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Doppler Ultrasonography for the Noninvasive Measurement of Uterine Artery Volume Blood Flow Through Gestation in the Pregnant Sheep

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Accurate noninvasive quantification of volume blood flow in the uterine arteries (UtAs) would have clinical and research benefits. We evaluated the correlation and agreement between uterine artery volume blood flow (UtABF) as calculated (cUtABF) from color/pulsed-wave Doppler acquisitions and that measured (mUtABF) by bilateral perivascular transit-time flow probes in 6 pregnant sheep at 2 gestational ages. Out of 22 Doppler acquisitions, 19 were successful. The overall correlation between cUtABF and mUtABF was 0.55 ($n = 19$, $P = .01$). Calculated UtABF and mUtABF were significantly correlated in late gestation ($n = 11$, $r = 0.71$, $P = .01$) but not at mid-gestation ($n = 8$, $r = .02$, $P = .96$). By Bland-Altman analysis, the mean cUtABF/mUtABF was 1.15 with 95% limit of agreement (−0.26 to 2.56), similar to results previously achieved using power/pulsed-wave Doppler. Despite the acceptable correlation, the limits of agreement between Doppler and transit-time flow probe measurements remain wide. This makes Doppler ultrasonography less than a desirable method to quantify UtABF in studies where accurate quantification is required.

Key words: Doppler, uterine artery, blood volume flow, agreement, correlation.

INTRODUCTION

Uterine artery blood flow is a major determinant of a healthy pregnancy. Pregnancies with an increased resistance to uterine artery blood flow are at a substantially higher risk of developing fetal growth restriction (FGR) and preeclampsia. Still, little is known about uterine artery volume blood flow (UtABF) in women with placental insufficiency because an accurate noninvasive method is yet to be available. Such a method could simplify preclinical research where an accurate estimation of UtABF is needed to examine the underlying pathophysiology and test for new therapeutic options.

Doppler ultrasonography is a noninvasive method that can be used to calculate the volume blood flow in a vessel using an estimate of the vessel area and blood flow velocity.[1] The luminal diameter is most commonly measured using color or power Doppler to delineate the vessel borders. Pulsed-wave Doppler is used to measure blood flow velocity at the same site.[2] Volume blood flow can then be estimated as a product of the blood flow mean velocity and vessel cross-sectional area. The quantification of UtABF through gestation using Doppler ultrasonography revealed lower UtABF in pregnancies complicated by FGR adding scientific veracity to these

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measurements.[3] The technique acquired further credibility when recent experiments in the pregnant sheep correlated the noninvasive Doppler measurement method for quantification of UtABF with measurements obtained using the invasive perivascular transit-time flow probe method.[4] Perivascular transit-time flow technology, which uses piezoelectric transducers positioned around a vessel during surgery, is considered a highly accurate method for volume blood flow estimation and has been already evaluated in vitro[5] and in vivo.[6] Thus the data in sheep experiments were interpreted as showing that Doppler ultrasonography can measure UtABF with reasonable accuracy, sufficient for it to be used in combination with maternal BP measurement, to estimate uterine vascular resistance.[4]

Encouraged by these results,[4] and taking into consideration the known limitations of Doppler ultrasonography,[7] we used Doppler ultrasound to estimate the short-term increase in UtABF in the pregnant sheep in response to local adenovirus-mediated vascular endothelial growth factor (VEGF) expression.[8] Because direct comparison of Doppler ultrasonography with an accurate reference method has been only recently reported in the uterine arteries (UtAs) of pregnant sheep,[4] we decided to further investigate the correlation and agreement between these 2 measurement methods as described in this article.

METHODS

This study was part of a larger experiment investigating the long-term effect of adenovirus vector–mediated local overexpression of VEGF on UtABF in pregnant sheep.[9] All work was conducted in accordance with the Animals (Scientific Procedures) Act (1986) under aseptic conditions.

