Thea Anette Andersen and Helene Caroline Arneberg

Blood flow calculated in the uterine arteries based on the PixelFlux technique

Graduate thesis in Medicine

Trondheim, June 2017

Supervisor:
Torbjørn Moe Eggebø, Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway and National Center for Fetal Medicine, Trondheim University Hospital (St. Olavs Hospital) Trondheim, Norway

Co-supervisors:
Hans Torp, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway
Thomas Scholbach, Leipzig Ultrasound Institute, Leipzig, Germany
Liv Lorås, National Center for Fetal Medicine, Trondheim University Hospital (St. Olavs Hospital) Trondheim, Norway

ACKNOWLEDGEMENTS

This article is submitted as our 5th year student thesis at the faculty of medicine, NTNU, June 2017. It has also been accepted for publishing in International Ultrasound Open.

We wish to thank our supervisors Torbjørn Moe Eggebø, Thomas Scholbach and Hans Torp for their enthusiasm, encouragement and readiness to help whenever needed during this work. The extent of this thesis would not be possible without your guidance.

We also wish to thank Liv Lorås and the fantastic midwifes at the National Center of Fetal Medicine, NTNU, for all their help collecting data. We could not have done this without your help.

Table of Content

CONFLICT OF INTEREST

Thomas Scholbach co-developed the PixelFlux software and is medical advisor as well as co-owner of the Chameleon-software company.

ABBREVIATIONS

PI, pulsatile index; RI, resistance index; TAmax, time-averaged peak velocity; BMI, body mass index; r, correlation coefficient

ABSTRACT

Introduction: We aimed to compare two Doppler methods which calculate blood flow in the uterine arteries. We also aimed to explore associations between blood flow with birthweight and with fetal weight-gain/day from sonography to delivery.

Material and methods: We conducted a prospective observational pilot study in pregnancy week 24-25 in women with risk pregnancies referred to the fetal medical centre at St. Olavs Hospital, Trondheim, Norway from March 2016 to June 2016. Blood flow in the uterine arteries was calculated based on time-averaged peak velocity (TAmax) and on the PixelFlux technique. PixelFlux is a new method based on pixelwise calculation of spatially angle-corrected velocities and areas of all pixels inside a vessel during a heart cycle.

Results: The mean flow calculated from PixelFlux was 811ml/minute (median 777, range 209-1988 ml/minute) and mean flow using calculation from TAmax was 787 ml/minute (median 710, range 179 – 2120 ml/minute). The intra class correlation coefficient was 0.83 (95% CI 0.72-0.90) and limits of agreement were -441 ml/minute (95% CI -558 to -324 ml/minute) to 489 ml/minute (95% CI 372 to 606 ml/minute). We observed a significant association between mean flow calculated from PixelFlux and birthweight ($r = 0.41$; $p < 0.01$) and between flow calculated from PixelFlux and weight gain/day ($r = 0.33$; $p = 0.02$). Calculation based on TAmax was significant associated to birthweight ($r = 0.34$; $p = 0.02$), but not to weight-gain/day. Pulsatile index was not associated to flow, birthweight or fetal weight-gain/day.

Conclusions: The PixelFlux method might be a promising tool in predicting fetal growth.

INTRODUCTION

The volume of the utero-placental blood flow is an important parameter in assessing normal placental function and fetal development[1]. By using ultrasound colour Doppler it is possible to assess the flow-resistance in the placenta[2]. Pulsatility index (PI) and resistance index (RI) are commonly used to quantify the ratio of blood flow velocities in the uterine artery and the placenta[3]. High resistance based on high PI values and notches in the uterine arteries are used as predictors of adverse maternal and fetal outcomes[1, 4-7]. It is assumed that a precise measurement of the total blood flow (Q) would be the best way to assess placental function and well-being of the foetus. Blood flow can be calculated from the diameter of the vessel and time-averaged maximum velocity (TAmax) or mean velocity (TAmean)[8-14]. It has recently been suggested to calculate flow using colour Doppler with PixelFlux-technique by examining the area and colour of each pixel in a vessel section [15, 16].

The aim of the study was to compare associations between blood flow volumes measured by the PixelFlux-Technique with measurements from TAmax, and to investigate association to PI and RI. We also aimed to explore associations between blood flow measurements in the uterine arteries in second trimester examinations with birthweight and with daily weight gain from sonography to delivery.

