Pixelwise Quantification of Placental Perfusion Visualized by 3D Power Doppler Sonography

Pixelweise Plazentaperfusionsmessung aus dreidimensionalen Power-Doppler-Daten

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Zusammenfassung

Ziel: Wir stellen eine neue, nicht invasive Methode vor, die eine pixelweise Bemessung der Perfusion verschiedener Plazentaschichten aus standardisierten dreidimensionalen Powerdoppler-Videos ermöglicht (PixelFlux-Methode).
Material und Methoden: Standardisierte Powerdoppler-Videos anterior und zentral lokalisierter Plazenten (13.–38. Schwangerschaftswoche) von 22 Schwangeren wurden unter definierten Bedingungen untersucht. Alle Schwangerschaften führten zur Geburt eines normalgewichtigen Kindes. Die Gewebsperfusionsintensität in 4 Plazentaschichten wurde mithilfe der PixelFlux-Software als Produkt der Amplitude der Powerdoppler-Signale und der perfundierten Fläche bezogen auf die Fläche der Untersuchungsregion automatisch berechnet. Die Perfusionsintensität wurde in Prozent der maximal möglichen Perfusion angegeben.
Ergebnisse: Zwischen der Uteruswand (6,6 %), der maternalen (2,4 %), der fetalen Schicht (1,6 %) und der Chorionplatte (9,3 %) konnten signifikante Perfusionsintensitätsunterschiede nachgewiesen werden. Im Verlauf der Schwangerschaft kam es zu einem kontinuierlichen Anstieg der Perfusionsintensität in der Uteruswand und den Plazentazotten.
Schlussfolgerung: Die PixelFlux-Methode erlaubt die Messung der Plazentaperfusion aus dreidimensionalen Powerdoppler-Ultraschallvideos und kann Unterschiede der Perfusion der Plazentaschichten darstellen.

Abstract

Purpose: We present a new method for noninvasive automatic measurement of perfusion intensity (PixelFlux method) in standardized 3 D power Doppler sonography to quantify differences of perfusion intensities among different placental layers.
Materials and Methods: Power Doppler sonographic videos of anterior and central placentas were recorded at various gestational ages (13 to 38 weeks) under defined conditions in 22 women with uncomplicated pregnancies which ended in the delivery of an appropriately grown fetus. Tissue perfusion intensity in four placental layers was calculated as the product of the Doppler amplitude and the perfused area encoded by power Doppler signals related to the area of the respective layer. Measurements are given as the percentage of maximal possible perfusion.
Results: Significant differences in placental perfusion intensities in the uterine wall (6.6 %), the maternal flow within the intervillous space (2.4 %), the fetal flow within placental villi (1.6 %) and the chorionic plate (9.3 %) were demonstrated with a continuous increase in the uterine wall and the placental villi.
Conclusion: Placental perfusion intensity was quantified noninvasively from 3 D power Doppler signal data in an easily accomplishable manner with a new software-based measurement procedure. There are significant differences in perfusion intensities among placental layers. Placenta perfusion measurement with the PixelFlux method is feasible and can discern significant perfusion differences among different placenta layers.

Introduction

Fetal development requires a sufficient exchange of oxygen and metabolites across the placenta during the whole duration of pregnancy. Regular perfusion of the placenta from the maternal as well as the fetal side is a vital and fundamental prerequisite for the exchange [1]. There are pathologies in which there is reduced blood supply from the mother or reduced placental perfusion on the fetal side. In both cases the fetus becomes hypoxic and there are associated morphological changes in the placenta [2]. A classic example including both alterations is intrauterine growth restriction (IUGR)/fetal growth restriction (FGR).

Currently, Doppler velocimetry of the uterine arteries is used to assess increased blood flow impedance, hence indirectly measuring placental perfusion from the maternal side. To date, it is the only noninvasive screening test for FGR [3]. Abnormal uterine artery Doppler indices at 20 – 24 weeks will identify 50 – 70 % of pregnancies complicated by fetal growth restriction for a 5 % false-positive rate. These figures are significantly worse when this screening is performed earlier in pregnancy [4]. At 11 – 14 weeks gestation for example this detection rate is only 27 % for a 5 % false positive rate [5, 6]. This screening tool, which indirectly assesses the perfusion of the placenta from the maternal side thus, has a poor sensitivity and predictive value, especially early in gestation.