Time-mated pregnant ewes at mid-gestation (n = 6, 80-96 days of gestation, term = 145 days) carrying singleton or twin lambs underwent an initial midline laparotomy. General anesthesia was induced with thiopental sodium 20 mg/kg intravenously (IV; Thiovet, Novartis Animal Health UK Ltd, Hertfordshire, United Kingdom) and maintained with 2% to 2.5% isoflurane in oxygen (Isoflurane-Vet, Merial Animal Health Ltd, Essex, United Kingdom) after intubation. The gestational age was confirmed using ultrasound examination of fetal size according to standard measurements. The UtAs were identified bilaterally and mobilized immediately proximal to the first bifurcation. A 6-mm 6-PS transit-time flow probe (Transonic Systems Inc, New York), which can measure blood flow within a ±10% limit of error,[6] was placed around each main UtA and the visceral peritoneum secured over it with 5-0 Prolene (Ethicon, Norderstedt, Germany). The cabling from each probe was tunneled subcutaneously, exteriorized through small incisions in the ewe's flank, and the skin buttons (CB12, Transonic Systems Inc) were secured to the skin. A 3-mL intramuscular (IM) injection of Penstrep (200 mg/mL procaine penicillin and 250 mg/mL dihydrostreptomycin; Norbrook Laboratories Ltd, County Down, United Kingdom) and an intraperitoneal injection of sodium benzylpenicillin G 3 g (Crystapen, Schering-Plough, Uxbridge, United Kingdom) + gentamicin 80 mg (Genticin Injectable, Roche Products Ltd, Hertfordshire, United Kingdom) were given at the end of the procedure for infection prophylaxis. The abdomen was then closed and buprenorphine 0.01 mg/kg IM (Vetergesic, Alstoe Animal Health, York, United Kingdom) was given every 6 to 12 hours for analgesia during the first 48 to 72 hours after the operation and the ewe was allowed to recover. Measurement of UtABF was possible once the amplitude of the signal reached 35%, which was achieved in all vessels within 24 hours of probe placement.

After 7 days, the sheep underwent a second general anesthetic and UtABF was measured on both sides using color/pulsed-wave Doppler ultrasound and the transit-time flow probe simultaneously before laparotomy for injection of adenovirus VEGF vector (Ark Therapeutics Plc, Kuopio, Finland) was performed. Uterine arteries Doppler ultrasound measurements were performed in the supine position using an Acuson 128 XP10 ultrasound scanner with a C3 3.5 MHz curvilinear transducer (Siemens, Bracknell, United Kingdom). All ultrasound measurements were performed by the same investigator (ALD). Ventilated ewes were maintained at steady state (maternal oxygen and carbon dioxide levels, pulse and respiratory rate, and temperature) during the Doppler examination. The time-averaged maximum blood velocity (TAMX-V) was measured in the main UtAs bilaterally proximal to the bifurcation and to the transit-time flow probe, using color/pulsed-wave Doppler. The insonation angle was kept as close as possible to 0° and always below 30°; angle corrections were made when the angle of insonation deviated from 0°. The time-averaged intensity-weighted mean velocity (TAMEAN-V) was calculated by multiplying TAMX-V by 0.6, which is the spatial velocity distribution coefficient derived in the sheep UtAs.[4] The vessel diameter, D was measured during systole using color Doppler. The vessel area (A) was

Table 1. Uterine Artery Volume Blood Flow (UtABF) Observations In Pregnant Sheep[a]

Uterine Artery	Uterine Horn	Singleton/Twin	GA (Days)	mUtABF mL/min	cUtABF mL/min	TAMX-V (cm/s)	*A* (mm^2)
Sheep 1L	Pregnant	Singleton	137	582	164	26.7	17.1
Sheep 1R	Empty	Singleton	137	148	339	34.7	27.0
Sheep 2R	Pregnant	Singleton	95	368	316	64.3	13.7
Sheep 2L	Empty	Singleton	140	30	253	26.7	26.4
Sheep 2R	Pregnant	Singleton	140	304	134	24.7	15.2
Sheep 3L	Pregnant	Singleton	102	201	275	30.0	25.5
Sheep 3R	Empty	Singleton	102	42	102	25.0	11.3
Sheep 3L	Pregnant	Singleton	140	286	185	38.3	13.4
Sheep 3R	Empty	Singleton	140	54	136	21.7	17.3
Sheep 4R	Pregnant	Twins	87	486	231	51.0	12.6
Sheep 4L	Pregnant	Twins	87	454	287	51.0	15.7
Sheep 4R	Pregnant	Twins	139	601	906	61.3	40.7
Sheep 4L	Pregnant	Twins	139	656	523	54.3	27.3
Sheep 5R	Pregnant	Singleton	88	748	139	46.0	8.4
Sheep 5R	Pregnant	Singleton	136	792	1181	55.3	59.0
Sheep 5L	Empty	Singleton	136	415	587	47.7	34.2
Sheep 6R	Pregnant	Twins	103	250	171	19.7	23.7
Sheep 6L	Pregnant	Twins	103	198	277	35.0	22.1
Sheep 6R	Pregnant	Twins	136	338	437	38.3	31.2

