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Feasibility of three dimensional power Doppler ultrasonography methods to assess placental perfusion

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ABSTRACT

Objectives: Given the crucial role of the placenta in establishing a healthy pregnancy, reliable non-invasive methods to measure placental perfusion are desirable. The aim of this study is to determine the reproducibility and potential bias in different three-dimensional power Doppler (3DPD) methods assessing placenta perfusion.

Methods: Ten singleton pregnancies around 16 weeks of gestation, with an anteriorly located placenta and centrally inserted umbilical cord were included in this study. Eight different combinations of a specific placental sweep and sonobiopsy method were used to evaluate placental perfusion. Vascularization index (VI), flow index (FI) and vascularization-flow index (VFI) were determined offline using the 4D-view program. Reproducibility and repeatability of the methods, expressed as correlation coefficients and Bland-Altman mean differences, were calculated. Differences between sampling methods were analyzed using *t*-test or Mann-Whitney *U* test.

Results: Intra- and inter-class correlation coefficient (CC) was highest when using a spherical centrally placed sonobiopsy of 2 cm^3 in a whole placenta sweep (method 1; IntraCC VI 0.985, FI 0.769, VFI 0.993, InterCC VI 0.986, FI 0.784, VFI 0.987). Overall, intraCCs were higher compared to interCCs. Lowest mean differences in VI and FI were found comparing spherical to manual sonobiopsies, whereas the mean differences in VFI were lowest when comparing central *versus* peripheral located sonobiopsies. Comparing the three vascular indices, best median intra- and interCC and lowest mean differences were found for VFI.

Conclusions: Three dimensional placental vascularization analysis showed best reproducibility using whole placental sweep volume and centrally located, spherical sonobiopsy of 2 cm^3.

Introduction

During pregnancy, the placenta develops as one of the most essential short-lived organs in the human body. In normotensive, early gestation, adequate maternal-fetal exchange is established by a physiological invasion of trophoblast cells into the walls of the spiral arteries. Trophoblast invasion causes the spiral arteries to lose their smooth muscle cells, thereby increasing their diameter and losing their vasoreactive properties and with it establishment of a continuous low-velocity blood flow into the intervillous space [1,2]. Complete transformation of the myometrial spiral arteries is thought to be more extensive in the central part compared to peripheral parts of the placenta [3]. Failure of this transformation is a significant component in the etiology of reduced placental perfusion that leads to many disorders collectively known as placenta syndrome (PS) [4,5]. Given the placenta's crucial role to enable adequate oxidative and nutrient fetal-maternal exchange, validly reproducible, non-invasive methods to measure placental insufficiency are desirable. The golden standard in determining placental insufficiency is postpartum histopathology [6]; however, this information is behind time to affect clinical decision and

Abbreviations: 3DPD, Three Dimensional Power Doppler; VI, Vascularization Index; FI, Flow Index; VFI, Vascularization-Flow Index; CC, correlation coefficient; PS, placental syndrome; CD, color Doppler; BMI, body mass index; VOCAL, Virtual Organ Computer-aided AnaLysis; WP, whole placenta; SP, segmental placenta; CM, centimeters; SD, standard deviation; IVF, *In vitro* fertilization; IQR, inter quartile range; STIC, spatiotemporal image correlation; CI, confidence interval.

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intervention. Therefore, researchers have started to focus on non-invasive, antepartum methods to measure utero-placental circulation, amongst color Doppler (CD) ultrasonography, that also allows to study the placental vascular development throughout gestation. Abnormal upstream CD measurements, representing high resistance in the uterine artery, are associated with an increased risk of preeclampsia and intra uterine growth restriction, both representors of maternal and fetal placental syndromes [7]. Nonetheless, uterine artery resistance is a surrogate for downstream placental perfusion and does not necessarily reflect the (patho-) physiological changes within the placenta [8]. There is a growing interest for 3D power Doppler (3DPD) ultrasonography during recent years, given the higher sensitivity for small vessels and low-velocity flow compared to CD [9–12]. Measures of placental perfusion, obtained by sonobiopsy sampling, are expressed as vascularization index (VI), flow index (FI) and vascularization flow index (VFI) and are calculated out of 3-dimensional volume units. Previous 3DPD-studies showed conflicting results on reproducibility that may depend on the chosen region of interest, sweep method and measurement method of the sonobiopsy sampled [13–17]. It is therefore challenging to interpret and extrapolate previous published data.

The aim of this study was to determine the validity and reproducibility of 3DPD methods assessing placental perfusion, focusing on the location and method of sonobiopsy sampling in combination with different 3D sweeps.

Methods

Study population

This study was performed between January and July 2019, as part of a longitudinal study, at the Department of Gynecology and Obstetrics, Maastricht University Medical Centre (MUMC+). A total of 10 Dutch speaking or understanding women with a singleton pregnancy around 16 weeks of gestation, with an anterior located placenta and centrally inserted umbilical cord were included in this study. Ethical approval for this study was given by the Ethics Committee of the MUMC+ (METC 15-4-026). Written informed consent was obtained for every participating woman. Exclusion criteria were fetal chromosomal or syndromal abnormalities, placental tumors, Body Mass Index (BMI) > 35 kg/m^2 and age < 18 years. All placental ultrasonography images were acquired by one sonographer (V.S.) using Voluson 730 Expert ultrasound machine (GE Healthcare) equipped with a 3,5 MHz abdominal transducer. Women were positioned in a semi-recumbent position (+/- 45°) and were asked to remain as still as possible during the examination. A baseline two-dimensional ultrasound scan was performed to confirm the viability of the pregnancy, position of the fetus and location of the placenta. Ultrasound scans were performed between 15.9 and 18.0 weeks of gestation as part of a longitudinal study.

Ultrasound settings

The position of the transducer was adjusted to provide a cross-sectional view of the placenta. During the placental sweep measurement, women were asked to hold their breath for up to 10 s. Basic settings were PRF 1.3 kHz, WMF low 2, frequency low, quality norm, gain 1 (CD) and -1.6 (PD), depth 10.7 cm, overall zoom 1.1, scan angle 55° and a 3D box size that was in line with the outer margins of the scan angle. However, depending on maternal BMI and the presence of artefacts, the CD gain was adjusted (to a maximum of 5) to reach individual optimal settings. Based on previous studies, eight different methods to evaluate placental perfusion were selected and used for analysis. Each method was a combination of a specific placental sweep (of the whole or segmental placenta) and sonobiopsy method (spherical or manual volume of 2 cm^3). The software program Virtual Organ Computer-aided AnaLysis (VOCAL) (GE) was used to calculate the 3DPD indices offline in every patient.