MATERIAL AND METHODS

We conducted a prospective observational pilot study in women with single pregnancies referred to the fetal medical centre at St. Olavs Hospital, Trondheim, Norway from March 2016 to June 2016. They were referred to have an examination of pulsatile index (PI) with Doppler ultrasound in the uterine arteries in gestational week 24-25 due to high-risk pregnancies. An extended examination was performed including fetal weight estimation and measurements of blood flow in the uterine arteries. All women gave written consent and the local ethics committee approved the study (Rek Midt 2015/2304). Outcome measures were birthweight, weight gain/day from the ultrasound examination to delivery and preterm deliveries.

Trans-abdominal ultrasound measurements were performed using Voluson E8 ultrasound equipment (GE Medical Systems, Zipf, Austria) with a 3.5–7.5-MHz three-dimensional curved multifrequency trans-abdominal transducer. Specially trained midwives did all examinations. Blood flow volume (ml/minute) in the uterine arteries (cQUtA) was first calculated as 0.5*TAmax (cm/s) * cross section area of the vessel (CSA) (cm^2) * 60. CSA was calculated as π * $(\text{diameter}/2)^2$ assuming that the uterine vessels were circular. The angle of the ultrasound waves to the vessels were angle corrected, but kept as close as possible to zero. The diameter of the vessel was measured in the systole on the colour Doppler image as published in previous studies[11, 17]. The blood flow in the two uterine arteries were summarized.

Thereafter the blood flow was calculated using the PixelFlux method[18]. PixelFlux is a new method based on pixelwise calculation of spatially angle-corrected velocities and areas of all pixels inside a vessel. Each pixel has an equal area, determined by the spatial resolution of the ultrasound machine, and all pixels are taken into account at their angle-corrected velocity and their angle-corrected area. Velocity is coded by colour, and by comparing the colour of each pixel in the cross-sectional area with colour bar delivered by the manufacturer with each image, it is possible to measure blood flow velocities directly. The flow volumes are calculated pixelwise and for each pixel the angle-corrected velocity is multiplied by the area. Then, all pixels' flow volumes inside a vessel's section are added to calculate the flow volume of the vessel during a heart cycle. Figure 1 illustrate variation during a heart cycle. All the calculations and measurements are done automatically by the PixelFlux program.

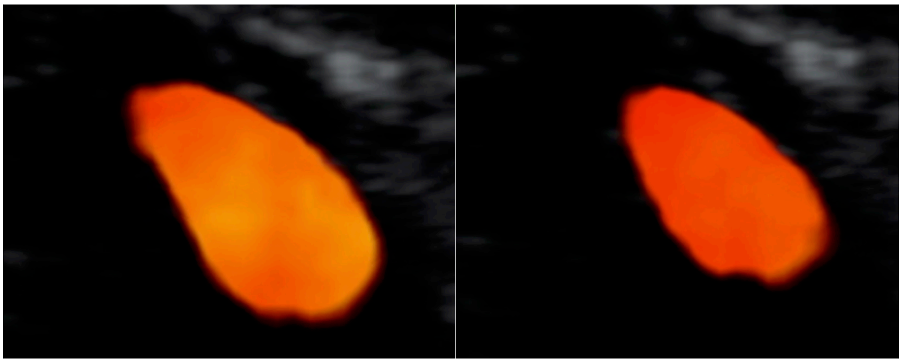

Figure 1: Variation in velocity (colour) in left uterine artery during a heart cycle (systole to left and diastole to right)

Fetal weight in the second trimester was estimated using biparietal diameter (BPD) and abdominal circumference (AC) and the mean of three measurements. We calculated fetal weight using the algorithm implemented in eSnurra [19] . The Norwegian Directory of Health recommends this method. The birthweight was obtained immediately after birth. Mean fetal weight gain/day was calculated as the difference between birthweight and estimated fetal weight in the second trimester, and thereafter related to the remaining days in pregnancy from the ultrasound examination to delivery.

Statistical analyses

Variables were compared using Chi-square test, Mann-Whitney U-test and linear regression. In the regression analyses we adjusted for possible confounders as for parity, maternal age, body mass index (BMI) and systolic blood pressure. Correlation between methods was analyzed using intra class correlations coefficient. If zero was inside the 95% CI of the mean difference between methods, no bias was assumed. The analysis of inter-method agreement was performed using limits of agreement as described by Bland and Altman. $P<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS Statistics for Mac, v. 23.0 Armonk, NY: IBM Corp.

RESULTS

In all, 60 women were included in the study, but seven were excluded due to suboptimal insonation angle, three because fetal weight estimation was not performed and three because we miss information about birthweight, leaving 47 women in the study population. Characteristics of the study population is presented in Table 1.