Abbreviations: *A*, vessel area; GA, gestational age; R, right UtA; L, left UtA; TAMX-V, time-averaged maximum velocity in the UtAs.
[a] UtA volume blood flow was measured by Doppler ultrasound (cUtABF) and transit-time flow probe (mUtABF).

calculated as $A = \pi(D/2)^2$ assuming the vessel to have a circular lumen. Time-averaged maximum blood velocity and *D* values from 3 separate consecutive Doppler acquisitions taken over 2 minutes was averaged and used for analysis. The calculated UtA volume blood flow (cUtABF) was derived using the formula: cUtABF (mL/min) = TAMEAN-V (cm/s) × *A* (cm^2) × 60.

The perivascular transit-time flow probe derived UtA blood flow (mUtABF) was recorded simultaneously during each Doppler acquisition via the skin buttons using the PhysioGear I telemetric transmitter system and the PhysioView Data Acquisition Software (Transonic Systems Inc) at a sampling rate of 128 Hz. The data acquired from the flow probes was analyzed using the Acqknowledge software (Biopac Systems Inc, California) to generate a mean UtABF measurement for each UtA over the period during which the Doppler examination was performed.

At the end of gestation (136 to 140 days), the sheep were anesthetized for the third time and the ultrasound and perivascular transit-time flow probe measurements of UtABF were recorded simultaneously again as above, before the sheep were euthanized using an IV overdose of pentobarbital sodium (Euthatal, Merial Animal Health Ltd, Essex, United Kingdom).

Linear regression was used to calculate the Pearson correlation coefficients between mUtABF and cUtABF, and its components TAMX-V and *A*. Multiple regression was used to generate an equation for mUtABF as the dependent variable. Statistical agreement between the calculated and measured UtABF was analyzed through a Bland-Altman Plot. All statistical calculations were performed using the MedCalc Software (MedCalc Software, Mariakerke, Belgium). A *P* value less than or equal to .05 was considered statistically significant. We also measured the ability of cUtABF to estimate mUtABF within ±20% to 40%. This was based on the observation that a 40% reduction in UtA volume blood flow will reduce fetal weight in pregnant sheep by approximately 30% to 35%.[10]

RESULTS

Of the 6 pregnant ewes, 4 had singleton and 2 had twin lambs. A total of 22 UtABF observations were attempted, whereby each UtA was examined on 2 occasions, once in mid-gestation and once at term; in sheep 1, the observation at mid-gestation was not attempted due to time constraints. There were 19 satisfactory observations (Table 1), of which 8 were made at mid-gestation and 11 at term; 14 measurements were obtained from the pregnant horn (twin or singleton), and 5 were obtained from the empty horn (singleton only). Satisfactory Doppler measurement of UtABF was not possible during 3 observations due to

Table 2. The Correlation of UtA Volume Blood Flow Measured by Transit Time Flow Probes With Measurement by Doppler Sonography, With Time-Averaged Maximum Velocity and With UtA Vessel Area, Using the Pearson Correlation Coefficient[a]

UtABF Measured by Transit-Time Flow Probes	n	UtABF Calculated From Sonography		Time-Averaged Maximum Velocity in Uterine Arteries		Uterine Artery Vessel Area	
		Correlation	*P* Value	Correlation	*P* Value	Correlation	*P* Value
All uterine arteries	19	0.55	**.013**	0.67	**.001**	0.32	.171
Pregnant uterine arteries	14	0.54	**.045**	0.55	**.040**	0.35	.214
Empty uterine arteries	5	0.93	**.018**	0.96	**.009**	0.75	.141
Mid-gestation uterine arteries	8	0.02	.957	0.61	.106	-0.53	.172
Late gestation uterine arteries	11	0.71	**.013**	0.74	**.009**	0.59	.056
Uterine arteries in singleton pregnancies	12	0.51	.085	0.60	**.038**	0.33	.298
Uterine arteries in twin pregnancies	7	0.64	.117	0.87	**.009**	0.27	.562