3DPD sweeps

After visualizing the area of interest and starting the Power Doppler (PD) function on the ultrasound machine, a 3D sweep was performed to require a 3DPD volume. Three different 3DPD sweeps were taken in every patient; one large sweep covering the whole placenta (WP) and two smaller sweeps of the central and peripheral segments of the placenta (SP), see Fig. 1. A WP sweep was acquired by adjusting the sweep angle to a maximum of 90° to ensure the entire length of the placenta was captured during the measurement (Fig. 1a). To acquire the SP sweeps, the sonographer zoomed in on the area of interest, including the chorionic and basal plate of the placenta. For central segments of the placenta, the 3D box was placed at the umbilical cord insertion (Fig. 1b). For peripheral segments of the placenta, the 3D box was placed in line with the lateral (maternal right side by convention) edge of the placenta (Fig. 1c).

VOCAL analysis sonobiopsies

The volumes were analyzed using VOCAL included in the 4D-View software (Version 10.5, BT12, GE Healthcare). Assessment of the 3DPD images was performed using the rotational sonobiopsy method of the VOCAL software, in which the volume was rotated 6 times over a 30° angle. The 3DPD volume was displayed in a multiplane view, consisting of orthogonal planes A, B, C and a fourth plane displaying the rendered volume. Plane A was displayed as coronal, plane B as sagittal and plane C as axial. For the 3DPD analysis, plane A was used as a workplane. Sonobiopsy was positioned in the center of the volume and blood vessels from the basal and chorionic plates were not included. Within each 3DPD sweep, two different sonobiopsy methods were performed: 1) a spherical sonobiopsy with a predefined volume of 2 cm^3 and 2)

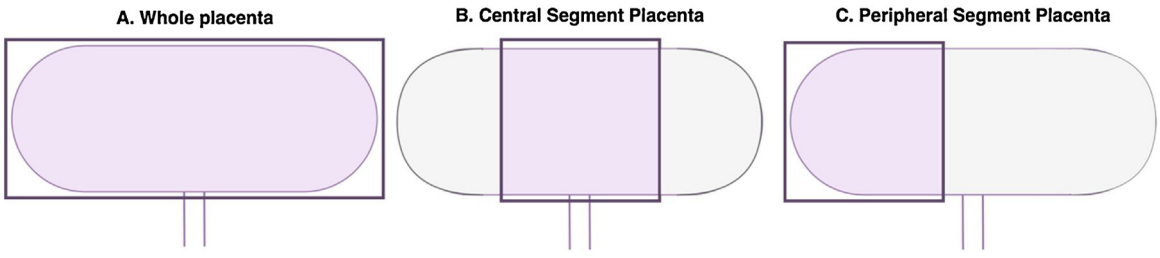

Fig. 1. Schematic overview of different sweep methods in a placenta with central insertion of the umbilical cord. A; 3D Power Doppler sweep of the whole placenta (WP), B; 3D Power Doppler sweep of the central segment of the placenta (SP) and C; 3D Power Doppler sweep of the peripheral segment of the placenta (SP).

a manual sonobiopsy. For the latter, VOCAL's manual mode enabled us to trace a specific volume of interest (sonobiopsy) of ± 2 cm^3 within the acquired 3DPD sweep and calculated the vascularization in this given volume. VOCAL automatically calculated 3DPD indices; *i.e.* Vascularization Index (VI), Flow Index (FI) and Vascularization-Flow Index (VFI). VI reflects the relative proportion of colored to grey voxels in the volume of interest and determines the quantitative amount of blood vessels. FI represents the mean power Doppler signal intensity. VFI is a combination of the two previous described indices, derived by their multiplication, providing information concerning vessel presence and the amount of flow [13,16].

Fig. 2 gives a summary of the eight sonobiopsy methods, using WP sweeps (method 1–4), central SP sweeps (method 5,7), peripheral SP sweeps (method 6,8) and spherical or manual sonobiopsies. All described eight methods were performed three times in every placenta volume, twice by observer JH and once by observer DP.

The Supporting information provides a protocol that describes obtaining the 3DPD sweep and the offline analysis to calculate VI, FI and VFI using method 1.

Statistical analysis

Statistical analysis was performed using SPSS version 20 (SPSS, Inc, Chicago, Illinois) and Graphpad Prism version 5.0. Normal distribution of the data was confirmed with the Shapiro- Wilk test. The intra-rater and inter-rater reproducibility, expressed as the intra-class correlation coefficient (ICC) and 95 % confident intervals was calculated using the two-way random model, single measures and absolute agreement. Values less than 0.50 were considered poor reproducibility, values between 0.50-0.75 as moderate reproducibility, values between 0.75-0.90 as good reproducibility and values greater than 0.90 as excellent reproducibility [18]. The difference between each pair of measurements was calculated by the value of the first measurement minus the value of the second measurement. Then, the Bland-Altman mean difference including standard deviation (SD) and the 95 % confidence intervals (mean difference plus or minus (1.96 x SD)) were calculated for each method and vascular index. The mean differences between the measurements were close to zero if the raters tend to agree in measurement. Last, independent *t*-test and Mann-Whitney *U* test were used as appropriate to compare between sampling methods.

Results

Characteristics of study group

A total of 10 pregnancies without fetal structural anomalies or pre-existing conditions at that moment were included. All women

Table 1
Maternal characteristics of study population (n = 10). Data are presented as mean with corresponding standard deviation, median with inter quartile range or number with percentage of the total population.