There is thus a need for an improved screening test for predicting FGR and/or monitoring placental tissue perfusion quantitatively in all pregnancies. Recently, modern ultrasound technology has been used to qualitatively describe the vascular ontogeny in the placenta during gestation [7]. Such a direct evaluation of placental perfusion may therefore be applied to investigate whether deviations from the normal placental perfusion pattern could result in an unfavorable outcome of pregnancy such as FGR.

This article describes a new method for using power Doppler sonographic videos to measure the intensity of placental perfusion and its application in the evaluation of placental perfusion during normal pregnancy.

Material and Methods

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Patients

This was a prospective longitudinal study of uncomplicated pregnancies from 13 to 38 weeks of gestation in 22 non-obese women (BMI 19 – 24 kg/m^2) aged 23 – 34 years. Each woman gave signed informed consent for the study which was approved by the University Hospitals of Leicester NHS Trust Research and Development Unit and the Leicestershire and Rutland Ethics Committee.

The pregnancies were accurately dated by crown-rump measurements performed by ultrasound scans between 8 and 12 weeks. The women had their detailed ultrasound scan at 20 weeks during which a uterine Doppler scan was performed. Only pregnancies in which the placenta had a central or anterior location and the uterine artery Doppler scans were normal as defined by Bower et al. [8] were included in the study. All pregnancies with complications such as FGR, preeclampsia, diabetes mellitus, antepartum hemorrhage or other maternal diseases were also excluded. Power Doppler sonography was used to study placentas in the women recruited into the study.

Power Doppler sonography

To minimize inter-operator variability, all placental imaging was performed by a single operator (JCK) with extensive experience using this tool in a clinical setting. Imaging was performed using a 3 D Advanced Technology Laboratory (ATL) High Definition Imaging (HDI) 5000 ultrasound machine (ATL, Washington, Seattle, USA). Scanning was first performed on grayscale to localize the placenta. The setting was then switched to power Doppler mode and imaging of the placenta was performed by sweeping from left to right for 15 to 20 sec. However, due to artifacts and unplanned fetal and maternal movements, this was not always possible, and repeated sweeps were performed. The maximum duration of the scans was 2 to 3 minutes and a satisfactory sweep was obtained from those meeting the inclusion criteria within this period. During imaging, the mothers were advised to limit their movements and the fetuses were in a state of apnea.

After the acquisition of the various sweeps, a 3-dimensional program was activated and the noise edges were trimmed. The program was then set to reconstruct a 3-dimensional image of the placenta in the power angiographic (PA) mode from 11 2 D frames acquired during the sweep. The power setting of the ultrasound machine was a spatial temporary average intensity of less than 92 mWatts/cm^2; the wall filter was set to 75 MHz, the color intensity was set to 75 % and the pulse repetitive frequency was set to 700 Hz. The power setting was set to this level based on the advice of the Professor of Medical Physics at the University of Leicester. This setting was specifically recommended by the application specialist from ATL (ATL, Seattle, Washington, USA). The sensitivity was set to the maximum level and the persistence was set in the middle range. The 3 D images were digitally captured for subsequent automatic Doppler signal intensity calculations.

Calculation of perfusion intensity

Power Doppler depiction of placental blood flow was read out from videotapes to specially designed software for perfusion measurements (PixelFlux, Chameleon-Software, Leipzig, Germany). The perfusion signal intensity was calculated as follows:

