. Statistical significance was assumed at $P<0.05$. Analyses were performed using Stata version 14.0.

Results

Description of the study population

A total of 8,028 women were eligible for inclusion of which 4,512 (56%) provided written informed consent (Figure 1). After excluding women who did not have a placental sample or a photograph of the placenta available and women who had a preterm birth or stillbirth, 2120 women remained in the analysis. There were 1980 images which allowed analysis of the fetal side of the placenta and 2059 images which allowed assessment of the presence of placental infarcts. The 140 women whose placental images were excluded from analyses of the fetal surface did not differ from the women included in the analysis in terms of maternal characteristics, the Doppler measurements or ACGV (all $p>0.05$). Placental weight was available for 2112 of the 2120 women. The characteristics of the study cohort are summarised in Supplementary Table 2. The study cohort was broadly representative of the full-term livebirths in the entire POP study cohort. The characteristics of the placentae are summarised in Supplementary Table 3 footnote.

Intra-observer variability

Intra-observer variability was analysed in a sub-sample of 51 pictures. The variation between the two measurements on the same placenta was generally very low, with CV ranging from 0.21% to 4.77% (Supplementary Table 4). The proportion of the variance in the second measurement that could be explained by the first measurements varied between 86% and 98%. Similarly, the CCC was high for all measurements, ranging from 0.93 to 0.99. Detection of infarcts also showed good repeatability (Kappa statistic=0.92).

Pairwise correlation of placental measurements

Placental area and perimeter, length and breadth, and solidity and circularity were highly correlated (Supplementary Table 3). Therefore we excluded perimeter, length, breadth and solidity from further analyses.

Transformation of placental measurements

Mean placental area and weight varied with GA in a linear fashion ($p<0.001$ and $R^2=0.03$ for both associations) and the standard deviation did not significantly change with GA (Breusch-pagan/Cook-Weisberg test $p>0.05$). Therefore, we obtained GA-adjusted z-scores as standardised residuals from the linear regression models for the placental area and weight. The other placental measurements did not vary with GA. Deciles were calculated from GA-adjusted z-scores or unadjusted measurements, as appropriate.

Adjustment for maternal characteristics

Of the maternal characteristics, only BMI was associated with both ultrasonic measurements and placental measurements, and it was considered a possible confounder. We report analyses adjusted for BMI in addition to unadjusted analyses.

Regression analysis results

Uterine artery PI

Small placental surface area and low placental weight were both associated with an increased UtA mean PI. However, the association was stronger for area (UtA mean PI 0.87 SD greater in the lowest vs. the highest decile) than it was for weight (0.38 SD) (Table 1, Figure 2A and 2D). The UtA flow velocity waveform was also associated with the shape of the placenta. Specifically, high resistance patterns of UtA flow were associated both with increased cord deviation (Table 1, Figure 2C) and, somewhat unexpectedly, higher circularity (i.e. UtA resistance was lower in more elongated placentae) (Table 1, Figure 2B). Adjustment for maternal BMI did not materially affect these results.

Umbilical artery PI

Small placental surface area and low placental weight were also both associated with an increased UmA PI. However, the different strengths of association with ultrasound measurements were reversed compared with UtA mean PI: the association was stronger for weight (0.73 SD) than it was for surface area (0.24 SD, Table 1, Figure 3A and 3D). Adjustment for BMI did not materially affect these

results. In contrast to the UtA Doppler, there was no association between the shape of the placenta and the UmA Doppler.

Abdominal circumference growth velocity (ACGV)

The ACGV was strongly associated with both placental area (0.68 SD lower) and placental weight (0.73 SD lower, Table 1, Figure 4A and 4D). Both associations were statistically significant after BMI adjustment. However, there was no association between the shape of the placenta and the ACGV.

In bivariate linear regression analysis, including both placental area and weight, placental area was only associated with UtA PI and placental weight was only associated with UmA PI. Placental area and weight were each independently associated with ACGV (Table 2). These associations persisted in multivariable linear regression analysis, where all placental measurements were mutually adjusted for (Table 3). Also, circularity and cord deviation were associated with UtA PI after adjustment for all other placental measures.

Discussion

The key findings of the present study were (1) small placental area was associated with higher uterine artery PI (20 weeks gestation), (2) low placental weight was associated with higher umbilical artery PI (36 weeks gestation), (3) both placental measures were associated with a slower fetal ACGV (20 and 36 weeks gestation), and (4) the shape of the placenta was associated with the flow velocity waveform in the uterine artery but not the umbilical artery. Previous studies have addressed the relationship between uterine artery PI, umbilical artery PI, fetal growth and the risk of adverse pregnancy outcome [13, 16, 17] and described the associations between utero-placental Doppler velocimetry and fetoplacental pathology [18]. Furthermore, multiple studies have shown that abnormalities of placental shape are associated with vascular abnormalities and reduced efficiency in placental function [19, 20]. However, the present study is the first, to our knowledge, to evaluate the relationship between ultrasonic measurements in fetal life and the size and shape of the placenta following birth.

The results of the present study are consistent with existing data, but also considerably expand the physiological and pathological understanding of different patterns of utero-placental Doppler flow velocity waveforms. Higher values of UtA and UmA Doppler flow velocity waveforms by the PI are thought to reflect increased resistance in the vascular bed supplied [21]. It is known that high resistance patterns of UtA Doppler are associated with defective trophoblast invasion of the placental bed arteries [22]. Furthermore, uterine artery velocimetry is a marker of defective remodelling of spiral arteries with consequent placental malperfusion [22, 23]. A key finding of the present study is that the maternal surface area of the placenta was also a major determinant of the UtA resistance. This suggests that the magnitude of the physiological decrease in the uterine arterial resistance is related both to the depth of invasion in any given area, and to the total area of the placental bed, presumably as this will determine the total number of vessels invaded.