Baseline characteristics	
Age (years)	33 ± 5
BMI (pre-conceptional) (kg/m^2)	27 ± 5
Gestational age at ultrasound (days)	116 ± 5
Nulliparous (n, %)	6 (60)
Smoking (n, %)	2 (20)
Caucasian (n, %)	10 (100)
Obstetric complicated history (n, %)	1 (10)
Pregnancy outcome	
Uncomplicated pregnancy (n, %)	9 (90)
Gestational age at delivery (days)	277 (269–286)
Birthweight (gram)	3495 (3004–3986)
Small for gestational age (n, %)	0 (0)
Male gender (n, %)	6 (60)
Severe blood loss (> 2000 ml) (n, %)	1 (10)

BMI; body mass index, kg; kilogram, m; meter, ml; milliliter.

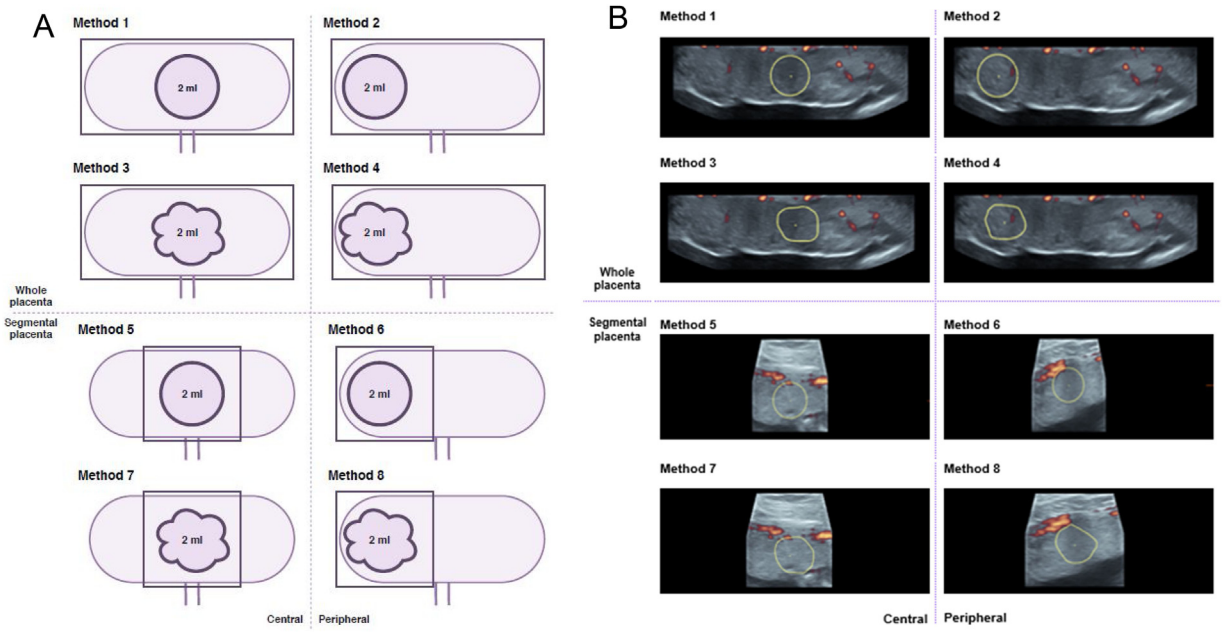

Fig. 2. A. Schematic overview of the eight different sonobiopsy methods in both whole placenta and segmental placental sweeps. The sonobiopsies are spherical or manually traced and placed in the central or peripheral part of the placenta.
B. Overview of the eight different sonobiopsy methods in both whole placenta and segmental placental sweeps. The sonobiopsies are spherical or manually traced and placed in the central or peripheral part of the placenta.

had an anterior-located placenta and centrally inserted umbilical cord. Average gestational age during ultrasound scan was 116 ± 5 days. The women had a mean age of 33 ± 5 years and a pre-conceptional BMI of 27 ± 5 kg/m^2, ***see*** Table 1. All women were Caucasian and did not use antihypertensive drugs at time of measurement. Six women were nulliparous and four women were multiparous. Three of them had no previous obstetric complications; one woman lost her child due to placental abruption. Furthermore, nine women had *in vitro* Fertilization (IVF) pregnancy and one woman had a spontaneous pregnancy. In the current pregnancy, nine women were uncomplicated, whereas one pregnancy ended again in intra-uterine fetal death due to recurrence of placental abruption. This woman gave birth at 32 weeks of gestation. Median gestational age at delivery was 277 (Inter quartile range (IQR): 269-286) days, median birthweight was 3495 (IQR: 3004-3986) gram and there were no small-for-gestational age neonates. Sixty percent of the women gave birth to a boy. One women had severe blood loss during delivery (4000 ml).

Intra-rater reproducibility

Intra-class correlation coefficient

Intra-rater reproducibility of the eight used methods, quantified as the ICC with 95 % confidence interval (CI) of the measured VI, FI and VFI, see Table 2. The intraCC for VI and VFI were > 0.7 in all methods, except for method 7 (ICC VI 0.54, VFI 0.67). An intraCC of > 0.9 for VI and VFI was found in method 1, 2 and 6, whereas VFI in method 4 also showed excellent reproducibility (ICC = 0.96) next to method 1, 2 and 6. However, taking into account the 95 % CI, only method 1 showed excellent intra-reproducibility for VI and VFI. The intraCC for FI was > 0,7 in method 1, 2, 5, and 7. An intraCC of > 0.9 for FI was only found in method 5, however the lower limit of the confidence interval was 0.87.

Regardless of the used method, best median intraCC was observed for VFI (0.91, 95 % CI 0.70-0.96). Median intraCC of VI was higher compared to median intraCC of FI (VI; 0.86, 95 % CI 0.79-0.96, FI; 0.69, 95 % CI 0.56-0.83).

Inter-rater reproducibility

Inter-class correlation coefficient

Inter-rater reproducibility of the used methods was evaluated using inter-rater correlation coefficients (interCC) with 95 % confidence interval (CI) of the measured VI, FI and VFI, see Table 3. InterCC was > 0.7 for VI in method 1, 2 and 3 (0.99, 0.90, 0.73), for FI in method 1, 5, and 7 (0.78, 0.90, 0.81) and for VFI in method 1, 2 and 4 (0.99, 0.97, 0.91). InterCC > 0.9 for VI was found in method 1 and for VFI in method 1, 2 and 4. However, taking into account the 95 % CI, only method 1 showed excellent inter-reproducibility for both VI and VFI. An interCC of 0.90 was found in method 5 for FI, however the lower limit of the confidence interval was 0.63.

