Factors Associated with Placental Vascularization Measured by 3D Power Doppler Ultrasonographic Sphere Biopsy between 11 and 14 Weeks of Gestation

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Abstract

Objective Preeclampsia is associated with placental vascularization disorders. Ultrasonographic sphere biopsy (USSB) of the placenta can estimate the vascularization of the placenta and potentially the risk of preeclampsia. We aimed to explore the factors related to placental vascularization measured with USSB in the first trimester.

Study Design A prospective cohort was conducted in women recruited at 11 to 14 weeks. Three-dimensional acquisition of the placenta with power Doppler was undertaken along with crown-rump length (CRL). Using USSB of the full placental thickness at its center, vascularization index, flow index, and vascular flow index were measured. Pearson's correlation coefficients and multivariate linear regression were used to correlate the vascularization indices with CRL and maternal characteristics.

Results A total of 5,612 women were recruited at a mean gestational age of 12.8 $\pm$ 0.6 weeks. We observed that vascularization indices increase with CRL. After adjustment, we observed that maternal age, ethnicity other than Caucasian, and body mass index were associated with lower vascularization indices, while diabetes, smoking, and assisted reproduction technology were not. We observed that parous women without history of preeclampsia had greater vascularization indices compared with nulliparous women.

Conclusion Placental vascularization indices assessed by USSB fluctuate with gestational age, ethnicity, maternal age, body mass index, and previous pregnancy history.

Keywords
- pregnancy
- preeclampsia
- placenta
- ultrasound
- Doppler

Preeclampsia is a multisystem disorder of pregnancy defined by the combination of new-onset hypertension and proteinuria that contribute substantially to perinatal morbidity and mortality worldwide.[1,2] The etiology of preeclampsia remains controversial, but it is now recognized that alterations in the growth and development of placental villi and their underlying vasculature play an important role in the pathogenesis of the disease.[3] The physiological transformation of uterine spiral arteries by cytotrophoblasts that invade the myometrium is typically missing in preeclampsia and mainly in the preterm forms of the disease.[4,5]

Placental size and vascularization have been evaluated by three-dimensional (3D) ultrasound in the first trimester of pregnancy. Small placental volume and low vascularity indices of the entire placenta measured by 3D power Doppler in the first trimester have been linked with preeclampsia and fetal growth restriction (FGR).[6–10] However, measurement of the volume and vascularization of the entire placenta

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requires manual drawing of the contour of the placenta in several planes, which is time-consuming and requires expertise, precluding its use in clinical practice. The ultrasonic sphere biopsy (USSB) technique, a shorter procedure, could be used to estimate the vascularization of the placenta or the subplacental myometrium.[8] Dar et al used the USSB technique to estimate the vascularization of the uteroplacental space in the first trimester and observed that women who subsequently developed preeclampsia had lower values of vascular index (VI), flow index (FI), and vascular FI (VFI) than controls.[11] More recently, vascularization of the placenta using USSB of the full placenta thickness was highly correlated with the vascularization of the entire placenta and associated with the risk of preterm preeclampsia.[12] However, most studies that evaluated USSB of the placenta were limited in their sample size.

Before studying the predictive values of those vascularization indices, we aimed to estimate the normal range for each of them (VI, FI, and VFI) using the USSB technique between 11 and 14 weeks and to explore their association with maternal characteristics.

Study Design

This was a prospective screening study for adverse obstetric outcomes in women attending their routine first hospital visit in pregnancy. At this visit, which is held at 11 to 13 weeks and 6 days of gestation, we recorded maternal characteristics and performed a transabdominal ultrasound scan to confirm gestational age from the measurement of the

Table 1 Participants characteristics

Maternal age (y)	31.2 ± 5.3
Gestational age (wk)	12.8 ± 0.6
Body mass index (kg/m^2)	25.9 ± 5.3
Smokers (%)	381 (6.8)
Parity (%)	
Nulliparous	2,639 (47.0)
Parous, no previous PE	2,833 (50.5)
Parous with previous PE	140 (2.5)
Ethnicity (%)	
White	4,276 (76.2)
Black	750 (13.4)
South Asian	282 (5.0)
East Asian	140 (2.5)
Mixed	164 (2.9)
Diabetes (%)	
No	5,562 (99)
Type 1 diabetes	26 (0.5)
Type 2 diabetes	24 (0.4)
Method of conception (%)	
Spontaneous	5,436 (96.9)
Ovulation drugs	30 (0.5)
In vitro fertilization	146 (2.6)

Abbreviation: PE, preeclampsia.
Note: Continuous variables are reported as mean ± standard deviation. Nominal variables are reported as *n* (%).