Abbreviation: UtABF, uterine artery volume blood flow.
[a] The *P*-values in bold represent statistically significant *P*-values (i.e. <0.05)

technical difficulties related to inadequate visualization of the UtA at mid- (n = 2, 1 on the empty horn of a singleton gestation and 1 in a twin gestation) and late gestation (n = 1, in a twin gestation).

The mean UtA diameter was 4.52 ± 0.88 mm at mid-gestation and 5.84 ± 1.35 mm in late gestation (*P* = .03). In late gestation, blood flow in the UtAs supplying a pregnant horn was significantly higher than in those supplying an empty horn (mUtABF = 508 ± 198 mL/min vs 161 ± 176 mL/min, *P* = .02). The blood flow in the UtAs supplying a twin gestation did not differ significantly from flow in those vessels supplying the pregnant horn of a singleton pregnancy at mid-gestation (mUtABF = 347 ± 144 mL/min vs 439 ± 280 mL/min, *P* = .59) and in late gestation (mUtABF = 531 ± 170 mL/min vs 491 ± 241 mL/min, *P* = .81). There was insufficient data available to compare mUtABF at mid-gestation in arteries supplying the pregnant and empty horns (Table 1).

The correlation coefficient between cUtABF and mUtABF when measured in all UtAs was *r* = 0.55 (*P* = .01, Table 2). In the UtAs supplying the empty horn, cUtABF and mUtABF were correlated (*r* = .93, *P* = .02) as well as in vessels supplying the pregnant horn (r = .54, *P* = .04, Table 2). When the observations were conducted at mid-gestation, mUtABF and cUtABF were not correlated (*r* = 0.02, *P* = .96) while in observations conducted in late gestation, mUtABF and cUtABF were well correlated (*r* = .71, *P* = .01, Table 2). When measured in all UtAs, mUtABF was significantly correlated with TAMX-V (*r* = .67, *P* = .001) but not with vessel area (*A*; Table 2).

To consider the other potential variables, we calculated multiple linear regression equations for mUtABF using the following independent variables: cUtABF (or its components TAMX-V and *A*), the gestational age at acquisition (GA), whether the UtA supplied the nonpregnant or pregnant horn (*P*), and whether the ewe was carrying a singleton or a twin gestation (*T*). The multiple regression equations are shown in Table 3. When all UtAs were considered together, multiple linear regression equations could be fitted (*P* = .001), although GA, *T*, or *A* were excluded from the final equation as their equation coefficients did not reach statistical significance. When considered according to gestational age, multiple regression was statistically significant in late gestation (*P* = .002 and .004, Table 3) but not at mid-gestation where a multiple linear regression equation between mUtABF (dependent variable) and cUtABF, *P*, and *T* (independent variables) could not be fitted.

Doppler derived UtABF, cUtABF was within ±20% of mUtABF in only 2 of the 19 satisfactorily completed measurements (10.5%) and within ±40% in 8 out of the 19 observations (42.1%). This low agreement was observed in both mid- and late gestation and was independent of pregnancy side. One cUtABF observation from Sheep 2L at 140 days was an obvious outlier; and it is of note that it had not been possible to achieve a satisfactory Doppler measurement from this artery at mid-gestation. After exclusion of this outlier observation and using Bland-Altman analysis, the mean cUtABF/mUtABF was 1.15 with a 95% limit of agreement (−0.26 to 2.56; Figure 1).