Comparing the three vascular indices regardless of the used method, best median interCC was observed for VFI (0.66, 95 % CI 0.24-0.97). Median interCC of FI was higher compared to median interCC of VI (FI; 0.59, 95 % CI 0.30-0.81, VI; 0.51, 95 % CI 0.34-0.90).

In addition to the interCCs, the mean difference and the 95 % limits of agreement from the vascular indices were calculated. Highest mean differences in VI, FI and VFI were found in method 4, 3 and 8 respectively. Lowest mean differences in VI and VFI were found in method 1 (VI; 0.06 ± 1.23, VFI; -0.11 ± 0.53), whereas lowest mean difference in FI was found in method 7 (0.24 ± 5.09).

Comparing the three vascular indices regardless of the used method, we found that in five out of the eight methods lowest mean differences and smallest limits of agreement were found in VFI. Method 1 showed lowest mean difference in VI and method 4 and 7 in FI.

Differences between methods

The first measurements of observer JH were used to investigate the differences between the location, sampling and sweep methods.

When comparing central sonobiopsies to peripheral sonobiopsies in the placental bed, no significant differences in VI were found in method 1 *vs.* (*versus*) 2, method 3 *vs.* 4, method 5 *vs.* 6 and method 7 *vs.* 8, see Table 4. Moreover, no differences in VI were

Table 2
Intra-rater reproducibility expressed as intra-rater correlation coefficients.

Method	Sweep	Location	Sonobiopsy	Vascular Index	Intra-CC	95 % CI Intra-CC
1	WP	C	S	VI	0.985	0.921 - 0.997
				FI	0.769	0.154 - 0.947
				VFI	0.993	0.965 – 0.999
2	WP	P	S	VI	0.959	0.846 – 0.990
				FI	0.722	0.248 – 0.922
				VFI	0.937	0.765 – 0.984
3	WP	C	M	VI	0.845	0.517 – 0.959
				FI	0.666	0.092 – 0.906
				VFI	0.804	0.380 - 0.948
4	WP	P	M	VI	0.863	0.536 – 0.964
				FI	0.542	−0.478 – 0.746
				VFI	0.955	0.818 – 0.989
5	SP	C	S	VI	0.855	0.545 – 0.961
				FI	0.966	0.872 – 0.992
				VFI	0.886	0.628 – 0.970
6	SP	P	S	VI	0.950	0.794 – 0.990
				FI	0.560	−0.169 – 0.893
				VFI	0.927	0.704 – 0.985
7	SP	C	M	VI	0.536	−0.131 – 0.862
				FI	0.834	0.481 – 0.955
				VFI	0.672	−0.195 – 0.853
8	SP	P	M	VI	0.788	0.299 – 0.953
				FI	0.619	0.012 – 0.906
				VFI	0.699	0.097 – 0.931

WP; whole placenta, SP; segmental placenta, C; central, P; peripheral, M; manually traced, S; spherically traced, VI; vascularization index, FI; flow index, VFI; vascularization-flow index, CI; confidence interval.

Table 3
Inter-rater reproducibility expressed as inter-rater correlation coefficients and mean differences.

Method	Sweep	Location	Sonobiopsy	Vascular Index	Inter-CC	95 % CI Inter-CC	Mean difference (SD)	95 % Limits of Agreement
1	WP	C	S	VI	0.986	0.931 - 0.997	0.064 ± 1.230	−2.347 – 2.475
				FI	0.784	0.208 – 0.954	−3.010 ± 3.910	−10.674 – 4.654
				VFI	0.987	0.942 – 0.997	−0.110 ± 0.530	−1.149 – 0.929
2	WP	P	S	VI	0.896	0.658 – 0.973	−1.389 ± 4.166	−9.554 – 6.776
				FI	0.624	0.079 – 0.889	−2.731 ± 7.196	−16.835 – 11.373
				VFI	0.965	0.867 – 0.991	−0.481 ± 1.465	−3.352 – 2.390
3	WP	C	M	VI	0.727	0.184 – 0.931	−0.947 ± 4.225	−9.228 – 7.334
				FI	0.352	−0.182 – 0.784	−4.007 ± 5.609	−15.001 – 6.987
				VFI	0.635	0.024 – 0.904	−0.566 ± 2.050	−4.584 – 3.452
4	WP	P	M	VI	0.339	−0.296 – 0.778	−8.076 ± 23.501	−54.138 – 37.986
				FI	0.557	−0.115 – 0.871	0.375 ± 7.071	−13.484 – 14.234
				VFI	0.914	0.692 – 0.978	−0.883 ± 2.417	−5.620 – 3.854
5	SP	C	S	VI	0.522	−0.113 – 0.865	−0.945 ± 2.513	−5.871 -3.981
				FI	0.900	0.633 – 0.976	−0.610 ± 2.930	−6.353 – 5.133
				VFI	0.295	−0.283 – 0.765	−0.428 ± 1.237	−2.852 -1.997
6	SP	P	S	VI	0.240	−0.280 – 0.749	−4.040 ± 5.809	−15.426 – 7.346
				FI	0.296	−0.435 – 0.801	−3.247 ± 9.232	−21.342 – 14.848
				VFI	0.010	−0.451 – 0.617	−1.676 ± 2.652	−6.874 – 3.522
7	SP	C	M	VI	0.497	−0.085 – 0.851	−1.622 ± 2.602	−6.722 – 3.478
				FI	0.807	0.338 – 0.953	−0.239 ± 5.089	−10.213 – 9.735
				VFI	0.688	−0.061 – 0.862	−0.701 ± 1.193	−3.039 – 1.637
8	SP	P	M	VI	0.424	−0.189 – 0.839	−4.296 ± 7.142	−18.294 – 9.702
				FI	0.672	0.081 – 0.922	−3.601 ± 5.805	−14.979 – 7.777
				VFI	0.238	−0.338 – 0.758	−1.828 ± 3.147	−7.996 – 4.340

WP; whole placenta, SP; segmental placenta, C; central, P; peripheral M; manually traced, S; spherically traced, VI; vascularization index, FI; flow index, VFI; vascularization-flow index, CI; confidence interval.