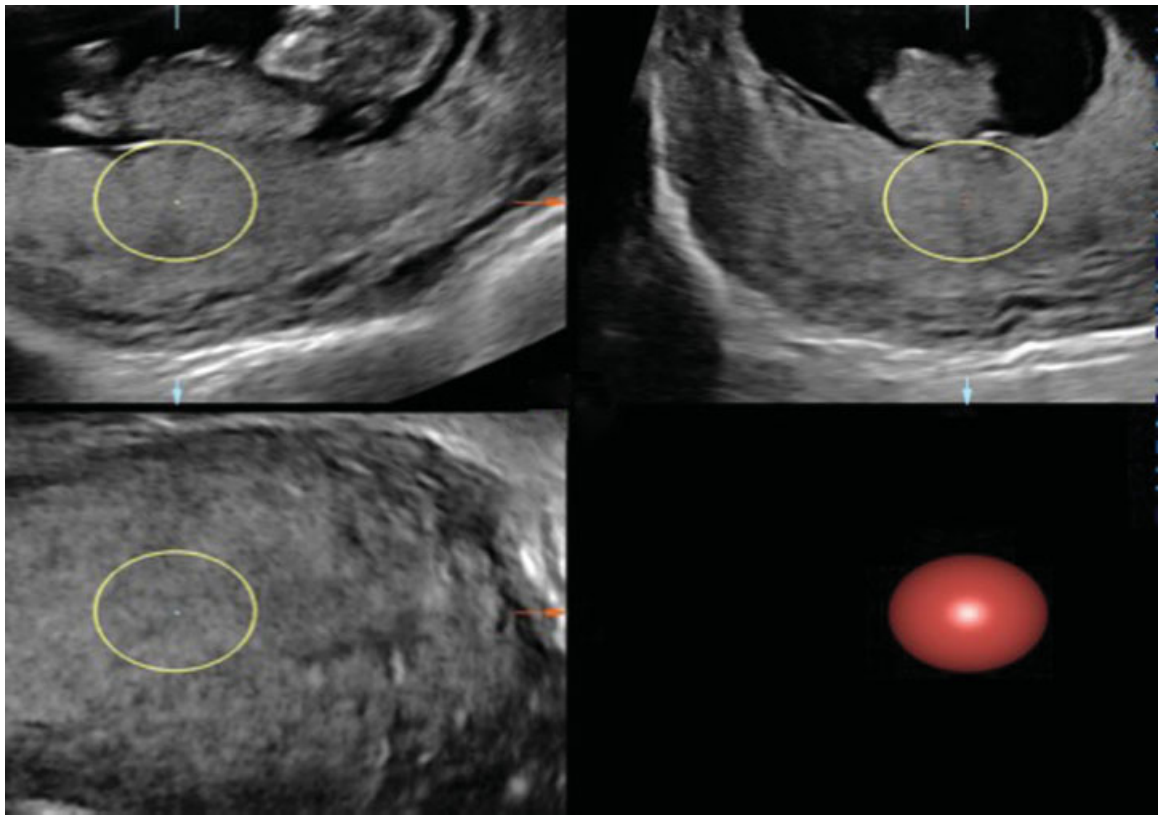

Fig. 1 Ultrasonographic sphere biopsy of the placenta.

fetal crown-rump length (CRL), to diagnose any major fetal abnormalities, and to measure fetal nuchal translucency.[13] Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee. Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, African, South Asian, East Asian, and mixed), cigarette smoking during pregnancy (yes or no), parity (nulliparous if there were no previous pregnancies beyond 23 completed weeks or parous), method of conception (spontaneous or assisted with ovulation drugs or in vitro fertilization), and diagnosis of pregestational diabetes (type 1 or type 2). The maternal weight in kilogram and height in centimeter were measured for calculation of body mass index (BMI).