Multiple studies have shown strong associations between absent umbilical artery end-diastolic flow velocity and FGR [24, 25]. Previous placental studies have tended to focus on the ultrastructural characteristics of the villi, and have shown

associations between abnormal UmA Doppler and maldevelopment of the villous vascular tree [26]. The present study indicates that placental weight is also a major determinant of the UmA artery flow velocity waveform. We speculate that the total number of villi in the placenta might increase with placental weight. Hence, in parallel with the previous arguments developed for the uterine arteries, the pattern of flow in the UmA is due both to the total number of villi and the vascular development within the villi.

One of the strengths of the present study is that it includes blinded ultrasonographic assessment in a large cohort through all trimesters of pregnancy. The methodological strength of this approach is that disclosure of the scan results could have led to differential treatment of the women based on the information from the scans, which could have biased the results. Another strength of this study is the consistency of the measurements of placental size and shape achieved by using a Matlab code. Evaluation of intra-observer reproducibility and reliability showed a high level of consistency between two sets of blinded measurements. The present study could be criticised on the grounds that it was confined to nulliparous women who were largely of white European ancestry. While this fact somewhat limits the external validity of the study, the analysis of a homogeneous population has some advantages for a study of human physiology, due to the potential for confounding by socio-economic or ethnic characteristics.

In conclusion, we found that both uterine and umbilical artery Doppler flow velocity waveforms are associated with different aspects of the size and shape of the placenta following birth. Both are associated with the rate of fetal growth. This study underlines the dependence of normal fetal growth on both the maternal supply of nutrients and the fetal uptake of nutrients within the placenta. It also indicates that vascular function on both the maternal and fetal side of the human placenta are related to the size and the shape of the placenta, as well as its microscopic and ultrastructural characteristics.

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Additional Information

Competing interests. The authors have no competing interests.

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Author contributions. GCSS and DSCJ designed the study and managed the teams who collected the data. NS, US and RPM analysed the data and all authors contributed to the interpretation of the data. NS, US and GCSS drafted the article and DSCJ and RPM revised it critically for important intellectual content. All authors approved the final version of the manuscript. We confirm that all persons designated as authors qualify for authorship and that all persons who qualify for authorship are listed as authors.

Legends for Figures

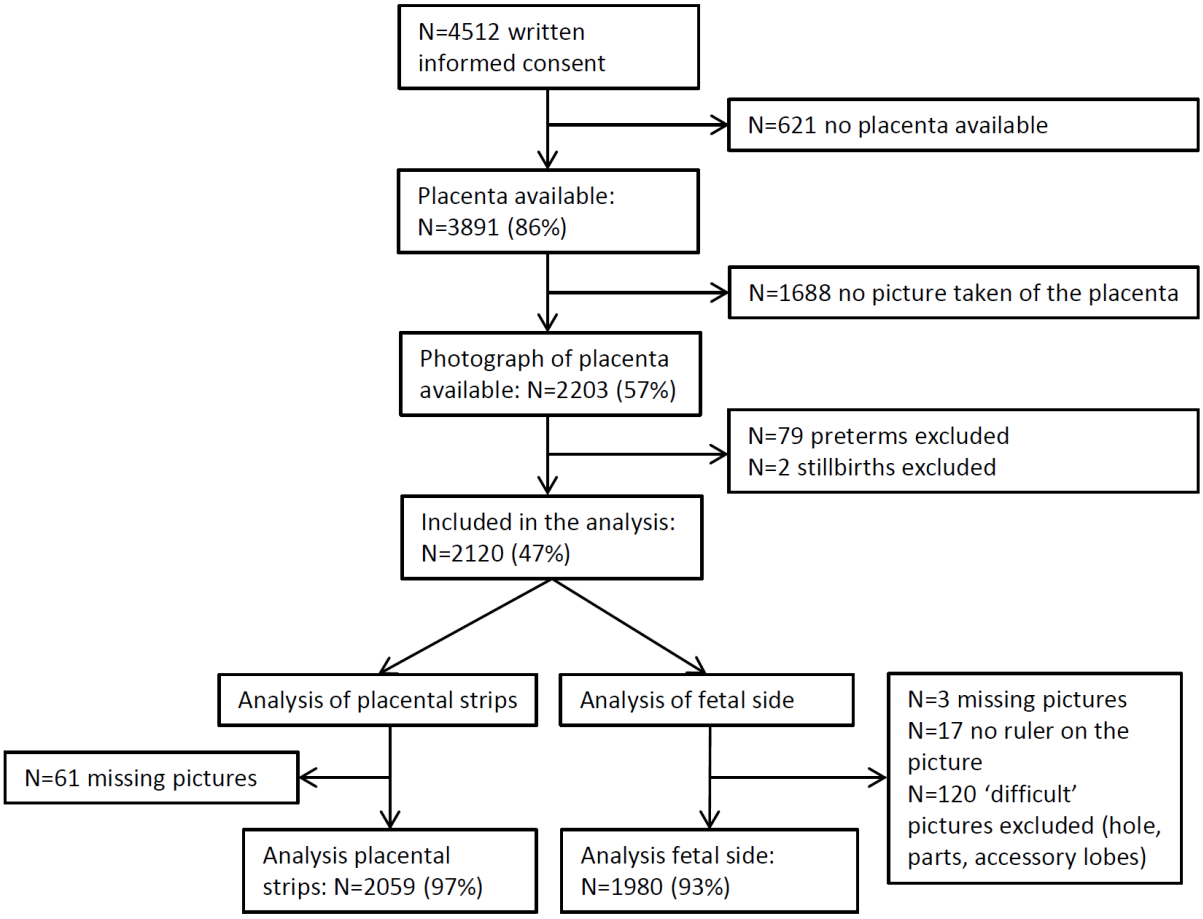
Figure 1. Flow chart of the study cohort.