Table 4
Differences in central *vs*. peripheral biopsies, spherical *vs*. manual biopsies and whole placenta sweeps *vs*. segmental placenta sweep for VI, FI, VFI.

	Methods	VI	FI	VFI
Location of sonobiopsy	**Central** *versus* **peripheral**			
	1 *vs*. 2	3.06 (1.20–7.46) *vs*. 0.51 (0.23–7.01)	31.63 (30.40–37.58) *vs*. 29.70 ± 8.76	1.04 (0.39–2.38) *vs*. 0.16 (0.59–2.40)
	3 *vs*. 4	2.38 (0.15–4.97) *vs*. 0.99 (0.48–6.56)	31.64 ± 5.81 *vs*. 34.37 ± 7.28	0.90 (0.20–1.53) *vs*. 0.27 (0.12–1.49)
	5 *vs*. 6	3.62 ± 2.83 *vs*. 4.44 ± 3.92	33.18 ± 6.21 *vs*. 31.86 ± 5.07	1.30 ± 1.18 *vs*. 1.44 ± 1.31
	7 *vs*. 8	3.61 ± 2.61 *vs*. 5.07 ± 5.59	31.07 (29.07–42.88) *vs*. 29.72 (27.65–39.73)	1.37 ± 1.20 *vs*. 0.99 (0.56–3.17)
Sonobiopsy method	**Spherical** *versus* **manual**			
	1 *vs*. 3	3.06 (1.20–7.46) *vs*. 2.38 (0.15–4.97)	31.63 (30.40–37.58) *vs*. 31.64 ± 5.81	1.04 (0.39–2.38) *vs*. 0.90 (0.20–1.53)
	2 *vs*. 4	0.51 (0.23–7.01) *vs*. 0.99 (0.48–6.56)	29.70 ± 8.76 *vs*. 34.37 ± 7.28	0.16 (0.59–2.40) *vs*. 0.27 (0.12–1.49)
	5 *vs*. 7	3.62 ± 2.83 *vs*. 3.61 ± 2.61	33.18 ± 6.21 *vs*. 31.07 (29.07–42.88)	1.30 ± 1.18 *vs*. 1.37 ± 1.20
	6 *vs*. 8	4.44 ± 3.92 *vs*. 5.07 ± 5.59	31.86 ± 5.07 *vs*. 29.72 (27.65–39.73)	1.44 ± 1.31 *vs*. 0.99 (0.56–3.17)
3D sweep method	**Whole placenta** *versus* **segmental placenta**			
	1 *vs*. 5	3.06 (1.20–7.46) *vs*. 3.62 ± 2.83	31.63 (30.40–37.58) *vs*. 33.18 ± 6.21	1.04 (0.39–2.38) *vs*. 1.30 ± 1.18
	2 *vs*. 6	0.51 (0.23–7.01) *vs*. 4.44 ± 3.92	29.70 ± 8.76 *vs*. 31.86 ± 5.07	0.16 (0.59–2.40) *vs*. 1.44 ± 1.31
	3 *vs*. 7	2.38 (0.15–4.97) *vs*. 3.61 ± 2.61	31.64 ± 5.81 *vs*. 31.07 (29.07–42.88)	0.90 (0.20–1.53) *vs*. 1.37 ± 1.20
	4 *vs*. 8	0.99 (0.48–6.56) *vs*. 5.07 ± 5.59	34.37 ± 7.28 *vs*. 29.72 (27.65–39.73)	0.27 (0.12–1.49) *vs*. 0.99 (0.56–3.17)

Data are presented as median with interquartile range or as mean with standard deviation. No statistically significant differences are found. Data are tested using Mann Whitney U or independent T-test.

found for spherical sonobiopsies *vs*. manual sonobiopsies in method 1 *vs*. 3, method 2 *vs*. 4, method 5 *vs*. 7 and method 6 *vs*. 8. Also, taking a whole placental sweep *vs*. a segmental placental sweep, showed no significant differences in VI, when comparing method 1 *vs*. 5, method 2 *vs*. 6, method 3 *vs*. 7 and method 4 *vs*. 8. Similar trends were found for FI and VFI.

Lowest mean differences in VI and FI were found comparing spherical to manual sonobiopsies, whereas the mean differences in VFI was lowest when comparing central *versus* peripheral located sonobiopsies, see Table 5.

Discussion

As far as we know, this is the first study to report on the most reproducible 3DPD method to measure placental vascularization at 16 weeks of gestation and that addresses the possible influence of the sonobiopsy location within the placenta. VI and VFI showed best intra- and inter reproducibility using a spherical centrally placed sonobiopsy in a whole placenta sweep (method 1). Furthermore, no significant influences were found of the location, sampling and sweep methods on the reproducibility of measurements.

Since the introduction of 3DPD ultrasound, a new era in measuring vascularization within the field of gynecology and obstetrics has begun. Using this non-invasive quantification technique, a virtually reconstructed vascular tree within a volume of interest can be assessed and the vascularization can be determined by the calculation of vascular indices [19]. Previous investigators have demonstrated that the use of 3DPD indices show good correlation with real blood flow, vessel number and erythrocyte density in experimental animal models and computer-driven flow phantom models [20,21]. Although 3DPD has been studied in measuring placental perfusion in both healthy and pathological pregnancies, it is challenging to encapsulate and

Table 5
Mean differences in central *versus* peripheral biopsies, spherical *versus* manual biopsies and whole placenta sweeps *vs.* segmental placenta sweep for VI, FI, VFI.