A 3D sweep of the entire placenta was acquired by Voluson E6 or Voluson E8 ultrasound equipment (GE Medical Systems, Milwaukee, WI) with a 4 to 8 MHz transducer. Identical preestablished instrument power settings were used in all cases (angio mode: cent; smooth: 4/5; free response question [FRQ]: normal quality; density: 7; enhance: 16; balance: 175; filter: 2; actual power: 100% decibel [dB]; pulse repetition frequency: 0.6 kHz; gain color: −7.2 dB; wall motion filter [WMF] = low 1). For each participant, the VI, FI, and VFI of a USSB taken at the center of the placenta and including its full thickness were assessed with virtual organ computer-aided analysis (VOCAL, ►**Fig. 1**)[14–16] by a single observer blinded to clinical data. Each volume voxel contains information on the power Doppler signal with intensity ranging from 0 to 100. VI represents the ratio of color voxels in the studied volume (expressed as percentages). FI is the average color value of all color voxels, representing average blood flow intensity (expressed as an absolute value: 0–100). VFI is the average color value of all voxels of the studied volume, both gray and color (expressed as an absolute value: 0–100).[17] Biomarkers were transformed to obtain a normalized distribution. For each biomarker, a regression model was fitted using CRL. The values obtained from the regression were used to compute the multiple of median (MoM) by dividing each woman's indices by the median value corresponding to her CRL at measurement.

Mann–Whitney test and Pearson's test were used to evaluate the association between vascularization indices collected from the placental USSB and maternal characteristics including maternal age, parity, ethnicity, BMI, smoking, diabetes, and conception methods. We conducted multivariate linear regressions to evaluate the association between maternal characteristics and each index. The statistical analyses were computed using SAS statistical software packages (Version 9.4, SAS Institute Inc., Cary, NC).

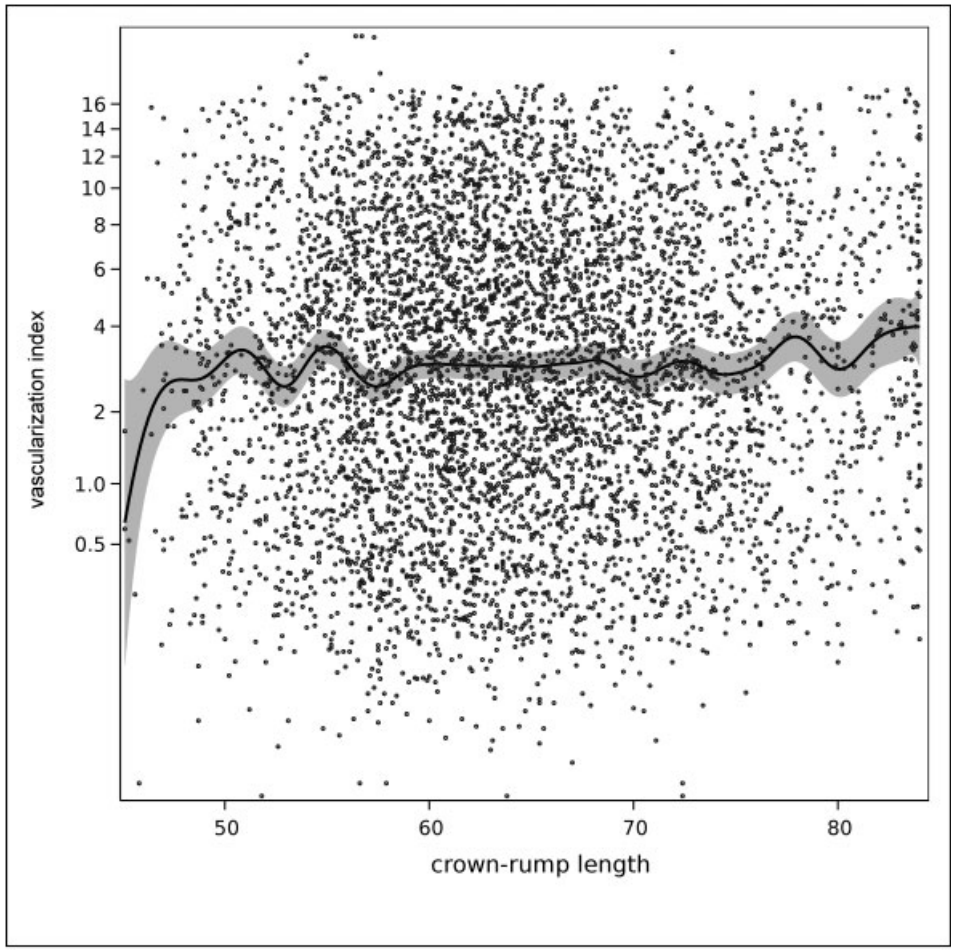

Fig. 2 Distribution of the vascularization index (VI) according to crown-rump length (CRL). The line represents penalized B-spline curve; and shaded area, the 95% confidence intervals of the means.