1. The distances within the image and the power Doppler energy were calibrated with the PixelFlux software. The amount of coloration which encoded the maximum Doppler energy was set to 100 %. All subsequent measurements used this calibration as a reference. Regions of interest (ROI) were defined using the orientation of blood flow in four distinct anatomical layers: uterine wall (representing blood flow within the placental bed), maternal flow within the intervillous space, fetal flow within placental villi, and chorionic plate (**Fig. 1**). The distinction of the four layers was based on our previous study on staging placental development using the comparison between 3 D power Doppler and morphology [7]. Two of the layers were reliably distinguishable by identifying the blood flow in the uterine wall and chorionic plate. A distinction between maternal and fetal flow in the placenta proper was made according to their comb-like interdigitation of flow signals. The maternal flow in the placenta appeared as arterial jets (aj in **Fig. 1**) from the uterine wall into the intervillous space (maternal flow). The fetal flow in the placenta is shown in the vessels of small tree-like structures or placental villi (pv in **Fig. 1**) emanating from the chorionic plate (fetal flow). The ROIs were chosen as horizontal slices within the borders described above (**Fig. 2a, b**): The uterine wall encompassed vessels in the myometrial part of the placental bed (**Fig. 2a**, layer 1). The maternal layer was defined by the flow of maternal blood within the intervillous space adjacent to the uterine layer (**Fig. 2a**, layer 2). The fetal layer was defined by the flow within the placental villi (**Fig. 2b**, layer 3). The chorionic plate was the layer on the bottom of the images with mostly horizontally oriented vessels (**Fig. 2b**, layer 4).
2. Each pixel inside the ROIs was quantified with respect to two parameters: 1. Power Doppler energy: The color hue of the coloration of each pixel was automatically compared with the color hues of the image color bar (**Fig. 2a, b**, upper right cor-

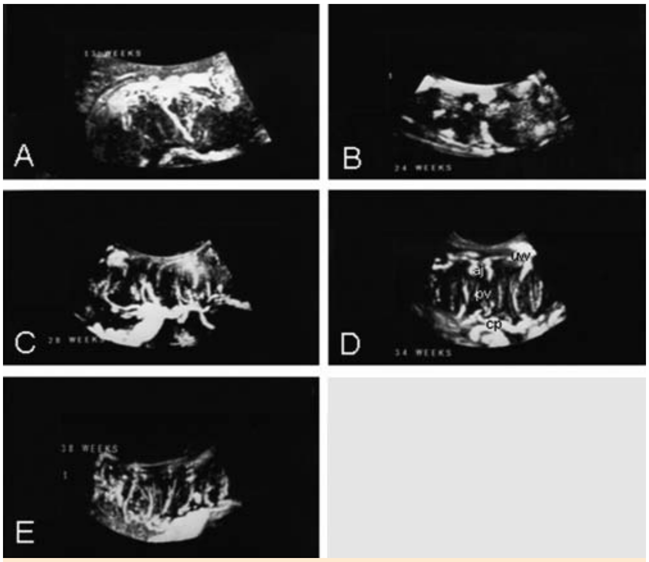

Fig. 1 Power Doppler ultrasonographic images from different placentae of 13, 24, 28, 34 and 38 weeks of gestation. Blood flow within the uterine wall (uw) is always on top of the images with inflow via arterial jets (aj) into the intervillous space. The flow of maternal blood within the intervillous space is depicted by grey shading. Flow within the placental villi (pv) can be seen best in 28 to 38 weeks. Flow within the chorionic plate (cp) is always on the bottom of the images; sometimes also the flow within the umbilical cord (uc) is visible.

Abb. 1 Powerdopplersonografische Bilder verschiedener Plazenten in der 13., 24., 28., 34. und 38. SSW. Der Blutfluss innerhalb der Uteruswand (uw) befindet sich oben im Bild mit dem Einstrom arterieller Jets (aj) in den intervillösen Raum. Der mütterliche Blutstrom innerhalb des intervillösen Raumes ist in Grautönen dargestellt. Der Blutfluss innerhalb der Chorionplatte (cp) befindet sich unten im Bild. Zuweilen ist auch der Blutfluss innerhalb der Nabelschnur (uc) sichtbar.

ner of images). From the zero point to the end of the bar, each hue was assigned an individual power Doppler energy value between 0 % and 100 %. 2. Occupied area: Each image was calibrated with respect to its dimensions. Then each pixel's area was calculated by measuring its length and width. In the next step the colored area inside the ROI and the mean power Doppler energy of the entire ROI were calculated by referring the sum of all colored pixel area values and their energy values to the entire ROI. These measurements and calculations were repeated for all images of a complete placenta sweep.