Figure 2. Mean Uterine Artery Doppler PI (20 weeks) in deciles of placental measurements (95% confidence intervals): A. Placental area, B. Placental circularity, C. Cord deviation, D. Placental weight. Kruskal-Wallis rank test p-values are given.

Figure 3. Mean Umbilical Artery Doppler PI (36 weeks) in deciles of placental measurements (95% confidence intervals): A. Placental area, B. Placental circularity, C. Cord deviation, D. Placental weight. Kruskal-Wallis rank test p-values are given.

Figure 4. Mean Abdominal Circumference Growth Velocity (ACGV) (20-36 weeks) in deciles of placental measurements (95% confidence intervals): A. Placental area, B. Placental circularity, C. Cord deviation, D. Placental weight. Kruskal-Wallis rank test p-values are given.

Figure 1.



In individual analyses the number varies due to missing measurements in the outcomes.

Figure 2.

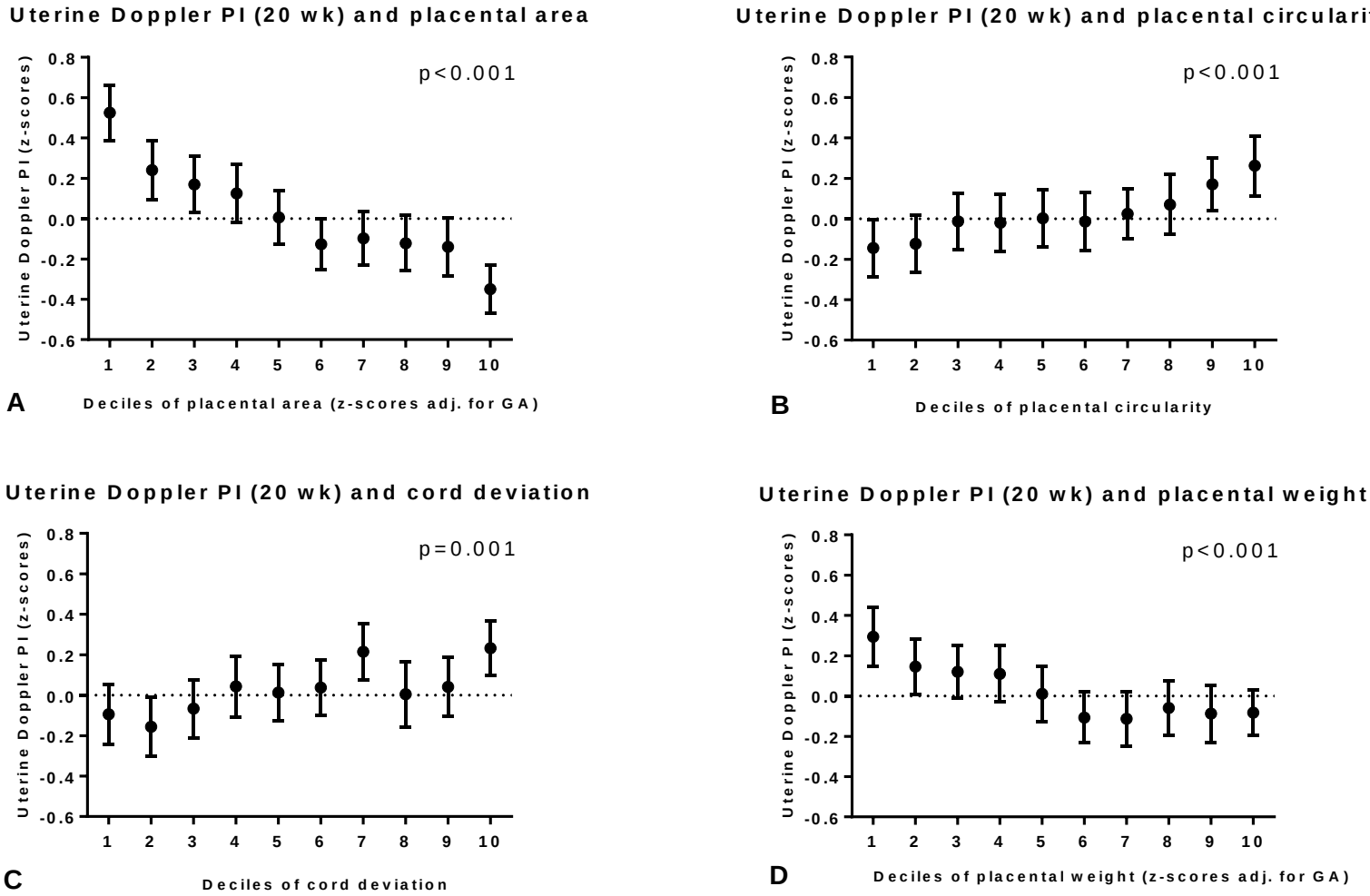

Uterine Doppler PI (20 wk) and placental area
p<0.001
Uterine Doppler PI (z-scores)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1 2 3 4 5 6 7 8 9 10
A
Deciles of placental area (z-scores adj. for GA)
Uterine Doppler PI (20 wk) and placental circularity
p<0.001
Uterine Doppler PI (z-scores)
1 2 3 4 5 6 7 8 9 10
B
Deciles of placental circularity
Uterine Doppler PI (20 wk) and cord deviation
p=0.001
Uterine Doppler PI (z-scores)
1 2 3 4 5 6 7 8 9 10
C
Deciles of cord deviation
Uterine Doppler PI (20 wk) and placental weight
p<0.001
Uterine Doppler PI (z-scores)
1 2 3 4 5 6 7 8 9 10
D
Deciles of placental weight (z-scores adj. for GA)

Figure 3.