Results

We recruited 5,612 singleton pregnancies at a mean gestational age of 12.8 ± 0.6 weeks of gestation. Participants' characteristics are reported in ►**Table 1**. ►**Figs. 2–4** illustrate the values of VI, FI, and VFI plotted against CRL. We observed a small but significant positive correlation between FI (cc = 0.05, $p < 0.001$), VFI (cc = 0.03, $p < 0.05$), and CRL, but not between VI (cc = 0.02, $p = 0.011$) and CRL. ►**Table 2** reported the 5th, 10th, 50th, 90th, and 95th centile of each index according to the CRL separated in four categories. Square root transformation was used to obtain normal distribution of each index.

In univariate analysis, we observed that advanced maternal age, greater BMI, East Asian ethnicity were all associated with lower vascularization indices, while smoking and method of conception were not significantly associated with VFI. We observed a negative correlation for all indices with maternal age (cc from –0.07 to –0.03, all with $p < 0.05$) and between BMI and FI and VFI (cc, –0.13 and –0.04, all with $p < 0.001$). Finally, we observed that parous women without history of preeclampsia had greater VFI, VI, and FI values compared with nulliparous women and greater FI values compared with parous women with previous preeclampsia (►**Table 3**). Parous women with previous preeclampsia had significantly lower FI than nulliparous women, but other vascularization indices showed no significance.

In multivariate linear regression analyses including all covariates (►**Table 4**), we observed that maternal age and BMI were associated with lower vascularization indices values (VI, FI, and VFI, all with $p < 0.01$). When compared with Caucasian women, all other ethnic groups had lower indices values ($p < 0.01$). In counterpart, pregestational diabetes, smoking, and assisted reproduction technology were not significantly associated with any of the indices. With regards to parity, we observed that parous women without history of preeclampsia had greater vascularization indices values compared with nulliparous women (VI, FI, and VFI, all with $p < 0.01$), while parous women with prior preeclampsia had nonsignificantly different indices values.