3. Perfusion intensity was calculated by multiplying the mean Doppler energy of all pixels and the area occupied by all colored pixels inside the ROI.
4. Perfusion intensity was expressed as a percentage. A perfusion intensity of 100 % represented an ROI filled with colored pixels at a maximum energy level throughout the duration of the sweep (i.e, from one edge of the placenta to the opposite edge).
5. The perfusion intensities of the layers defined above were calculated separately and compared to each other.

The data analysis with the PixelFlux software of a sweep took about 10 sec.

Statistical analysis

The perfusion intensity of the placental layers was compared using the Mann-Whitney-U test. P-values less than 0.05 were regarded as significant. Data are presented as means and standard deviation.

Results

A total of 29 subjects were recruited at the outset but only 22 were included in the analyses. 7 were excluded because (a) the placenta was not central (2), (b) the patient developed preeclampsia (3), (c) the patient had antepartum hemorrhage (1) or (d) the patient delivered before 37 completed weeks (1). The mean gestational age at delivery and the birth weights of the babies were 38.0 ± 1.8 (range 37 to 42) weeks and 3364 g ± 380 g (range 2856 g to 4013 g), respectively. The mean number of times each placenta was examined was 8 (± 3). In total 508 videos were recorded during placenta sweeps. From these videos 16 248 sonographic images were used for quantification.

Fig. 1 shows examples of still images of the power Doppler sonographic sweeps of the placentas at different gestational ages. The maternal inflow into the intervillous space is outlined by arterial jets (aj in **Fig. 1 d**). The fetal flow within the placental villi (pv in **Fig. 1 d**) is difficult to detect early in gestation due to the fact that this method is unable to detect vessels of diameters less than 200 µm [7]. The flow within the chorionic plate steadily increased during pregnancy (cp in **Fig. 1 d**).

Videos were used to generate systematic still flow images, which then allowed quantification of perfusion intensities within different regions of the placenta. **Fig. 2a, b** show the same still image at the same time point but with different frames in which perfusion intensities were quantified. **Fig. 2c, d** show the perfused areas and the respective ROIs are indicated in **Fig. 2a, b**. **Fig. 2e, f** show the power Doppler energy of each layer along the placenta sweep. **Fig. 2 g, h** show the calculated perfusion intensities of each layer. In **Fig. 2i** the mean perfusion intensities of both layers are compared with each other.

Using these ROIs, the perfusion intensities of four different regions were quantified (**Fig. 3**): (1) maternal blood flow in the uterine wall, (2) maternal flow within the intervillous space, termed maternal layer (containing mostly maternal blood flow within the intervillous space), (3) fetal flow within placental villi, termed fetal layer (containing mostly fetal blood flow within villous vessels) and, (4) fetal flow in the chorionic plate.

Fig. 3 shows a summary of the blood flow intensities of the four different layers. These layers were defined by their typical vascular and morphological patterns. Only typical patterns led to an assignment of a certain layer to one of the abovementioned four layers. The highest intensity was recorded in the chorionic plate, which was clearly distinguishable in 24 % of the placentas, followed by the uterine wall, which was clearly discernable in 60 % of the placentas, while the least intensity was recorded in the fetal layer. The intensities in the chorionic plate were always statistically different from those of the maternal and fetal layers ($p < 0.05$) but not from those of the uterine wall ($p > 0.05$). The intensities of the uterine wall were significantly higher than those of the maternal layer ($p = 0.04$) and fetal layer ($p = 0.04$) in the placenta.

An analysis of the changes in flow intensities with gestation is shown in **Fig. 4** and **Table 1**. The perfusion intensity of the uterine wall and the maternal layer remained constant after 13 weeks of gestation until term while the perfusion intensity of the chorionic plate and the fetal layer increased during gestation (**Fig. 4**, **Table 1**).