Table 1 Characteristics of study population

	Median	Range
Maternal characteristics		
Maternal age (years)	32	21-45
BMI	26	20-48
Systolic blood pressure (mmHg)	114	90-141
Diastolic blood pressure (mmHg)	70	46-91
Pregnancy characteristics		
Pregnancy duration (days)	280	186-293
Characteristics of the new-born		
Weight gain per day (g)	26	13-37
Birthweight (g)	3445	875-5000
Apgar score 1 minute	9	3-10
Apgar score 5 minutes	10	4-10
Apgar score 10 minutes	10	8-10
pH in umbilical artery	7.24	7.06-7.38

The mean flow calculated from PixelFlux was 811ml/minute (median 777, range 209-1988 ml/minute) and mean flow using calculation from TAmax was 787 ml/minute (median 710, range 179 – 2120 ml/minute). The mean difference was 24 ml/minute (95% CI -45; 94 ml/minute) and we did not observe any significant difference between the two methods due to the fact that the CI intervals were crossing zero. The association between mean flow from the two methods is presented as a scatter plot in Figure 2 and as a Bland Altman plot in Figure 3. The intra class correlation coefficient was 0.83 (95% CI 0.72-0.90) and limits of agreement were -441 ml/minute (95% CI -558 to -324 ml/minute) to 489 ml/minute (95% CI 372 to 606 ml/minute). Details are presented in Figure 4.