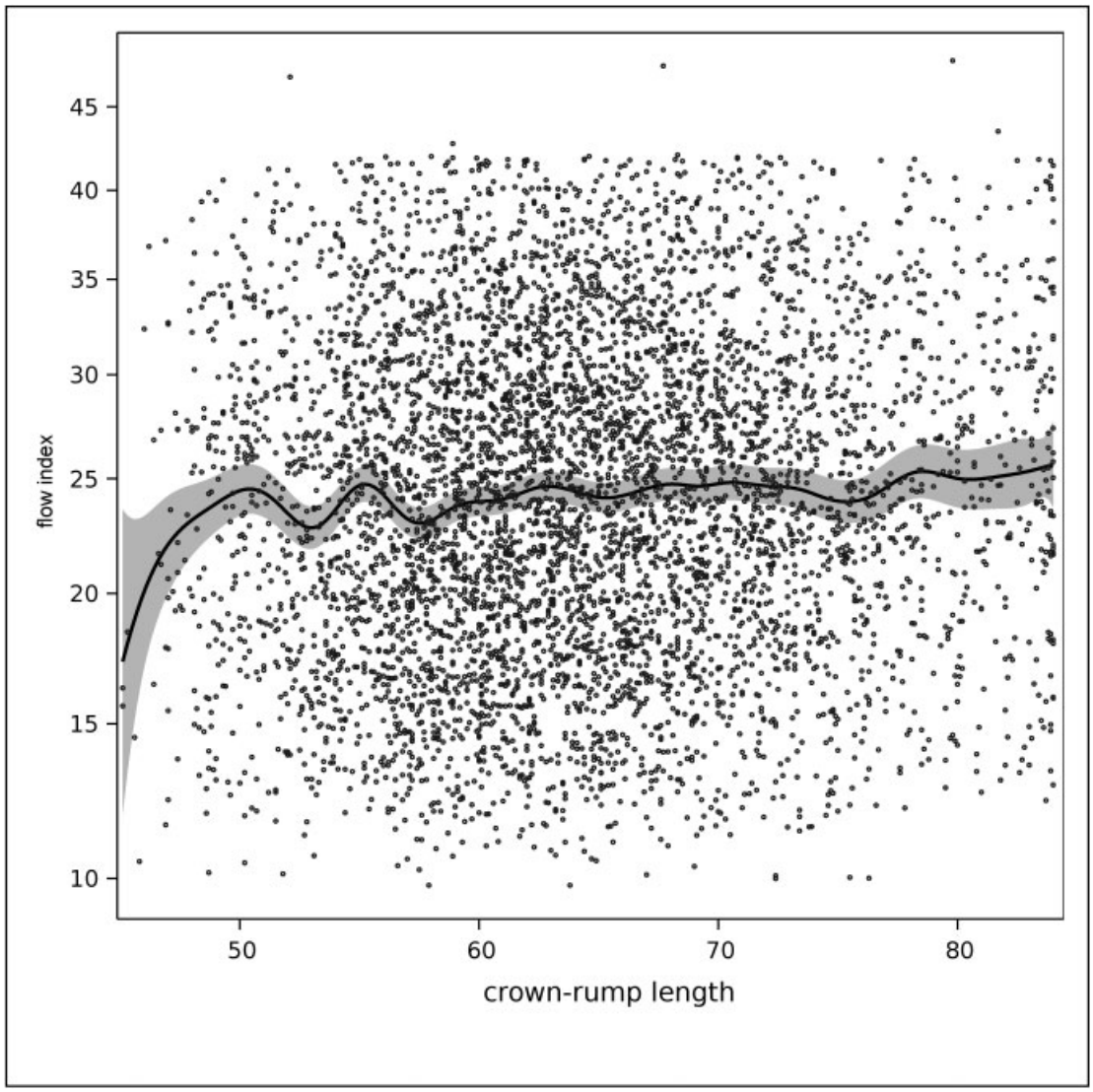

Fig. 3 Distribution of the flow index (FI) according to crown-rump length (CRL). The line represents penalized B-spline curve; and shaded area, the 95% confidence intervals of the means.