	Methods	VI			FI			VFI		
		Absolute value	Mean difference (SD)	95 % limits of agreement	Absolute value	Mean difference (SD)	95 % limits of agreement	Absolute value	Mean difference (SD)	95 % limits of agreement
Location of sonobiopsy	**Central** *versus* **peripheral**									
	1 vs. 2	3.06 (1.20–7.46) *vs.* 0.51 (0.23–7.01)	−0.50 ± 5.86	*−11.99- 10.99*	31.63 (30.40–37.58) *vs.* 29.70 ± 8.76	4.11 ± 5.16	*−6.00-14.22*	1.04 (0.39–2.38) *vs.* 0.16 (0.59–2.40)	−0.25 ± 1.98	*−4.14-3.62*
	3 vs. 4	2.38 (0.15–4.97) *vs.* 0.99 (0.48–6.56)	−0.77 ± 5.15	*−10.86-9.32*	31.64 ± 5.81 *vs.* 34.37 ± 7.28	−2.73 ± 6.36	*−15.20-9.74*	0.90 (0.20–1.53) *vs.* 0.27 (0.12–1.49)	−0.38 ± 1.90	*−4.10-3.34*
	5 vs. 6	3.62 ± 2.83 *vs.* 4.44 ± 3.92	−0.38 ± 5.16	*−10.49-9.73*	33.18 ± 6.21 *vs.* 31.86 ± 5.07	1.81 ± 9.64	*−17.08-20.70*	1.30 ± 1.18 *vs.* 1.44 ± 1.31	0.03 ± 1.97	*−3.83-3.89*
	7 vs. 8	3.61 ± 2.61 *vs.* 5.06 ± 5.59	−1.32 ± 6.27	*−13.60-10.97*	31.07 (29.07–42.88) *vs.* 29.72 (27.65–39.73)	2.92 ± 9.09	*−14.90-20.74*	1.37 ± 1.20 *vs.* 0.99 (0.56–3.17)	−0.22 ± 2.35	*−4.83-4.39*
Sonobiopsy method	**Spherical** *versus* **manual**									
	1 *vs.* 3	3.06 (1.20–7.46) *vs.* 2.38 (0.15–4.97)	1.06 ± 3.29	*−5.39-7.51*	31.63 (30.40–37.58) *vs.* 31.64 ± 5.81	1.06 ± 3.29	*−5.39-7.51*	1.04 (0.39–2.38) *vs.* 0.90 (0.20–1.53)	0.58 ± 1.85	*−3.05-4.21*
	2 *vs.* 4	0.51 (0.23–7.01) *vs.* 0.99 (0.48–6.56)	0.59 ± 2.77	*−4.84-6.02*	29.70 ± 8.76 *vs* 34.37 ± 7.28	−4.67 ± 7.80	*−19.96-10.62*	0.16 (0.59–2.40) *vs.* 0.27 (0.12–1.49)	0.32 ± 1.36	*−2.35-2.99*
	5 *vs.* 7	3.62 ± 2.83 *vs.* 3.61 ± 2.61	0.02 ± 2.04	*−3.98-4.02*	33.18 ± 6.21 *vs.* 31.07 (29.07–42.88)	−1.68 ± 3.93	*−9.38-6.02*	1.30 ± 1.18 *vs.* 1.37 ± 1.20	−0.08 ± 0.86	*−1.77-1.61*
	6 *vs.* 8	4.44 ± 3.92 *vs.* 5.06 ± 5.59	−0.63 ± 2.74	*−6.00-4.74*	31.86 ± 5.07 *vs.* 29.72 (27.65–39.73)	0.20 ± 7.26	*−14.03-14.43*	1.44 ± 1.31 *vs.* 0.99 (0.56–3.17)	−0.21 ± 1.12	*−2.41-1.99*
3D sweep method	**Whole placenta** *versus* **segmental placenta**									
	1 *vs.* 5	3.06 (1.20–7.46) *vs.* 3.62 ± 2.83	2.05 ± 1.24	*−0.38-4.48*	31.63 (30.40–37.58) *vs.* 31.64 ± 5.81	1.88 ± 9.23	*−16.21-19.97*	1.04 (0.39–2.38) *vs.* 1.30 ± 1.18	0.97 ± 3.02	*−4.95-6.89*
	2 *vs.* 6	0.51 (0.23–7.01) *vs.* 4.44 ± 3.92	1.32 ± 8.33	*−15.01-17.65*	29.70 ± 8.76 *vs.* 31.86 ± 5.07	−0.85 ± 9.96	*−20.37-18.67*	0.16 (0.59–2.40) *vs.* 1.44 ± 1.31	0.86 ± 3.88	*−5.06-6.78*
	3 *vs.* 7	2.38 (0.15–4.97) *vs.* 3.61 ± 2.61	−0.26 ± 1.20	*−2.61-2.09*	31.64 ± 5.81 *vs.* 31.07 (29.07–42.88)	−3.22 ± 11.54	*−25.84-19.40*	0.90 (0.20–1.53) *vs.* 1.37 ± 1.20	−0.20 ± 1.70	*−3.53-3.13*
	4 *vs.* 8	0.99 (0.48–6.56) *vs.* 5.06 ± 5.59	−0.01 ± 6.51	*−12.77-12.75*	34.37 ± 7.28 *vs.* 29.72 (27.65–39.73)	5.04 ± 6.70	*−8.09-18.17*	0.27 (0.12–1.49) *vs.* 0.99 (0.56–3.17)	0.26 ± 2.61	*−4.86-5.38*

Data are presented as median with interquartile range or as mean with standard deviation. No statistically significant differences are found. Data are tested using Mann Whitney U or T-test.

interpret the available data on 3DPD methods and to implement it in the clinic without using a standardized protocol.

This study addresses eight different combinations of a specific placental sweep and sonobiopsy method at 16 weeks of gestation. Previous studies investigated placental perfusion at different gestational ages, ranging from 12 to 35 weeks of gestation, resulting in a wide variance of 3DPD VI, FI and VFI data. Performing a 3DPD placental sweep too early (<12 weeks of gestation) could give an underestimation of the vascularization because of still incomplete adaptation of the utero placental circulation [22], whereas measuring during later gestational age could give difficulties in performing the measurements due to the growing placenta and fetus. If, in future, 3DPD is used as a screening tool for development of clinical placental syndromes, the beginning of the second trimester would be an optimal time to affect clinical decision making.