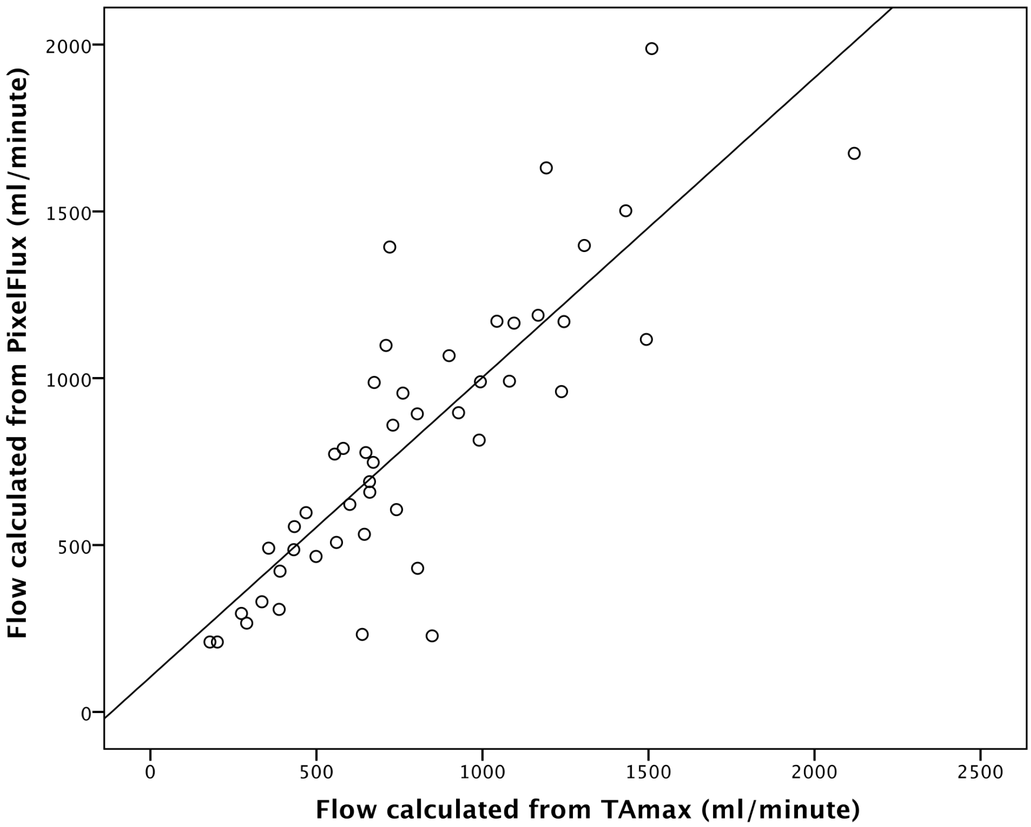

Figure 2: Association between flow calculated from TAmax and PixelFlux

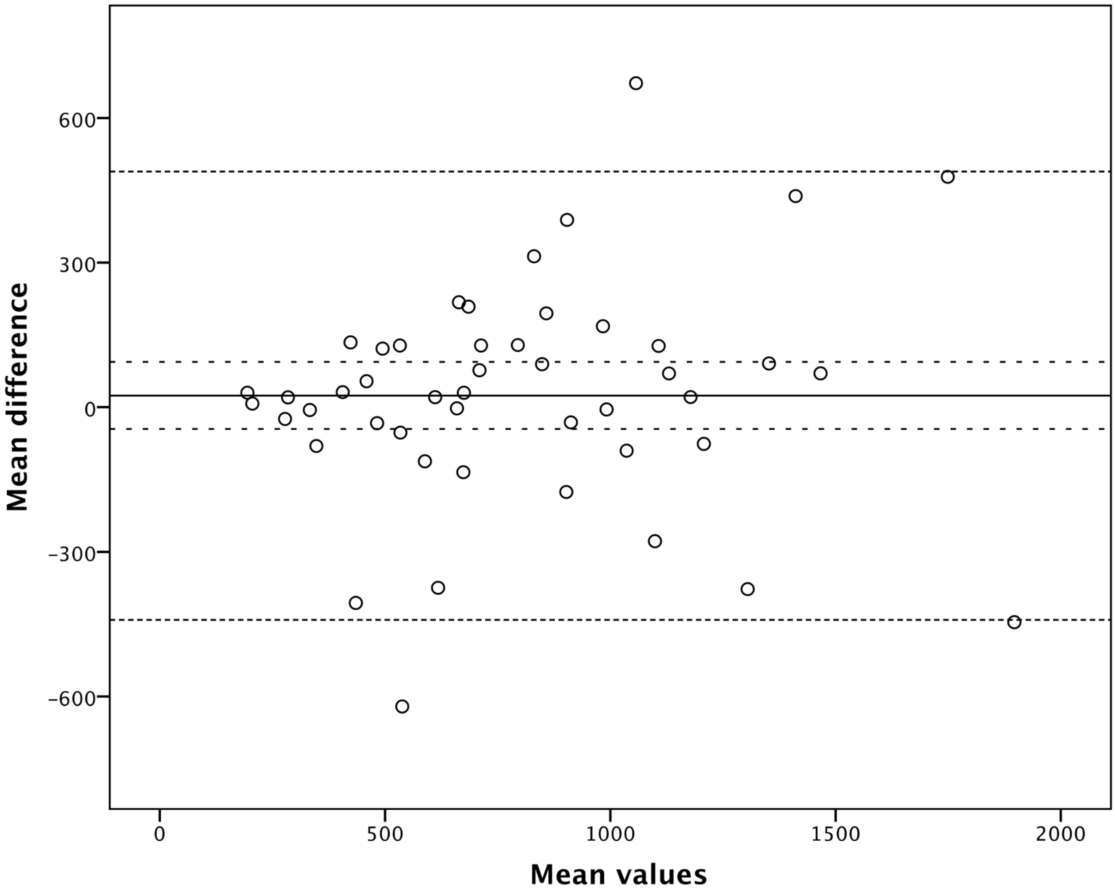

Figure 3: Bland-Altman plot for inter-method agreement of total uterine artery flow based on time-averaged peak velocity and the PixelFlux technique. Mean difference in ml/min (continuous line) with 95% confidence interval (separated dots) and 95% limits of agreement (tight dots) (i.e., mean difference ± 1.96 SD) are shown.

					Difference between the two observers				
Inter method agreement	*Mean*	*Median*	*Range*	*ICC (95% CI)*	*Mean (95% CI)*	*1.96 SD*	*Lower limit (95% CI)*	*Upper limit (95% CI)*	*Range*
	799	709	(194 to 1897)	0.83 (0.72-0.90)	24 (-45 to 94)	465	-441 (-558 to -324)	489 (372 to 606)	(-621 to 672)

Mean, median and range are calculated from the mean results from the two methods; ICC, intraclass correlation coefficient; SD, standard deviation.

Figure 4: Inter-method agreement of mean flow in uterine arteries calculated from TAmax and from PixelFlux

We observed a significant association between mean flow calculated from PixelFlux and birthweight (r =0.41; p <0.01) as presented in Figure 5 and between flow calculated from PixelFlux and weight gain/day (r =0.33; p =0.02) as presented in Figure 6. The correlations remained significant after adjusting for maternal age, BMI, parity and systolic blood pressure (p =0.01 and p =0.02, respectively). The correlations between flow calculated from TAmax and birthweight or weight gain/day is presented in Table 2. We did not observe any significant correlation between flow calculated from PixelFlux and PI (r = -0.03; p =0.83) as illustrated in Figure 7, or between PI and RI with birthweight or weight gain/day (Table 2).