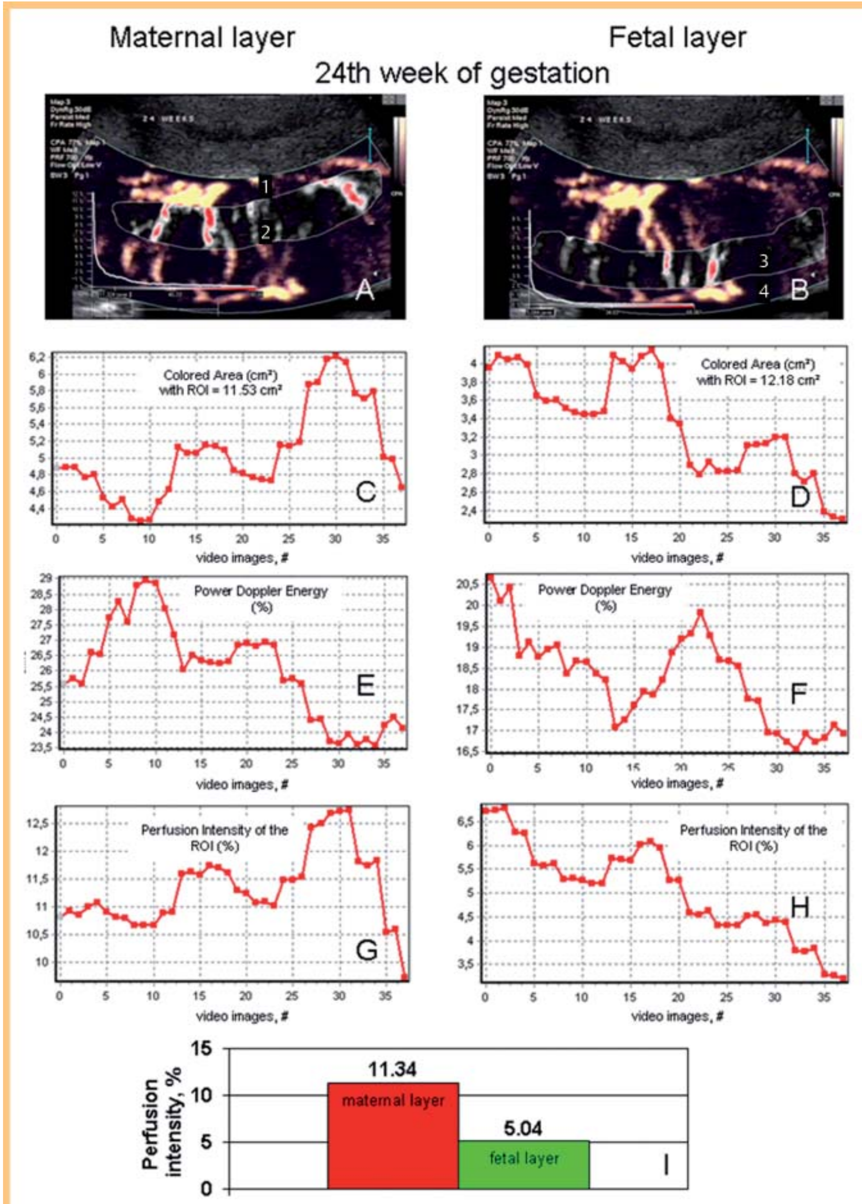

Fig. 2 **A, B** Image examples of Power Doppler videos from a placenta of 24 weeks of gestation. **A** Two layers are indicated by numbers, uterine wall was named 1, maternal flow in the intervillous space was named 2. **B** Two layers are indicated by numbers, fetal flow in the placental villi was named 3, blood flow in the chorionic plate was named 4. From a sweep through the placenta, blood flow intensities within the maternal (left) and fetal layers (right) were quantified. Perfusion maps and intensity distribution curves of the maternal and fetal layer are shown. Quantification of perfusion was calculated from an ultrasonographic video with perfused areas **C, D**, Power Doppler energies **E, F**, perfusion intensities **G, H** and direct comparison of mean Power Doppler intensities of both layers **I**. Perfusion intensity of the maternal flow within the intervillous space was 11.3 % vs. 5.0 % intensity of the fetal flow within placental villi.