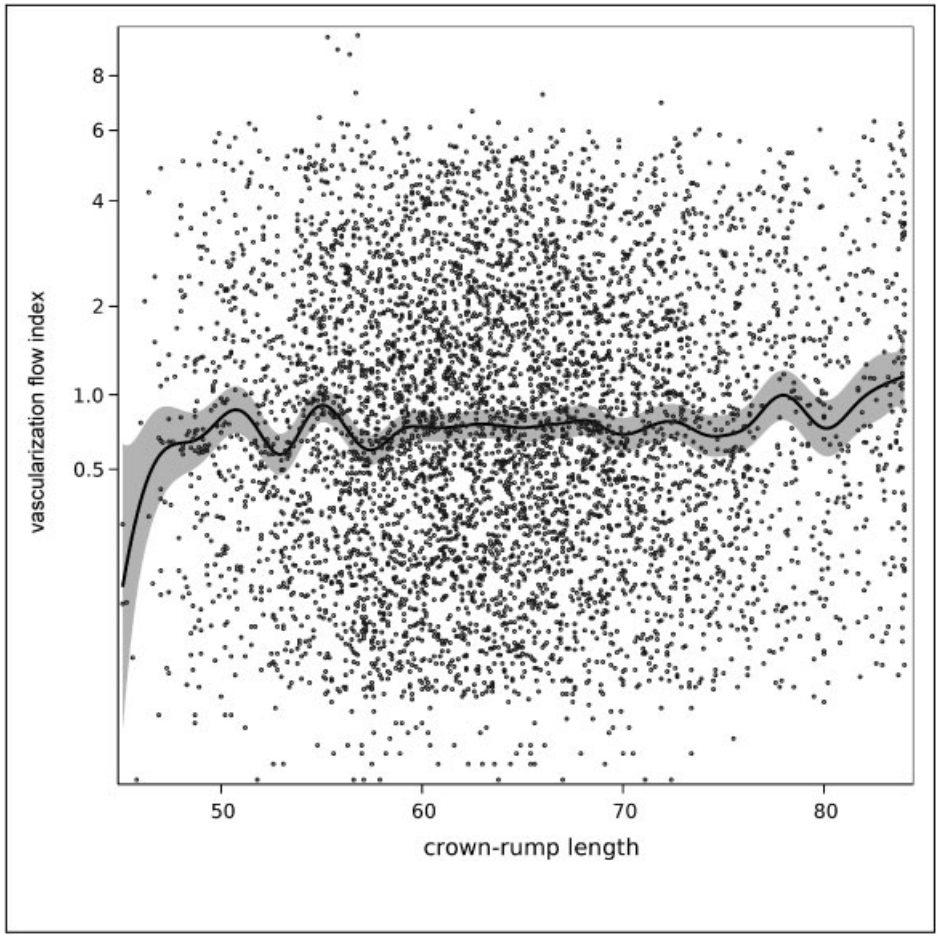

Fig. 4 Distribution of the vascularization flow index (VFI) according to crown-rump length (CRL). The line represents penalized B-spline curve; and shaded area, the 95% confidence intervals of the means.

Table 2 Vascularization indices distribution according to CRL

	CRL (mm)	Centiles				
		5th	10th	50th	90th	95th
VI	45–54	0.21	0.39	3.13	10.65	14.01
	55–64	0.17	0.32	3.08	11.02	13.54
	65–74	0.20	0.39	2.96	10.76	12.90
	75–84	0.28	0.50	3.63	11.55	13.70
FI	45–54	13.41	14.98	23.72	34.23	37.16
	55–64	13.56	15.12	24.05	35.27	37.87
	65–74	13.72	15.67	24.65	35.69	38.09
	75–84	14.18	15.81	24.69	35.33	38.35
VFI	45–54	0.04	0.07	0.67	3.23	4.45
	55–64	0.03	0.06	0.74	3.38	4.32
	65–74	0.03	0.08	0.76	3.25	4.16
	75–84	0.05	0.09	0.91	3.76	4.52

Abbreviations: CRL; crown-rump length; FI, flow index; VFI, vascularization flow index; VI, vascularization index.

Table 3 Vascularization indices according to parity and history of preeclampsia

Indices	Subgroups			*p*-Values		
	A. Nulliparous	B. Parous, no previous PE	C. Parous with previous PE	A vs. B	A vs. C	B vs. C
VI	0.97 (0.75–1.19)	1.03 (0.79–1.23)	1.01 (0.81–1.18)	< 0.01	0.52	0.26
FI	1.00 (0.88–1.07)	1.01 (0.89–1.12)	0.96 (0.83–1.09)	0.03	0.02	0.004
VFI	0.97 (0.71–1.22)	1.03 (0.76–1.28)	0.97 (0.76–1.19)	< 0.01	0.95	0.07

Abbreviations: FI, flow index; PE, preeclampsia; VFI, vascularization flow index; VI, vascularization index.
Note: Indices are reported as multiple of the median
(MoM) by interquartile after square root transformation. Median and interquartile range reported. *p*-Values based on Wilcoxon rank sums test.

Discussion

We observed that vascularization indices measured using USSB slightly increased with gestational age between 11 and 14 weeks of gestation. Moreover, we observed that maternal age, ethnicity other than Caucasian, and BMI were also associated with lower vascularization indices. We observed that parous women with no history of preeclampsia have greater vascularization indices values, while parous women with history of preeclampsia have vascularization indices

Table 4 Association between maternal characteristics and vascularization indices in multivariate linear regression models

	VFI[a]		VI[a]		FI[b]	
	Coefficient (SE)	*p*-Value	Coefficient (SE)	*p*-Value	Coefficient (SE)	*p*-Value
Maternal age	−0.006 (0.001)	< 0.0001	−0.005 (0.001)	< 0.0001	−0.001 (0.0004)	0.004
Race		0.0004		0.0002		< 0.0001
Caucasian	Reference					
African	−0.039 (0.014)		−0.037 (0.012)		−0.015 (0.006)	
East Asian	−0.079 (0.0290)		−0.064 (0.025)		−0.032 (0.013)	
South Asian	−0.042 (0.020)		−0.030 (0.018)		−0.033 (0.009)	
Mixed	−0.052 (0.026)		−0.056 (0.023)		−0.019 (0.012)	
BMI	−0.004 (0.001)	< 0.0001	−0.003 (0.001)	0.0007	−0.004 (0.0004)	< 0.0001
Smoking	−0.014 (0.018)	0.43	−0.007 (0.016)	0.64	−0.015 (0.008)	0.06
Parity and PE		< 0.0001		< 0.0001		< 0.0001
Nulliparous	Reference					
Parous, no previous PE	0.0648 (0.009)		0.057 (0.008)		0.017 (0.004)	
Parous with previous PE	0.021 (0.029)		0.035 (0.025)		−0.001 (0.013)	
Diabetes		0.78		0.73		0.99
No	Reference					
Type 1 diabetes	0.018 (0.065)		−0.014 (0.057)		−0.0004 (0.029)	
Type 2 diabetes	−0.077 (0.073)		−0.045 (0.059)		−0.005 (0.031)	
Conception		0.65		0.73		0.57
Spontaneous	Reference					
Ovulation drugs	−0.055 (0.060)		−0.035 (0.053)		−0.026 (0.027)	
In vitro fertilization	−0.014 (0.028)		−0.011 (0.025)		0.006 (0.013)	