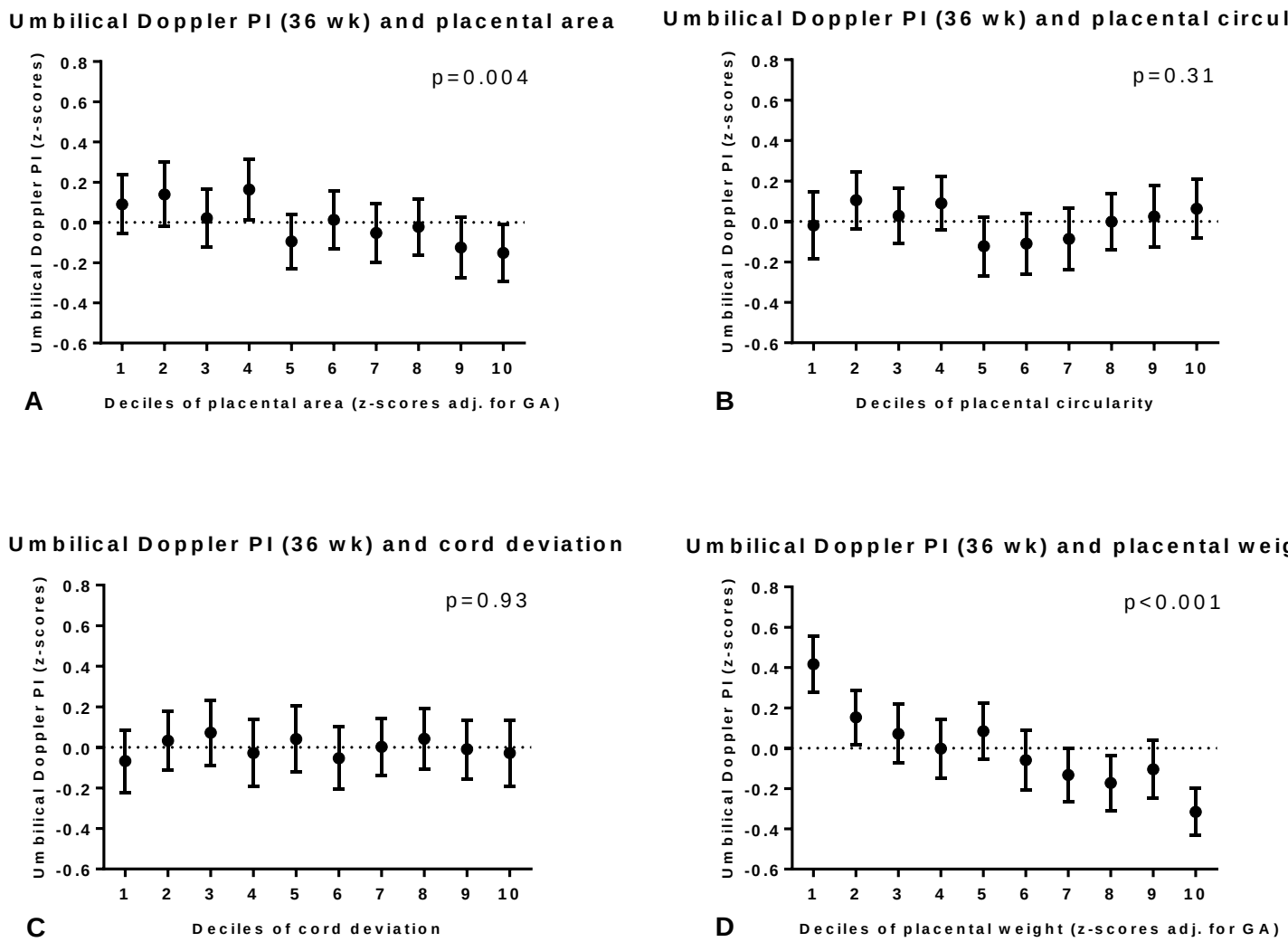

Umbilical Doppler PI (36 wk) and placental area
p=0.004
Umbilical Doppler PI (z-scores)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1 2 3 4 5 6 7 8 9 10
A
Deciles of placental area (z-scores adj. for GA)
Umbilical Doppler PI (36 wk) and placental circularity
p=0.31
Umbilical Doppler PI (z-scores)
B
Deciles of placental circularity
Umbilical Doppler PI (36 wk) and cord deviation
p=0.93
Umbilical Doppler PI (z-scores)
C
Deciles of cord deviation
Umbilical Doppler PI (36 wk) and placental weight
p<0.001
Umbilical Doppler PI (z-scores)
D
Deciles of placental weight (z-scores adj. for GA)

Figure 4.