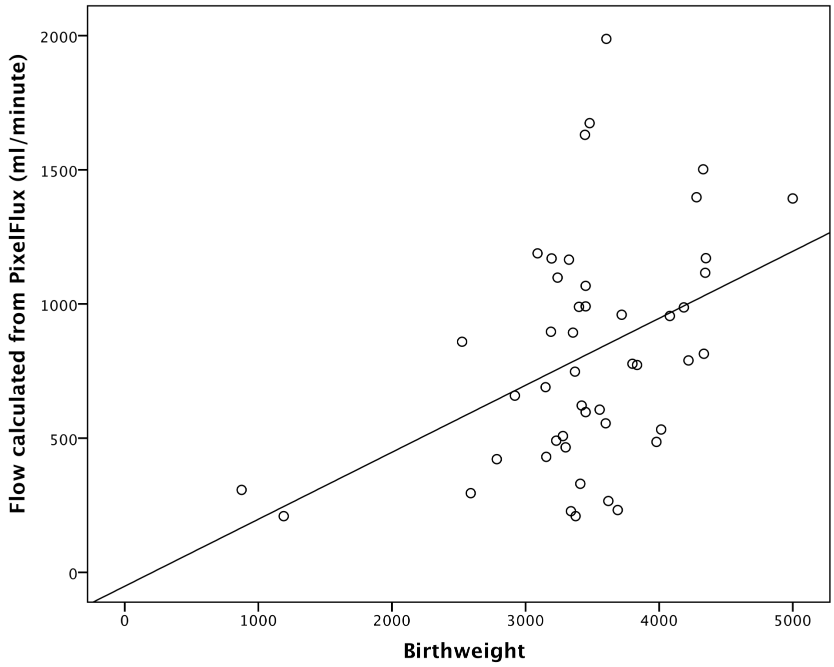

Figure 5: Association between flow calculated from PixelFlux and birthweight

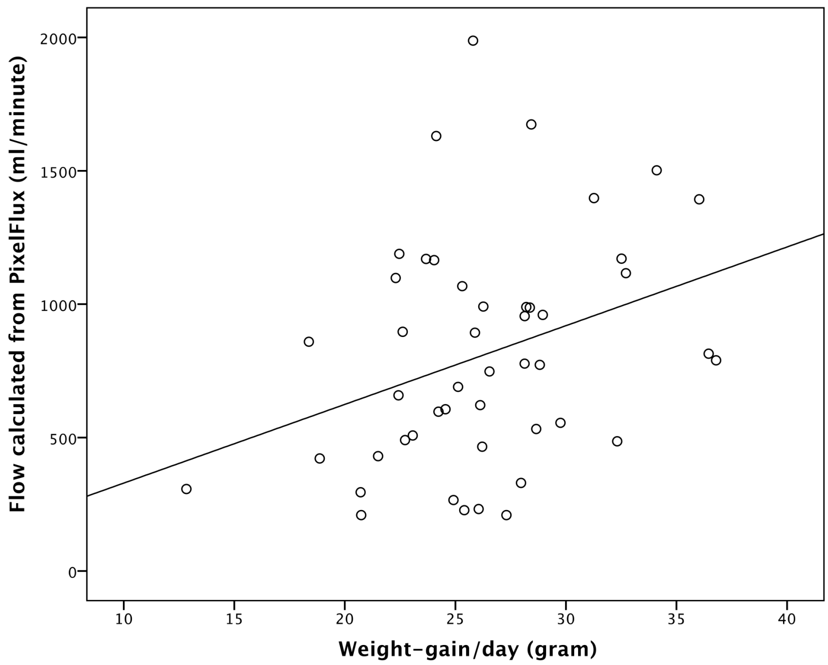

Figure 6: Association between flow calculated from PixelFlux and weight-gain/day

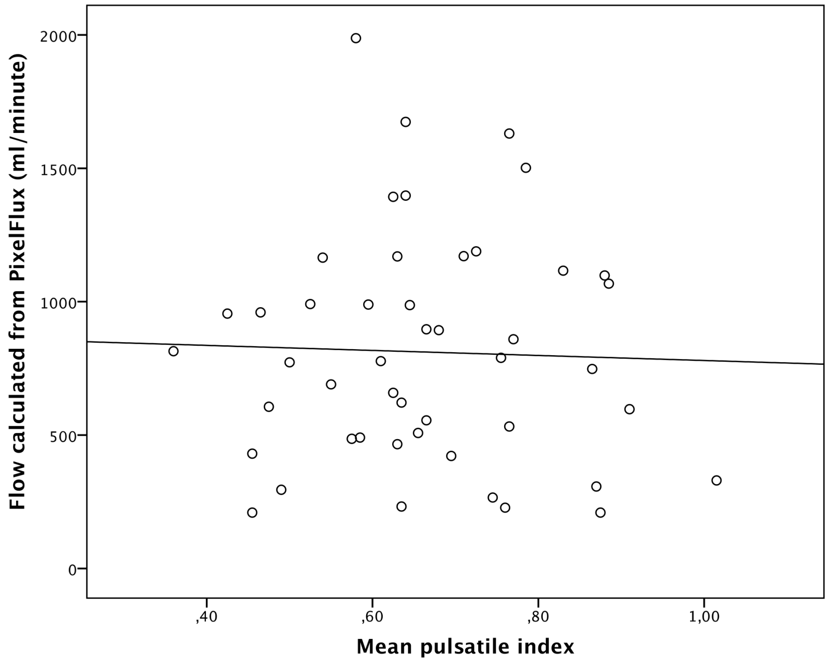

Figure 7: Association between flow calculated from PixelFlux and pulsatile index

Table 2: Correlations between fetal growth and mean of ultrasound parameters from the two uterine arteries

Correlation to	PixelFlux		TAmax		PI		RI	
	r	p-value	r	p-value	r	p-value	r	p-value
Birthweight	0.42	< 0.01	0.34	0.02	-0.25	0.09	-0.23	0.13
Weight-gain/day	0.33	0.02	0.29	0.05	-0.18	0.22	-0.15	0.33

TAmax, time-averaged peak velocity; PI, pulsatile index; r, correlation coefficient

Two women delivered preterm; one in week 26 and one in week 27; both due to spontaneous contractions. Flow calculated from PixelFlux in these women were 307 and 209 ml/minute, flow calculated from TAmax were 388 and 201 ml/min, and mean PI was 0.87 and 0.88, respectively.

DISCUSSION

The main findings in the study were a good correlation between the two methods estimating blood flow in the two uterine arteries in pregnancy week 24-25. The PixelFlux-measurements yielded significant correlations to both birthweight and weight gain/day, whereas TAmax calculations achieved significance to birthweight and PI and RI measurements turned out to be not significantly correlated.