This study concludes that using a spherical centrally placed sonobiopsy in a whole placenta sweep is the best reproducible method. Previous studies have examined placental vascularization by obtaining a sweep of the whole placenta and selecting all the placental tissue as volume of interest [11,23,24], whereas others selected specific areas of interest in the placenta by the use of a partial sweep or a sonobiopsy in a specific part of the placenta [13]. Selecting all the visible placental tissue could be challenging during later gestation given the difficulty of capturing the whole placenta [25]. Pomorski et al. found that this was impossible in 1 in 3 patients with a gestational age between 30 and 35 weeks and even 2 in 3 patients with a gestational age between 36 and 40 weeks [15]. Since the evaluation of the entire placenta was challenging during later gestation, researchers started to use a sonobiopsy method to measure placental vascularization [16,17,26]. Subsequently, Tuuli et al. confirmed that placental sonobiopsy vascular indices have a good correlation with those from evaluation of the entire placenta [27]. Therefore, this research did not included 3DPD methods that manually trace the entire placenta.

Given the previously published studies showing differences between centrally located and peripherally located spiral arteries and the physiology of spiral artery remodeling, we investigated these potential differences by comparing different methods [3,5,28]. Comparing 3D indices in the central parts of the placenta to the peripheral parts, we found no significant differences, suggesting the vascularization is equally allocated throughout the placental bed. However, the observed non-significance could also be explained by a type 2 error (*i.e.* a false-negative finding), given the small sample size and hence, low statistical power. Another explanation for the non-significance could be that at 16 weeks of gestation trophoblastic remodeling of spiral arteries from the central part to the peripheral parts is still occurring and differences in intensity are too small to be significant [29]. Sonobiopsy methods were also analyzed and no significant differences in VI, FI or VFI were found when comparing spherical sonobiopsies to manual biopsies. In the manually traced biopsies, the observers were able to subjectively exclude artifacts in the volume, however this did not result into observed differences. Moreover, no significant differences were found in using a whole placenta sweep compared to a partial placental sweep. This could be explained by the fact that the actual location of the sonobiopsy, regardless of sweep-method, is more or less the same.

In this study, FI showed the lowest intra- and inter-reproducibility compared to VI and VFI indices. It has been stated before that the FI may be a less reliable measure [30,31]. Most examiners believe that the FI is representing blood flow at a given time-point that is calculated out of the intensity of the colored voxels in the given volume. Yet, Raine-Fenning et al. observed that a greater distance between the transducer and the 'furthest' vessel decreased signal intensity and could thereby influence the PD signal, making it assumable that a higher BMI of the mother or fetal movements can have an influence on the FI-results [21]. Furthermore, the phase of cardiac cycle, and thus maternal heartrate, influences the measured amplitude in any portion of the resulting 3D volume, as constituent frames may vary from systole to diastole [32]. The variation in intensity of the PD signal throughout the cardiac cycle may be addressed through the use of Spatiotemporal image correlation (STIC). Using this promising technique, it creates multiple volumes representative of different moment of the cardiac cycle and thereby circumvents the inherent systematic errors of 3DPD indices [28]. Moreover, Inubashiri et al. highlighted the possible usefulness of STIC to evaluate spiral artery jets [33]. Although it might be a tool that can improve the repeatability of VOCAL, future studies need to be executed to confirm the clinical applicability given the time-consuming process [28].

Limitations of this study include that our study population was a moderate to high risk population, possibly influencing the measurements. Second, we did not take into consideration the reproducibility of the ultrasound scan itself, since the research question of this article focused on the offline 3DPD analysis, but these data could also provide promising insights. Third, the study has modest statistical power for hypothesis testing. As small group size increases the type-II error probability, precaution should be taken when interpreting the obtained p-values [34]. However, there is upcoming criticism that the use of statistical significance in a dichotomous way is misleading and blurring nuance. The interpretation of the size and precision of results may reflect much more detail and is currently given increasing priority than interpretation of p-values dichotomized into significant and not significant [35,36]. In addition, the aim of this study was to determine the validity and reproducibility of 3DPD methods assessing placental perfusion and not to investigate the differences between methods.

It is of utmost importance that a standardized protocol on how to measure 3D placental vascularization will be used to minimize measurement errors and to improve reproducibility [37]. Based on our results, the highest intra- and inter-observer reproducibility was observed in method 1, using a whole placental sweep volume and spherical sonobiopsy of 2 cm^3.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Not applicable.

References

[1] Pijnenborg R, Vercruysse L, Brosens I. Deep placentation. Best Pract. Res. Clin. Obstetrics Gynaecol. 2011;25(3):273–85.
[2] Pijnenborg R, Vercruysse L, Hanssens M. The uterine spiral arteries in human pregnancy: facts and controversies. Placenta 2006;27(9–10):939–58.
[3] Matijevic R, Meekins JW, Walkinshaw SA, Neilson JP, McFadyen IR. Spiral artery blood flow in the central and peripheral areas of the placental bed in the second trimester. Obstetrics Gynecol. 1995;86(2):289–92.
[4] Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am. J. Obstet. Gynecol. 2011;204(3):193–201.
[5] Hsieh YY, Chang CC, Tsai HD, Lee CC, Tsai CH. Longitudinal doppler sonographic measurements of vascular impedance in the central and peripheral spiral arteries throughout pregnancy. J. Clin. Ultrasound: JCU 2000;28(2):78–82.
[6] Khong TY, Mooney EE, Ariel I, et al. Sampling and definitions of placental lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch. Pathol. Lab. Med. 2016;140(7):698–713.