DISCUSSION

In this study, we observed a significant positive correlation between UtABF measured by perivascular transit-time

Table 3. Multiple Linear Regression Equations for mUtABF as the Dependent Variable

Dependent Variable	Independent Variables	Independent Variables Excluded by Multiple Linear Regression	Final Multiple Linear Regression Equation	R^2	P Value
mUtABF (all)	cUtABF, *T*, *P*, GA	*T* and GA	mUtABF= 25 + 0.40cUtABF+274P	0.53	.001
mUtABF (all)	TAMX-V, *A*, *T*, P, GA	*A*, *T* and GA	mUtABF= −137 + 8.81TAMX-V + 209P	0.55	.001
mUtABF (late gestation)	cUtABF, *T*, *P*	*T*	mUtABF= 22 + 0.42cUtABF + 272P	0.73	.002
mUtABF (late gestation)	TAMX-V, *A*, *T*, *P*	*A* and *T*	mUtABF = −175+10.31TAMX-V+243P	0.69	.004

Abbreviations: *A*, vessel area (mm^2); GA, gestational age (days); TAMX-V, time-averaged maximum velocity in the UtAs (cm/s); UtA, uterine arteries; UtABF, uterine artery volume blood flow; mUtABF, UtA volume blood flow measured by transit-time flow probes (mL/min); cUtABF, UtA volume blood flow calculated by sonography (mL/min); $T = 0$ if the pregnancy is a singleton gestation and $T = 1$ if the pregnancy is a twin gestation, $P = 0$ if the UtA is supplying an empty horn and $P = 1$ if the UtA is supplying a pregnant horn.

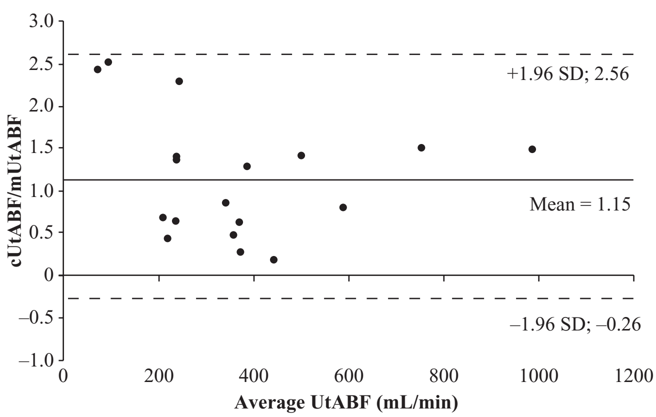

Figure 1. Bland-Altman plot of the agreement between calculated uterine artery volume blood flow (cUtABF) and measured UtABF (mUtABF) with 95% limits of agreement. Average UtABF = (cUtABF + mUtABF)/2. cUtABF indicates UtA volume blood flow calculated by Doppler; mUtABF = UtA volume blood flow measured by transit-time flow probes.

flow probe and by color/pulsed-wave Doppler ultrasonography in late gestation pregnant sheep. Time-averaged maximum blood velocity was also significantly correlated with mUtABF in late gestation. The correlation coefficients observed in this study are similar to those obtained previously with power/pulsed-wave Doppler.[4] This is to be expected, as both methods are measuring the same entity, the UtA.[11] In this regard, the absence of a correlation between mUtABF and cUtABF at mid-gestation is surprising and suggests a significant limitation in the ability of Doppler ultrasonography to quantify UtA volume blood flow at this gestational time period. The results of the multiple regression and the correlation coefficients suggest that the measurement of both the blood flow velocity and the vessel area contributed to the poor correlation at mid-gestation. It has been our observation that the UtA in the mid-gestation sheep has a more tortuous pathway than that at late gestation. This could partly explain the poor correlation between TAMV-X and mUtABF at mid-gestation since the corrections in the angle of insonation would not be adequate enough to accurately reflect the direction of blood flow. In addition, there is a larger relative error in vessel diameter estimation associated with the smaller vessel diameter at mid-gestation when compared to late gestation measurement.

Multiple regression showed that mUtABF was related to whether the UtA supplied a pregnant or a nonpregnant horn but was not related to singleton or twin gestation. This could be due to the observation that UtABF is significantly higher in those vessels supplying a pregnant horn as compared to vessels supplying an empty horn.