Abb. 2 **A, B** Bildbeispiele aus Powerdoppler-Videos einer Plazenta in der 24. SSW. **A** Die Uteruswand ist mit 1, der intervillöse Raum mit 2 bezeichnet. **B** Fetaler Blutfluss in den Plazentazotten mit 3, Blutfluss in der Chorionplatte mit 4 bezeichnet. Die Blutflussintensitäten innerhalb der mütterlichen (links) und der fetalen Schichten (rechts) wurden während eines Schwenkes durch die Plazenta quantifiziert. Die Perfusionskarten und die Intensitätsverteilungskurven sowohl der mütterlichen als auch der fetalen Schicht sind dargestellt. Die Perfusion wurde anhand der perfundierten Areale **C, D** eines Ultraschall-Videos quantifiziert. Die Powerdoppler-Energie **E, F**, die Perfusionsintensitäten **G, H** und der direkte Vergleich der mittleren Powerdoppler-Intensitäten in beiden Schichten **I** sind dargestellt. Die Perfusionsintensität des mütterlichen Flusses innerhalb des intervillösen Raumes lag bei 11,3 %, der des fetalen Flusses innerhalb der Plazentazotten bei 5 %.

Table 1 Mean values of blood flow intensities in three different groups of placentae during gestation are shown. Values are presented as mean and SEM with the number of placentae in brackets.

	Blood flow intensities			
Weeks of gestation	**Chorionic plate**	**Fetal layer**	**Maternal layer**	**Uterine wall**
13 – 19	16.10 ± 10.74 (3)	1.53 ± 1.13 (7)	4.10 ± 3.27 (7)	12.85 ± 7.24 (6)
20 – 29	23.48 ± 11.57 (5)	5.01 ± 2.48 (6)	5.95 ± 3.71 (6)	12.10 ± 8.05 (6)
30 – 38	42.00 ± 9.76 (2)	7.03 ± 3.44 (4)	6.77 ± 1.77 (4)	13.17 ± 7.27 (3)

Discussion

Three-dimensional Doppler imaging is now generally available in many obstetrical departments [9, 10]. The quantification of blood flow volumes, however, is demanding [11] and still in its infancy. Only recently a novel method of 3 D flow measurements inside the umbilical vein was introduced [12] which was extended to a model with automatic spatial angle correction [13]. In recent years, a number of groups have worked on the evaluation of placental blood flow using 3 D power Doppler. On one hand phantom studies were performed using a simple plastic tube or an in-vitro perfused placenta after delivery [14, 15]. Studies on placentas in-vivo mostly measured vascularization, flow and vascularization flow indices in the villous part of the placenta [16 – 19]. Few compared the flow in normal pregnancies to that in pregnancies complicated by pathologies such a fetal growth restriction or trisomies or examined the effect of cigarette smoking on placental vascularization [17, 19, 20]. Only a few groups have evaluated the flow in the placenta and the uterine wall [21 – 23]. To the best of our knowledge, this is the first study to examine flow in different compartments of the placenta and the placental bed such as maternal flow in the uterine wall and intervillous space and fetal flow in the chorionic villi and chorionic plate.

There is currently no simple method to quantitatively evaluate the perfusion of the placenta in-vivo although Doppler ultrasound can produce qualitative information [7]. A technique that has been developed in recent years is magnetic resonance imaging (MRI). MRI is specifically used for the diagnosis and characterization of placenta accreta, increta and percreta [24]. However, beside the use of classic MRI for the diagnosis of altered placental anchoring and invasion, this technique has not been applied to measure the perfusion of the placenta.

However, one clinical technique that has been developed from classic MRI for the noninvasive measurement of placental perfusion is perfusion-sensitive high-speed echoplanar imaging (EPI), which is a fast method for generating magnetic resonance images [25 – 27]. The first in-vivo measurements using the non-selective/selective inversion recovery echo-planar imaging sequence were performed in 1998 [27]. Using EPI, the placental perfusion was measured from 20 weeks until term with an average value of perfusion of 176 ml/100 mg tissue/min. (SD 96 ml/100 mg/min.). With the signal-to-noise ratio of this technique, the expected variability was calculated to be 71 % with a maximum scanning time of 400 sec.