[7] Velauthar L, Plana MN, Kalidindi M, et al. First-trimester uterine artery Doppler and adverse pregnancy outcome: a meta-analysis involving 55,974 women. Ultrasound Obstetrics Gynecol. 2014;43(5):500–7.
[8] Olofsson P, Laurini RN, Marsal K. A high uterine artery pulsatility index reflects a defective development of placental bed spiral arteries in pregnancies complicated by hypertension and fetal growth retardation. Eur. J. Obstetrics Gynecol. Reprod. Biol. 1993;49(3):161–8.
[9] Yamasato K, Zalud I. Three dimensional power Doppler of the placenta and its clinical applications. J. Perinat. Med. 2017;45(6):693–700.
[10] Sun W, Yin S, Wei Q, et al. Three-dimensional power Doppler ultrasound evaluation of placental blood flow in normal monochorionic diamniotic twin pregnancies. BMC Pregnancy Childbirth 2018;18(1):443.
[11] de Paula CF, Ruano R, Campos JA, Zugaib M. Quantitative analysis of placental vasculature by three-dimensional power doppler ultrasonography in normal pregnancies from 12 to 40 weeks of gestation. Placenta 2009;30(2):142–8.
[12] Hudson-Dixon CM, Long BW, Cox LA. Power Doppler imaging: principles and applications. Radiologic Technol. 1999;70(3):235–43.
[13] Merce LT, Barco MJ, Bau S. Reproducibility of the study of placental vascularization by three-dimensional power Doppler. J. Perinat. Med. 2004;32(3):228–33.
[14] Lai PK, Wang YA, Welsh AW. Reproducibility of regional placental vascularity/perfusion measurement using 3D power Doppler. Ultrasound Obstetrics Gynecol. 2010;36(2):202–9.
[15] Pomorski M, Zimmer M, Fuchs T, et al. Quantitative assessment of placental vasculature and placental volume in normal pregnancies with the use of 3D Power Doppler. Adv. Med. Sci. 2014;59(1):23–7.
[16] Noguchi J, Hata K, Tanaka H, Hata T. Placental vascular sonobiopsy using three-dimensional power Doppler ultrasound in normal and growth restricted fetuses. Placenta 2009;30(5):391–7.
[17] Guiot C, Gaglioti P, Oberto M, Piccoli E, Rosato R, Todros T. Is three-dimensional power Doppler ultrasound useful in the assessment of placental perfusion in normal and growth-restricted pregnancies? Ultrasound Obstetrics Gynecol. 2008;31(2):171–6.
[18] Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J. Chiropr. Med. 2016;15(2):155–63.
[19] Alcazar JL. Three-dimensional power Doppler derived vascular indices: what are we measuring and how are we doing it? Ultrasound Obstetrics Gynecol. 2008;32(4):485–7.
[20] Morel O, Pachy F, Chavatte-Palmer P, et al. Correlation between uteroplacental three-dimensional power Doppler indices and true uterine blood flow: evaluation in a pregnant sheep model. Ultrasound Obstetrics Gynecol. 2010;36(5):635–40.
[21] Raine-Fenning NJ, Nordin NM, Ramnarine KV, et al. Determining the relationship between three-dimensional power Doppler data and true blood flow characteristics: an in-vitro flow phantom experiment. Ultrasound Obstetrics Gynecol. 2008;32(4):540–50.
[22] Coppens M, Loquet P, Kollen M, De Neubourg F, Buytaert P. Longitudinal evaluation of uteroplacental and umbilical blood flow changes in normal early pregnancy. Ultrasound Obstetrics Gynecol. 1996;7(2):114–21.
[23] Larsen ML, Naver KV, Kjaer MM, Jorgensen FS, Nilas L. Reproducibility of 3-dimensional ultrasound measurements of placental volume at gestational ages 11-14 weeks. Facts Views Vision ObGyn 2015;7(4):203–9.
[24] Zalud I, Shaha S. Evaluation of the utero-placental circulation by three-dimensional Doppler ultrasound in the second trimester of normal pregnancy. J. Maternal-Fetal Neonatal Med. 2007;20(4):299–305.
[25] Deurloo K, Spreeuwenberg M, Rekoert-Hollander M, van Vugt J. Reproducibility of 3-dimensional sonographic measurements of fetal and placental volume at gestational ages of 11–18 weeks. J. Clin. Ultrasound: JCU 2007;35(3):125–32.
[26] Merce LT, Barco MJ, Bau S, Kupesic S, Kurjak A. Assessment of placental vascularization by three-dimensional power Doppler "vascular biopsy" in normal pregnancies. Croatian Med. J. 2005;46(5):765–71.
[27] Tuuli MG, Houser M, Odibo L, Huster K, Macones GA, Odibo AO. Validation of placental vascular sonobiopsy for obtaining representative placental vascular indices by three-dimensional power Doppler ultrasonography. Placenta 2010;31(3):192–6.
[28] Miyague AH, Raine-Fenning NJ, Polanski L, et al. Assessing repeatability of 3D Doppler indices obtained by static 3D and STIC power doppler: a combined in-vivo/in-vitro flow phantom study. Ultrasound Obstetrics Gynecol. 2013;42(5):571–6.
[29] Pijnenborg R, Bland JM, Robertson WB, Dixon G, Brosens I. The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy. Placenta 1981;2(4):303–16.
[30] Cabezas Lopez E, Martinez-Payo C, Engels Calvo V, San Frutos Llorente L, Perez-Medina T. Reproducibility of first trimester three-dimensional placental measurements. Eur. J. Obstetrics Gynecol. Reprod. biol. 2016;201:156–60.
[31] Jones NW, Raine-Fenning NJ, Mousa HA, Bradley E, Bugg GJ. Evaluating the intra- and interobserver reliability of three-dimensional ultrasound and power Doppler angiography (3D-PDA) for assessment of placental volume and vascularity in the second trimester of pregnancy. Ultrasound Med. Biol. 2011;37(3):376–85.
[32] Martins WP, Welsh AW, Lima JC, Nastri CO, Raine-Fenning NJ. The "volumetric" pulsatility index as evaluated by spatiotemporal imaging correlation (STIC): a preliminary description of a novel technique, its application to the endometrium and an evaluation of its reproducibility. Ultrasound Med. Biol. 2011;37(12):2160–8.
[33] Inubashiri E, Watanabe Y, Akutagawa N, Sugawara M, Kuroki K, Maeda N. Visualization of the spiral artery jet in fetal growth-restricted pregnancy by utilizing spatiotemporal image correlation with high-definition flow. J. Med. Ultrason. 2001;42(4)601–3 2015.
[34] Rothman KJ. Curbing type I and type II errors. Eur. J. Epidemiol. 2010;25(4):223–4.
[35] Amrhein V, Greenland S, McShane B. Scientists rise up against statistical significance. Nature 2019;567(7748):305–7.
[36] Wasserstein RL, Lazar NA. The ASA statement on p-values: context, process, and purpose. Taylor & Francis; 2016.
[37] Bhide A, Acharya G, Bilardo CM, Brezinka C, Cafici D, Hernandez-Andrade E, et al. ISUOG practice guidelines: use of Doppler ultrasonography in obstetrics. Ultrasound Med. Biol. 2013;41(2):233–9.