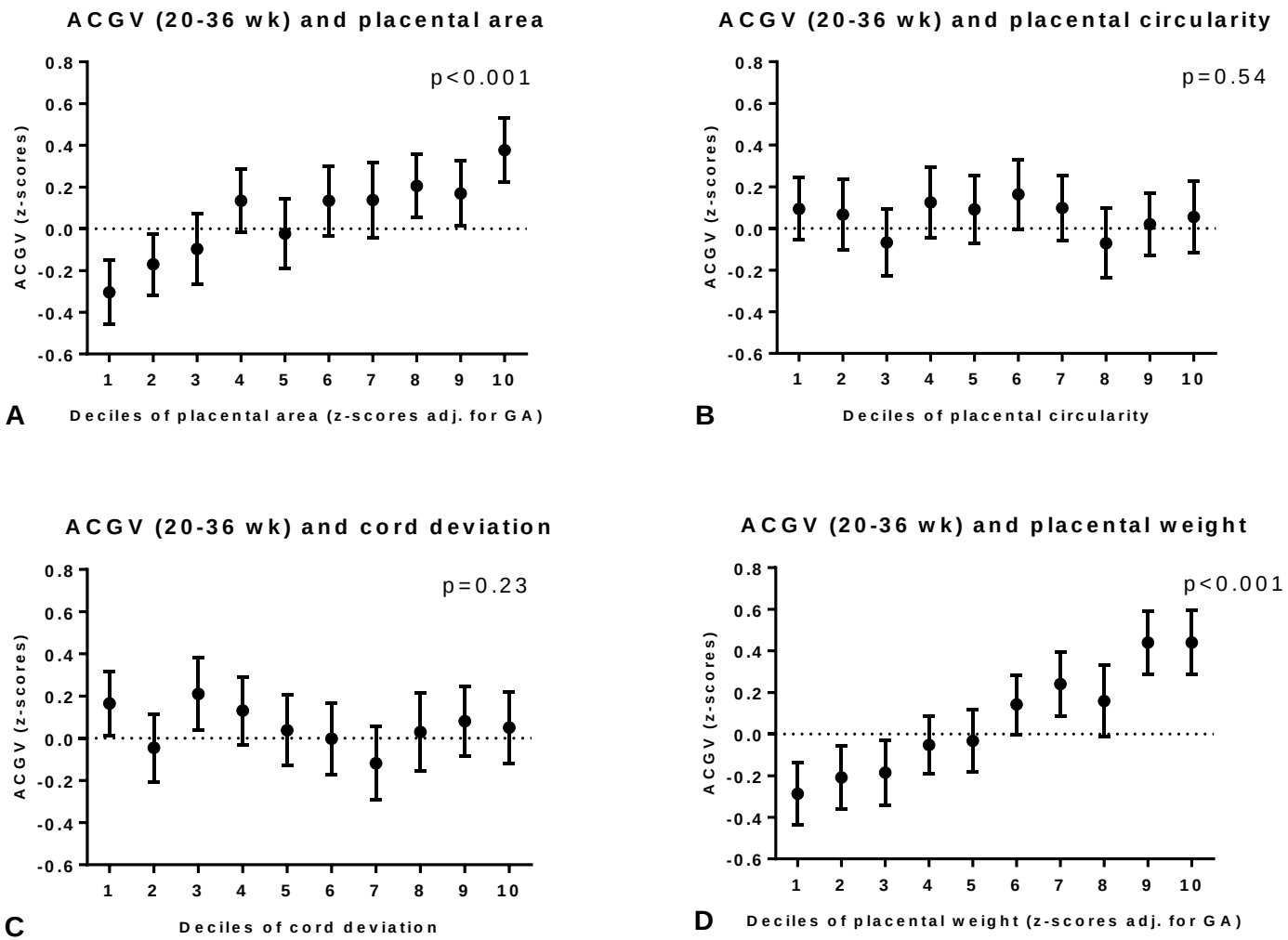

ACGV (20-36 wk) and placental area
p<0.001
ACGV (z-scores)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1 2 3 4 5 6 7 8 9 10
A
Deciles of placental area (z-scores adj. for GA)
ACGV (20-36 wk) and placental circularity
p=0.54
ACGV (z-scores)
1 2 3 4 5 6 7 8 9 10
B
Deciles of placental circularity
ACGV (20-36 wk) and cord deviation
p=0.23
ACGV (z-scores)
1 2 3 4 5 6 7 8 9 10
C
Deciles of cord deviation
ACGV (20-36 wk) and placental weight
p<0.001
ACGV (z-scores)
1 2 3 4 5 6 7 8 9 10
D
Deciles of placental weight (z-scores adj. for GA)

Table 1. Placental measurements in relation to uterine artery Doppler PI, umbilical artery Doppler PI and abdominal circumference growth velocity.

		Unadjusted analysis		**Adjusted for BMI**	
Analysis	N	Coeff (95% CI)*	P	Coeff (95% CI)*	P
UtA PI (20 weeks)					
Area	1948	0.87 (0.68 to 1.07)	<0.001	0.87 (0.68 to 1.07)	<0.001
Circularity	1949	-0.41 (-0.60 to -0.21)	<0.001	-0.41 (-0.61 to -0.21)	<0.001
Cord deviation	1780	-0.33 (-0.53 to -0.12)	0.002	-0.33 (-0.53 to -0.12)	0.002
Weight	2081	0.38 (0.19 to 0.57)	<0.001	0.40 (0.20 to 0.59)	<0.001
Infarcts	2029	-0.07 (-0.16 to 0.02)	0.14	-0.07 (-0.16 to 0.02)	0.13
Weight/area	1941	0.01 (-0.19 to 0.21)	0.92	0.01 (-0.19 to 0.21)	0.92
UmA PI (36 weeks)					
Area	1906	0.24 (0.04 to 0.45)	0.021	0.24 (0.04 to 0.45)	0.021
Circularity	1907	-0.08 (-0.29 to 0.12)	0.43	-0.08 (-0.29 to 0.13)	0.44
Cord deviation	1743	-0.04 (-0.26 to 0.18)	0.72	-0.04 (-0.26 to 0.18)	0.71
Weight	2032	0.73 (0.54 to 0.93)	<0.001	0.73 (0.54 to 0.93)	<0.001
Infarcts	1984	-0.01 (-0.10 to 0.09)	0.86	-0.01 (-0.10 to 0.09)	0.88
Weight/area	1898	-0.00 (-0.21 to 0.20)	0.96	-0.01 (-0.21 to 0.20)	0.96
ACGV (20-36 weeks)					
Area	1909	-0.68 (-0.91 to -0.46)	<0.001	-0.68 (-0.91 to -0.46)	<0.001
Circularity	1910	0.04 (-0.19 to 0.27)	0.74	0.02 (-0.20 to 0.25)	0.83
Cord deviation	1745	0.11 (-0.12 to 0.35)	0.35	0.12 (-0.11 to 0.36)	0.31
Weight	2039	-0.73 (-0.94 to -0.51)	<0.001	-0.66 (-0.88 to -0.44)	<0.001
Infarcts	1990	0.01 (-0.09 to 0.12)	0.83	-0.01 (-0.11 to 0.10)	0.99
Weight/area	1901	-0.10 (-0.33 to 0.13)	0.39	-0.10 (-0.32 to 0.13)	0.40