Pulsatile index is the preferred variable in clinical practice because it is easy to measure, and independent of insonation angle[20]. A high PI in the uterine arteries during pregnancy reflects high resistance in the placenta, and high PI in the second trimester is associated to increased risk of fetal growth restriction[2], preeclampsia[1, 3], placental infarction[7] and adverse fetal outcome[6]. Nevertheless, we did not observe any association between PI and fetal growth in our study. PI and blood flow was not associated, and placental flow might be more important for fetal growth than placental resistance.

Calculation of volume blood flow is desirable, but non-invasive techniques are challenging, especially in small vessels and in pulsating arteries[17]. Using standard calculations from TAmax, it is assumed that the mean cross-sectional velocity is 0.5 x TAmax. The constant 0.5 is used to calculate flow in tubes with parabolic velocity profile[8].

The PixelFlux method calculates flow through at least one entire heart cycle, and an automatic spatial angle correction in both the sagittal and frontal plane is implemented in the computer program[18], assuming circular vessel geometry. The main advantage is that simple 2-dimensional imaging is sufficient to calculate 3-dimensional flow volumes (so called "PixelFlux 243"-technique). An oblique vessel section is made and a short video clip of the vessel is recorded. Asymmetric flow distribution as well as the changing vessel area and flow velocities during the heart cycle are detected automatically by the PixelFlux technique. However, turbulence is still a problem and it is important to adjust pulse repetition frequency to avoid aliasing.