In the current study, we used Bland-Altman analysis to assess the adequacy of color/pulsed-wave Doppler to quantitatively replace an established and accurate method of UtABF measurement, perivascular transit-time flow probes. Bland-Altman analysis showed that the mean cUtABF/mUtABF was 1.15 with wide 95% limits of agreement (–0.26 to 2.56). This suggests that UtABF calculated by color/pulsed-wave Doppler is not an accurate alternative to measurement by perivascular transit-time flow probes. In another study, measurement of UtABF in the late gestation pregnant horn by power/pulsed-wave Doppler produced results similar to ours with a mean cUtABF/mUtABF of 1.14 and 95% limits of agreement (0.57-2.27).[4] An advantage of our study, as compared to what has been previously done, is that we have assessed UtABF in each animal at 2 different time points in gestation, and in a way so that each pair of data sets was obtained on a different occasion. This is more similar to the situation in practice. Repeating the measurement 6 times on a single occasion[4] may lead to an overestimation or underestimation of the performance of a certain technique. In addition, we have examined the UtA supplying both horns of the ewe's bicornuate uterus and not just that supplying the pregnant horn. We thus assessed the correlation and agreement at wider ranges of UtABF than was achieved previously.

Bland and Altman[11] suggest that 2 measurement methods can be used interchangeably provided the differences within the mean $\pm$ 2 SD are not clinically or experimentally important. This is not the case for measurement of UtABF as the 95% limits of agreement remain wide for both color/pulsed-wave and power/pulsed-wave Doppler when compared to a reference method. In our study, color/pulsed-wave Doppler could estimate UtABF within $\pm$40% in 42.1% of the successful attempts. For comparison, power/pulsed-wave Doppler could estimate UtABF within $\pm$40% in about 60% of the cases.[4] Thus, both color/pulsed-wave and power/pulsed-wave Doppler are not sufficiently accurate to replace the perivascular transit-time flow probe method for quantification of UtABF in animal experiments.

Although it has been suggested that power Doppler can overcome some of the inaccuracies of color Doppler in measuring vessel diameter because of its better sensitivity, there remain substantial limitations.[12] For volume blood flow estimation, both color and power Doppler require the concurrent use of pulsed-wave Doppler. For accurate velocity measurements, the pulsed-wave Doppler insonation angle should be as close as possible to zero because angles of insonation less than 30° will underestimate the maximal velocity[13] by less than 15%, while large insonation angles can induce substantial errors in the measurement of maximal velocity.[14] Factors that can limit the accuracy of pulsed-wave Doppler in estimating blood velocity also include nonlaminar flow, vessel shape, and estimation of mean velocity.[7] There are also inaccuracies inherent in the pulsed-wave technique because pulsed-wave Doppler systems are designed to achieve high spatial resolution rather than uniformity of insonation. There is also overweighting of the higher velocity components at the center of the vessel, resulting in flow overestimation.[15] When measuring vessel diameter, the blooming effect is common to both color and power Doppler, which results in overwriting of the vessel walls in an attempt to maximize vessel filling.[12] Errors in measuring vessel diameter have a large impact on cUtABF since the measurement is squared when calculating the vessel area $A = \pi(D/2)$.[2]

Despite using relatively conventional ultrasound technology, the correlation and agreement between color/pulsed wave UtABF and mUtABF in late gestation were similar to results previously obtained using power/pulsed wave Doppler, a method that is believed to be more accurate.[2,4] This suggests that the inaccuracy is more related to errors inherent in the ultrasonographic method rather than the use of specific equipment. Technological solutions may improve the ability of ultrasound to accurately estimate UtABF. For example, vector Doppler systems are better able to estimate the blood flow direction,[16] and B-flow imaging, a non-Doppler ultrasonographic technology, eliminates the blooming and aliasing effects seen with power and color Doppler.[17] The role of 3-dimensional Doppler for the quantitative assessment of uteroplacental blood flow is still not clear.[18] Until more accurate noninvasive methods become available, Doppler should not be used in an experimental setting where an accurate estimate of UtABF is desired, such as in the investigation of the physiology of placental insufficiency or placental substrate exchange.

CONCLUSION

Measurement of UtABF by color/pulsed-wave Doppler correlates with measurement by perivascular transit-time flow probes in the late gestation pregnant sheep. The correlation is almost as good as that achieved using power/pulsed-wave Doppler.[4] Both techniques however have such wide limits of agreement with transit-time flow probes to render them unsuitable for use in an experimental setting where an accurate estimation of UtABF is desired.

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