*Coefficients are given for the lowest decile (lowest 10% of the population) of the given continuous placental measurement compared with the highest decile, i.e. positive values indicate higher levels of UtA or UmA PI in the lowest decile of the placental measurement. These are expressed as z-scores, i.e. the unit for the coefficient is one standard deviation (SD). The coefficients are also given for the presence of any infarcts referent to cases with no visible infarcts.
UtA denotes uterine artery, UmA denotes umbilical artery, PI denotes pulsatility index and ACGV denotes abdominal circumference growth velocity.

Table 2. Placental area and weight in relation to uterine artery Doppler PI, umbilical artery Doppler PI and abdominal circumference growth velocity.

	Bivariate analysis		**Adjusted additionally for BMI**	
Analysis	Coeff (95% CI)*	P	Coeff (95% CI)*	P
UtA PI (20 weeks)				
Area	0.82 (0.62 to 1.03)	<0.001	0.82 (0.61 to 1.03)	<0.001
Weight	0.14 (-0.07 to 0.35)	0.19	0.15 (-0.06 to 0.36)	0.16
UmA PI (36 weeks)				
Area	-0.01 (-0.23 to 0.20)	0.92	-0.01 (-0.22 to 0.20)	0.93
Weight	0.80 (0.58 to 1.01)	<0.001	0.79 (0.58 to 1.01)	<0.001
ACGV (20-36 weeks)				
Area	-0.50 (-0.74 to -0.26)	<0.001	-0.52 (-0.76 to -0.29)	<0.001
Weight	-0.59 (-0.83 to -0.35)	<0.001	-0.51 (-0.75 to -0.27)	<0.001

*Coefficients are the difference in z-score of the given Doppler measurement in the lowest decile of the given placental measurement compared with the highest decile, i.e. positive values indicate higher levels of UtA or UmA PI in the lowest decile of weight or area.
Bivariate analysis is where the coefficient for area is adjusted for placental weight and *vice versa*.
UtA denotes uterine artery, UmA denotes umbilical artery, PI denotes pulsatility index and ACGV denotes abdominal circumference growth velocity.

Table 3. Placental measurements in relation to uterine artery Doppler PI, umbilical artery Doppler PI and abdominal circumference growth velocity. Multivariable analysis.

	Multivariable analysis		**Adjusted additionally for BMI**	
Analysis	Coeff (95% CI)*	P	Coeff (95% CI)*	P
UtA PI (20 weeks)				
Area	0.72 (0.49 to 0.94)	<0.001	0.72 (0.49 to 0.94)	<0.001
Circularity	-0.40 (-0.62 to -0.19)	<0.001	-0.40 (-0.62 to -0.19)	<0.001
Cord deviation	-0.25 (-0.46 to -0.05)	0.016	-0.25 (-0.46 to -0.05)	0.016
Weight	0.22 (-0.01 to 0.45)	0.061	0.22 (-0.01 to 0.45)	0.061
Infarcts	-0.09 (-0.19 to 0.00)	0.057	-0.09 (-0.19 to 0.00)	0.056
Weight/area	0.03 (-0.17 to 0.24)	0.77	0.03 (-0.17 to 0.24)	0.77
UmA PI (36 weeks)				
Area	0.00 (-0.24 to 0.24)	0.99	0.00 (-0.23 to 0.24)	0.97
Circularity	-0.13 (-0.36 to 0.10)	0.26	-0.13 (-0.36 to 0.10)	0.26
Cord deviation	-0.09 (-0.31 to 0.13)	0.43	-0.09 (-0.31 to 0.13)	0.43
Weight	0.85 (0.61 to 1.09)	<0.001	0.84 (0.60 to 1.09)	<0.001
Infarcts	0.03 (-0.07 to 0.14)	0.53	0.03 (-0.07 to 0.14)	0.52
Weight/area	-0.01 (-0.22 to 0.21)	0.94	-0.01 (-0.22 to 0.21)	0.94
ACGV (20-36 weeks)				
Area	-0.47 (-0.73 to -0.21)	<0.001	-0.50 (-0.76 to -0.24)	<0.001
Circularity	-0.04 (-0.29 to 0.21)	0.76	-0.05 (-0.30 to 0.20)	0.72
Cord deviation	0.02 (-0.22 to 0.27)	0.86	0.03 (-0.21 to 0.27)	0.83
Weight	-0.60 (-0.87 to -0.34)	<0.001	-0.52 (-0.78 to -0.25)	<0.001
Infarcts	0.01 (-0.10 to 0.12)	0.86	0.01 (-0.11 to 0.12)	0.94
Weight/area	-0.04 (-0.28 to 0.19)	0.73	-0.05 (-0.28 to 0.19)	0.68

*Coefficients are given for the lowest decile (lowest 10% of the population) of the given continuous placental measurement compared with the highest decile, i.e. positive values indicate higher levels of UtA or UmA PI in the lowest decile of the placental measurement. These are expressed as z-scores, i.e. the unit for the coefficient is one standard deviation (SD). The coefficients are also given for the presence of any infarcts referent to cases with no visible infarcts.
UtA denotes uterine artery, UmA denotes umbilical artery, PI denotes pulsatility index and ACGV denotes abdominal circumference growth velocity.