The total blood flow volumes in the uterine arteries increase during pregnancy[21]. Previous studies present varying blood flow volumes in the second trimester[11, 22, 23]. The vessel diameter has great influence on calculated flow based on TAmax or TAmean, and it is difficult to measure the diameter of the uterine arteries using two-dimensional transabdominal sonography. Measuring the vessel diameter on colour Doppler images might be preferred[11, 17]. The variation might also be related to the insonation angle when the vessel diameter was measured. Acharya et al. compared Doppler measurements of blood flow with actual flow in sheep and found good correlation[17]. They measured the vessel diameter on power Doppler images during the systole. Konje et al. calculated the total uterine artery flow to be around 500 ml/min in pregnancy week 20 with increasing flow of 39 mL/min per week from week 20 to 24[11]. These findings correlate well to our study. We found total uterine artery blood flow to be around 800 ml/min and the mean gestation length at the ultrasound examinations was 24 weeks and 4 days. In colour Doppler acquisitions the insonation angle should be as close as possible to zero degree and we measured the vessel diameter on these images. This insonation angle is not optimal measuring vessel diameter because the lateral resolution in ultrasound images is lower than the axial resolution. Thus, we might have overestimated the vessel diameter. Our main aims were not to calculate absolute flow, but to compare methods and to investigate associations between flow and fetal growth.

Strengths of the study were that all women were examined in pregnancy week 24-25 and that different examiners calculated flow from the two methods. The examiner using PixelFlux was blinded and not informed about clinical indications or about labour outcomes. Limitations of the study are a small study population and that it was a pilot study without a power calculation. It was sometimes challenging to achieve an optimal insonation angle of the uterine arteries and we had to exclude seven cases due to insufficient insonation angles.

In conclusion, we found a significant association between total blood flow based on PixelFlux in the uterine arteries in pregnancy week 24-25 and fetal weight-gain/day in remaining pregnancy. The PixelFlux method might be a promising tool in predicting pregnancy outcome, however, new and larger studies are necessary.

REFERENCES

1. Khalil A, Garcia-Mandujano R, Maiz N, Elkhouli M, Nicolaides KH. Longitudinal changes in uterine artery Doppler and blood pressure and risk of pre-eclampsia. Ultrasound Obstet Gynecol. 2014; 43:541-547.

2. 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 Obstet Gynecol Reprod Biol. 1993; 49:161-168.

3. van Asselt K, Gudmundsson S, Lindqvist P, Marsal K. Uterine and umbilical artery velocimetry in pre-eclampsia. Acta Obstet Gynecol Scand. 1998; 77:614-619.

4. Thuring A, Laurini R, Marsal K. Uterine venous blood flow in normal and complicated pregnancies: a methodological study. Ultrasound Obstet Gynecol. 2010; 35:462-467.

5. Hernandez-Andrade E, Brodszki J, Lingman G, Gudmundsson S, Molin J, Marsal K. Uterine artery score and perinatal outcome. Ultrasound Obstet Gynecol. 2002; 19:438-442.

6. Poon LC, Volpe N, Muto B, Yu CK, Syngelaki A, Nicolaides KH. Second-trimester uterine artery Doppler in the prediction of stillbirths. Fetal Diagn Ther. 2013; 33:28-35.

7. Orabona R, Donzelli CM, Falchetti M, Santoro A, Valcamonico A, Frusca T. Placental histological patterns and uterine artery Doppler velocimetry in pregnancies complicated by early or late pre-eclampsia. Ultrasound Obstet Gynecol. 2016; 47:580-585.

8. Flo K, Wilsgaard T, Acharya G. Agreement between umbilical vein volume blood flow measurements obtained at the intra-abdominal portion and free loop of the umbilical cord. Ultrasound Obstet Gynecol. 2009; 34:171-176.

9. Flo K, Wilsgaard T, Acharya G. Longitudinal reference ranges for umbilical vein blood flow at a free loop of the umbilical cord. Ultrasound Obstet Gynecol. 2010; 36:567-572.

10. Flo K, Wilsgaard T, Acharya G. A new non-invasive method for measuring uterine vascular resistance and its relationship to uterine artery Doppler indices: a longitudinal study. Ultrasound Obstet Gynecol. 2011; 37:538-542.

11. Konje JC, Kaufmann P, Bell SC, Taylor DJ. A longitudinal study of quantitative uterine blood flow with the use of color power angiography in appropriate for gestational age pregnancies. Am J Obstet Gynecol. 2001; 185:608-613.