3 D histograms have been used to describe the pattern of vascular distribution of tissues and to calculate (1) the vascularization index (VI), measuring the percentage of color voxels inside the tissue under investigation, (2) the volume, which represents the blood vessels within the tissue, and (3) the flow index (FI), which is the average color value of all color voxels. The 3 D histograms show the average blood flow intensity and the vascularization flow index (VFI), which is the average color value of all gray and color voxels of the tissue volume representing both blood flow and vascularization [28]. Our approach is very similar to quantitative 3 D sonograms. Nevertheless, there are some differences. We used a sweep of the entire placenta, whereas quantitative 3 D histograms with the VOCAL™ technique use a spherical volume which is usually selected from a placental region with the highest coloration [28]. This sphere encompasses the full thickness of the placenta [18]. A differentiation of several placental layers has not yet been carried out. With our method the investigator is not restricted in terms of the geometry of the ROI or definition of sub-ROIs. Moreover, our method is not restricted to a specific ultrasound system but can be applied to any 3 D ultrasound system.

Color Doppler and power Doppler sonography allow an estimation of perfusion while a reliable evaluation of relevant flow

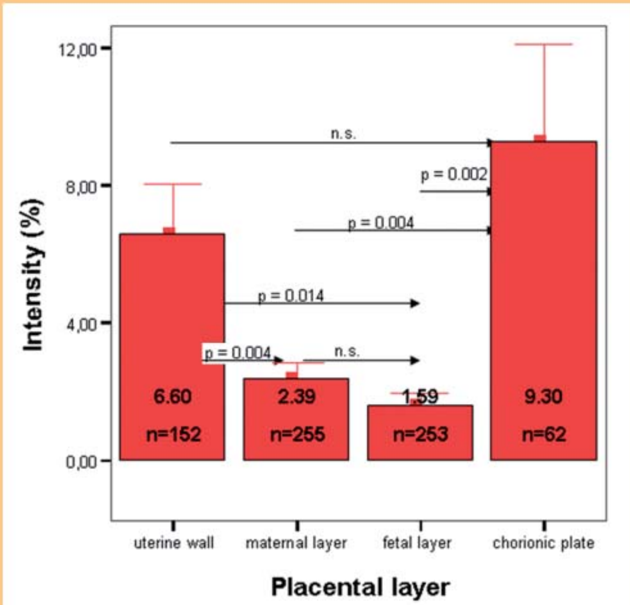

Fig. 3 This diagram displays perfusion intensities in the four layers of the placenta that were used for quantification. The columns represent perfusion intensity within the uterine wall, the maternal flow within the intervillous space, the fetal flow within the placental villi and the chorionic plate regardless of gestational age.

Abb. 3 Das Diagramm zeigt die Perfusionsintensitäten in den 4 Plazentaschichten, die zur Quantifizierung herangezogen worden sind. Die Säulen stellen die Perfusionsintensität innerhalb der Uteruswand, den mütterlichen Fluss innerhalb des intervillösen Raumes, den fetalen Fluss innerhalb der Plazentazotten und die Chorionplatte dar, wobei die Schwangerschaftsdauer nicht berücksichtigt wurde.