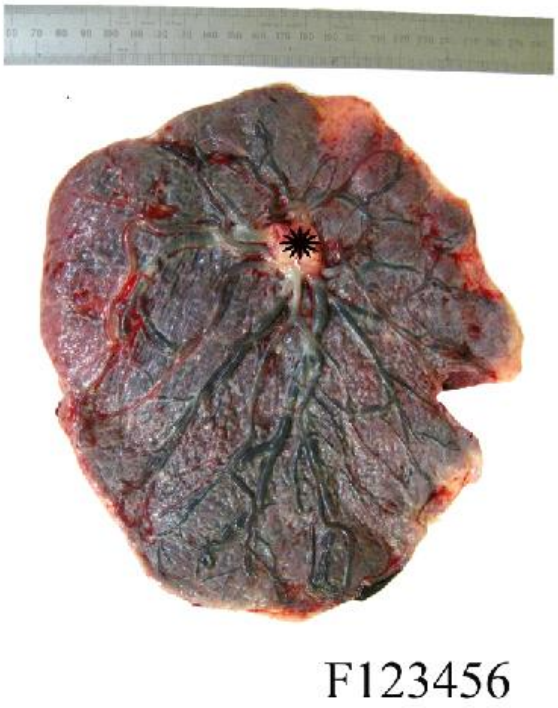
The relationship between human placental morphometry and ultrasonic measurements of utero-placental blood flow and fetal growth.

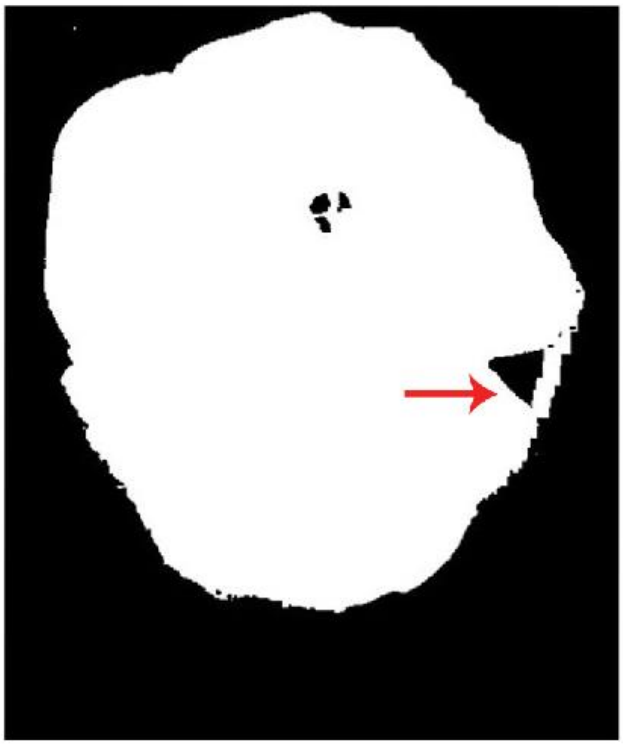
N. Salavati
Dr. U. Sovio
R. Plitman Mayo
Prof. D.S. Charnock-Jones
Prof. G.C.S. Smith

Supplementary material

Supplementary Figure 1. Placental measurements obtained with Matlab. ‘F123456’ is a (dummy) ID, where ‘F’ stands for fetal side and ‘123456’ for the actual ID-number.
Red arrow indicating the correction for the biopsy previously taken. Black asterisk indicating the umbilical cord insertion.

Supplementary Figure 1.





Supplementary Table 1. Placental variables obtained via Matlab.

Variable (unit)	Description
Area (cm^2)	Surface area of the placenta.
Perimeter (cm)	Perimeter of the placenta.
Length (cm)	The major axis of the placenta through the centre of mass of the placenta with a 90° angle between the axes.
Breadth (cm)	The minor axis of the placenta through the centre of mass of the placenta with a 90° angle between the axes.
Circularity	Calculated from the formula: $(4\times\pi\times Area)/(Perimeter^2)$. The value is between 0 and 1, (1 for a perfect circle). As the value approaches 0, it indicates an increasingly elongated shape.
Solidity	Solidity specifies the proportion of the pixels in the convex area (the smallest convex set that contains the placental area) that are also in the region and is computed as placental area/convex area.
Cord deviation	The centrality of the cord insertion (cm) was obtained by measuring the distance between the cord insertion point and the centre of mass of the placenta. Subsequently this was divided by the sum of length and breadth of the placenta to normalise the cord deviation in relation to the size of the placenta. The value of the normalised cord deviation is unit-less and can vary between 0 and <1, where 0 indicates perfect centrality higher values indicate more peripheral cord insertion.
Weight (g)	Weight of the placenta, measured after blood drainage and without cord and membranes.
Infarcts	Recognised as an area of ischemic necrosis. Only older infarctions were recorded, recognised as white/pale regions, since fresh infarcts were harder to distinguish from simple clots and blood. The infarction size was measured and assessed during the evaluation of the pictures of the placental strips in Matlab, in relation to the total volume of the placenta, which was calculated in Matlab.
Weight/area	Weight of the placenta divided by the surface area of the placenta.

Supplementary Table 2. Characteristics stratified by inclusion to the study cohort.