Abbreviations: BMI, body mass index; FI, flow index; PE, preeclampsia; SE, standard error; VFI, vascularization flow index; VI, vascularization index.
Note: Shown are the regression coefficients (and their SE) representing the change in transformed vascularization indices values (adjusted for crown-rump length) with one-unit increment in continuous variables or change of categories compared with the reference for nominal variables.
[a]Multiple of the median of the fourth root of VFI or VI.
[b]Multiple of the median of the square root of FI.

values similar to nulliparous women. Overall, lower values of vascularization indices measured using USSB in the first trimester are associated with risk factors for preeclampsia.

Our study is in agreement with smaller studies that evaluated vascularization of the entire placenta using 3D power Doppler according to gestational age. Moran et al observed a nonsignificant positive trend between the indices and gestational age between 12 and 35 weeks, but the analyses were limited to 250 participants with only 32 recruited before 20 weeks of gestation.[18] Similar results were observed by Pomorski et al between 20 and 40 weeks of gestation.[14] Few studies evaluated the association between maternal characteristics and placental vascularization indices in the first trimester. Gonzalez et al and Moran et al reported that women with pregestational diabetes had lower indices values (VI, FI, and VFI) as early as the first trimester but they did not stratify their results according to the type of pregestational diabetes and they did not adjust for BMI.[15,18] In the current study, we observed that lower vascularization indices were specifically observed in women with type 2 diabetes but the association did not remain significant after adjustment for confounding factors including BMI. Interestingly, Rizzo et al observed that in type I diabetic mothers, placental vascular indices were increased and these modifications were more evident in pregnancies with poor first trimester glycemic control.[16] We observed no significant difference in women with pregestational diabetes. However, the small number of participants with pregestational diabetes and the absence of data on the severity of the disease limited our analyses. A recent study reported that women with type 1 diabetes had a risk of preeclampsia and preterm preeclampsia 5 to 10 times greater compared with women without diabetes and women with type 2 diabetes had a risk 3 to 4 times greater.[16] Further studies are required to explain the mechanisms of disease between pregestational diabetes and preeclampsia, but our data suggest that maternal BMI should be considered in the equation.

Rizzo et al also observed lower vascularization indices of the entire placenta with smoking of greater than 10 cigarettes per day.[19] We did not confirm this observation with vascularization indices measured through USSB, again being limited by the precision of our variable that did not capture the strength of the smoke exposure. Finally, our finding that vascularization indices are greater in parous women without history of preeclampsia is in agreement with those of Gonzalez et al.[15]

Our study has some limitations, including the small number of participants with pregestational diabetes or those reporting the use of ovulation drugs, limiting our capacity of studying such effect on vascularization indices. Furthermore, although we considered multiple baseline variables, residual confounding cannot be excluded. However, our strengths include the overall large number of participants allowing adjustment of each index for gestational age.

Overall, we observed that gestational age contributes significantly but minimally to vascularization indices and they were only slightly influenced by maternal characteristics after transformation and adjustment for gestational age (CRL) into MoM. We observed that factors associated with lower values were typically related with the risk of preeclampsia. Therefore, the next step should be the evaluation of the predictive values of such indices for preeclampsia. Small studies already suggested that USSB VFI of the full placental thickness or the subplacental region, and the placental thickness itself, could be useful for the prediction of preeclampsia and preterm preeclampsia in the first trimester of pregnancy.[11,12,20] Our study demonstrated that such study should include adjustment of vascularization indices according to gestational age or CRL.

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Conflict of Interest
None.

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