12. Konje JC, Howarth ES, Kaufmann P, Taylor DJ. Longitudinal quantification of uterine artery blood volume flow changes during gestation in pregnancies complicated by intrauterine growth restriction. BJOG. 2003; 110:301-305.
13. Bower S, Vyas S, Campbell S, Nicolaides KH. Color Doppler imaging of the uterine artery in pregnancy: normal ranges of impedance to blood flow, mean velocity and volume of flow. Ultrasound Obstet Gynecol. 1992; 2:261-265.
14. Dickey RP, Hower JF. Ultrasonographic features of uterine blood flow during the first 16 weeks of pregnancy. Hum Reprod. 1995; 10:2448-2452.
15. Scholbach T, Heien C, Eggeb OT. Umbilical vein vasomotion detected in vivo by serial three-dimensional pixelwise spatially angle corrected volume flow measurements. Ultrasound Obstet Gynecol. 2015;
16. Scholbach T. M. NF, J. Stolle. Significant differences of fetal blood supply in fetuses of different weight classes demonstrated by the novel method of three-dimensional pixelwise fetal volume flow measurements (PixelFlux-method). Ultrasound Obstet Gynecol. 2012; 40:118.
17. Acharya G, Sitras V, Erkinaro T, Makikallio K, Kavasmaa T, Pakkila M, et al. Experimental validation of uterine artery volume blood flow measurement by Doppler ultrasonography in pregnant sheep. Ultrasound Obstet Gynecol. 2007; 29:401-406.
18. Scholbach J. Scholbach T. PixelFlux. Chameleon Software, Available online at: http://www.chameleon-software.de/en/home.php (accessed December 7, 2016).
19. Gjessing HK, Grottum P, Okland I, Eik-Nes SH. Fetal size monitoring and birth-weight prediction: a new population-based approach. Ultrasound Obstet Gynecol. 2017; 49:500-507.
20. Li N, Ghosh G, Gudmundsson S. Uterine artery Doppler in high-risk pregnancies at 23-24 gestational weeks is of value in predicting adverse outcome of pregnancy and selecting cases for more intense surveillance. Acta Obstet Gynecol Scand. 2014; 93:1276-1281.
21. Thoresen M, Wesche J. Doppler measurements of changes in human mammary and uterine blood flow during pregnancy and lactation. Acta Obstet Gynecol Scand. 1988; 67:741-745.
22. Ferrazzi E, Rigano S, Padoan A, Boito S, Pennati G, Galan HL. Uterine artery blood flow volume in pregnant women with an abnormal pulsatility index of the uterine arteries delivering normal or intrauterine growth restricted newborns. Placenta. 2011; 32:487-492.

23. Rigano S, Ferrazzi E, Boito S, Pennati G, Padoan A, Galan H. Blood flow volume of uterine arteries in human pregnancies determined using 3D and bi-dimensional imaging, angio-Doppler, and fluid-dynamic modeling. Placenta. 2010; 31:37-43.

REVIEWERS´ COMMENTS TO AUTHOR AND RESPONSE

Dear Prof. Deepak Bedi

Thank you very much for the positive responds to our manuscript.

Reviewer: 1

Why was femur length and head circumference not used in weight estimation? Also using best measurement with proper landmarks would have been more accurate for weight estimation than mean of three measurements.

Answer:

Several methods for weight estimations are used worldwide. Hadlock published the most used method in the 1980’s based on less than 200 women.

In Norway the eSnurra algorithm is used for estimating day of delivery[1] and also for weight estimations[2]. It is a population based method and calculations based on 40,000 women. eSnurra is a simple method only using BPD and abdominal circumference (AC). Our group has recently validated fetal weight estimation using eSnurra and we found good accuracy[3]. We used the eSnurra algorithm because it is the recommended method by the Norwegian Directory of Health.

The best measurement with proper landmarks might be used, however, we followed the eSnurra algorithm exactly, and this method uses the mean of three measurements.

It is not possible for us to change the method of fetal weight estimation retrospectively. We added a sentence about Norwegian guidelines in the revised manuscript.

Reviewer: 2

Thanks for the very pleasant article

Answer:

Thank you very much for this comment.

Best regards

Helene Caroline Arneberg, Thea Anette Andersen and Torbjørn Eggebø

1. Gjessing HK, Grottum P, Eik-Nes SH. A direct method for ultrasound prediction of day of delivery: a new, population-based approach. Ultrasound Obstet Gynecol. 2007; 30:19-27.
2. Gjessing HK, Grottum P, Okland I, Eik-Nes SH. Fetal size monitoring and birth-weight prediction: a new population-based approach. Ultrasound Obstet Gynecol. 2017; 49:500-507.
3. Eggebo TM, Klefstad OA, Okland I, Lindtjorn E, Eik-Nes SH, Gjessing HK. Estimation of fetal weight in pregnancies past term. Acta Obstet Gynecol Scand. 2017; 96:183-189.