Characteristics	Included N=2120	Not included N=2087	P
Maternal			
Age (years)	31 (27 - 33)	30 (26 - 33)	<0.001
<20	64 (3.0)	97 (4.7)	
20 - 24.9	256 (12)	301 (14)	
25 - 29.9	637 (30)	666 (32)	<0.001
30 - 34.9	825 (39)	725 (35)	
35 - 39.9	298 (14)	257 (12)	
≥ 40	40 (1.9)	41 (2.0)	
Age stopped FTE (years)	21 (18 - 23)	21 (18 - 23)	0.70
<19	681 (32)	708 (34)	
19-22	745 (35)	698 (33)	0.72
≥ 23	615 (29)	624 (30)	
Missing	79 (3.7)	57 (2.7)	
White ethnicity (%)	1967 (93)	1877 (90)	0.64
Missing	28 (1.3)	98 (4.7)	
Any alcohol consumption	80 (3.8)	106 (5.1)	0.04
Missing	1 (<0.1)	2 (0.1)	
Smoker, number (%)	97 (4.6)	112 (5.4)	0.24
BMI, kg/m^2	24 (22 - 27)	24 (22 - 28)	0.04
<25	1269 (60)	1188 (57)	
25 - 29.9	596 (28)	574 (28)	
30 - 34.9	181 (8.5)	219 (10)	0.01
35 - 39.9	49 (2.3)	67 (3.2)	
≥ 40	25 (1.2)	27 (1.3)	
Missing	0 (0)	12 (0.6)	
Fetal or neonatal			
Birth weight (grams)	3440 (3150 - 3750)	3440 (3130 - 3750)	0.54
Gestational age (weeks)	40.4 (39.4 - 41.3)	40.3 (39.3 - 41.1)	0.02

Data are median (interquartile range) or n (%). Maternal age was defined as age at recruitment. BMI at the 12 week scan was used. All other maternal characteristics were defined by self-report at the 20 weeks questionnaire or from examination of the clinical case record. Women who withdrew formally (n=67), delivered a stillborn baby (n=12) or delivered before 37 weeks (n=230, including 4 stillbirths) were excluded from both groups to ensure comparability. P-values are for difference between groups calculated using the two-sample Wilcoxon rank-sum (Mann-Whitney) test for

continuous variables and the Pearson Chi-square test for categorical variables, with trend test as appropriate. The missing category was not included in statistical tests. For variables without a "Missing" category, data were 100% complete. The women included in the study cohort were on average slightly older than the women who were not included in the study cohort, which was due to the secular trend in maternal age. Other differences were very minimal. FTE= full time education, BMI= body mass index.

Supplementary Table 3. Pairwise Spearman correlation of placental and ultrasonic measurements.

	Area	Perimeter	Length	Breadth	Cord deviation	Circularity	Solidity	Weight	UtA Doppler PI (20 weeks)	UmA Doppler PI (36 weeks)	ACGV (20-36 weeks)
Area	1.00										
Perimeter	0.96	1.00									
Length	0.85	0.89	1.00								
Breadth	0.86	0.75	0.50	1.00							
Cord deviation	-0.18	-0.19	-0.17	-0.16	1.00						
Circularity	-0.09	-0.36	-0.33	0.13	0.08	1.00					
Solidity	-0.15	-0.37	-0.28	-0.019	0.10	0.81	1.00				
Weight	0.51	0.43	0.39	0.47	0.04	0.13	0.12	1.00			
UtA Doppler PI (20 weeks)	-0.23	-0.25	-0.22	-0.18	0.10	0.12	0.15	-0.12	1.00		
UmA Doppler PI (36 weeks)	-0.08	-0.08	-0.06	-0.08	0.002	-0.01	-0.0008	-0.17	0.04	1.00	
ACGV (20-36 weeks)	0.17	0.16	0.15	0.15	-0.03	-0.01	-0.03	0.23	-0.08	-0.04	1.00

UtA denotes uterine artery, UmA denotes umbilical artery, PI denotes pulsatility index and ACGV denotes abdominal circumference growth velocity. Placental measurements are expressed as median (interquartile range):
Area (cm^2): 290 (252 - 336); Perimeter (cm): 65.1 (60.2 – 70.3); Length (cm): 21.6 (19.9 – 23.5); Breadth (cm): 17.4 (16.0 – 18.8); Cord deviation (normalised): 0.09 (0.05 – 0.13); Circularity: 0.87 (0.83 – 0.90); Solidity: 0.97 (0.96 – 0.98); Placental weight (g): 450 (394 – 515).

Supplementary Table 4. Analysis of intra-observer repeatability and reliability of placental measurements.

Placental measurement	N	R^2	CCC	CV (%)	Difference: mean (95% LA)
Area (cm^2)	51	0.94	0.97	1.61	188.8 (-3509, 3886)
Perimeter (cm)	51	0.94	0.97	1.16	0.81 (-48.9, 50.5)
Length (cm)	51	0.97	0.98	0.88	0.78 (-12.6, 14.1)
Breadth (cm)	51	0.95	0.98	0.84	0.46 (-9.03, 9.95)
Circularity	51	0.86	0.93	1.15	0.004 (-0.053, 0.061)
Solidity	51	0.92	0.96	0.21	0.001 (-0.012, 0.014)
Cord deviation (cm)	47	0.98	0.99	4.77	0.57 (-5.80, 6.94)

R^2: Pearson Correlation Coefficient squared i.e. proportion of variance explained
CCC: Concordance Correlation Coefficient
CV: Coefficient of Variation
LA: Limits of Agreement
For cord deviation there are n=4 missing. These pictures were declared by the observer as ‘no cord visible’. Cord deviation is not normalised (divided by breadth+length) in these analyses.