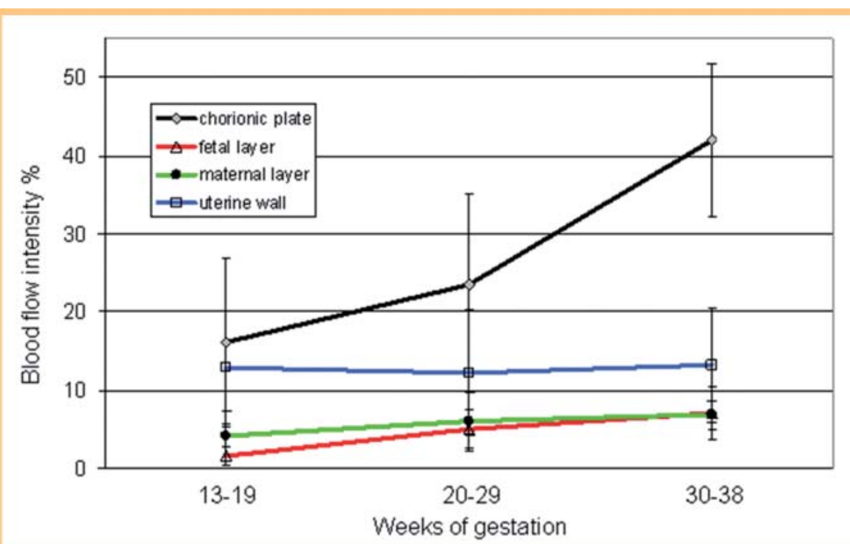

Fig. 4 This diagram displays perfusion intensities in the four layers of the placenta that were used for quantification, the uterine wall, the maternal layer, the fetal layer and the chorionic plate. For each layer the intensities during pregnancy were grouped into three stages, 13 – 19 weeks, 20 – 29 weeks and 30 – 38 weeks of gestation.

Abb. 4 Dieses Diagramm zeigt die Perfusionsintensitäten in den 4 Plazentaschichten, die zur Flussquantifizierung genutzt wurden, die Uteruswand, die mütterliche Schicht, die fetale Schicht und die Chorionplatte. In jeder dieser Schichten wurden im Schwangerschaftsverlauf die Perfusionsintensitäten in 3 Stadien zusammengefasst, der 13. – 19. SSW, der 20. – 29. SSW und der 30. – 38. SSW.

parameters in tissues is still not achieved. Recently, Konje et al. [7] used 3-dimensional power Doppler to assess placental perfusion throughout gestation. They were able to correlate ultrasonographic imaging with vessel architecture as seen in histological sections. They described harmonic developmental growth of villous trees with gestational age. Morphological criteria such as the relation of stem villi to villous branches, density of villous trees, and length of trees were suitable to describe a normal pattern of placental development. However, since the histological assessment of placental morphology can only be performed after delivery, we were looking for a quantitative tool to estimate placental perfusion in ongoing pregnancies.

In our series we focused mainly on anterior placentas because of technical difficulties with bidirectional scanning of posterior and lateral placentas, whereas others using the 3 D technique were able to evaluate whole placentas irrespective of their location [18].

We used a perpendicular sweep across the placentas recorded in power Doppler mode. Such a sweep is not standardized with respect to sweeping velocity and duration of sweep. It may therefore be useful to standardize these potential confounders in future studies and to follow up this study using machines that perform automatic 3 D reconstruction. It may also be useful to check more thoroughly whether the use of color instead of power Doppler may result in greater differences between the layers. Power Doppler depicts flow signals more precisely. However, it is not directional (in contrast to color Doppler) and more vulnerable to movement artifacts. Power Doppler sweeps do not allow the collection of perfusion pulsation data. Results from similar studies in tumors and renal transplants stress the specific value of perfusion pulsatility to describe mechanical properties of the intervascular space [29]. Due to a lack of in-vivo functional data, a comparison of results presented here is possible only with morphologic investigations [30, 31].

However, a significant correlation of tissue perfusion and tissue oxygenation has already been demonstrated [29].

In this pilot study, we restricted our Doppler studies to one experienced operator. However, we believe that with appropriate training and supervision, experienced sonographers should be able to perform such measurements. Additionally, with the advent of 3 – 4 D ultrasound, this should even become easier. We also restricted our measurements to the anterior placenta and to non-obese women. This was purely to minimize confounders in the acquired signals. However, we did apply this technique to obese women and similar results were obtained (Konje, unpublished results).

We could demonstrate a distinct decline in perfusion intensity from maternal to fetal flow within the placenta. In the future this difference as well as the absolute perfusion signal intensities may be used to detect early changes in perfusion that might help to predict fetal growth restriction. Maternal hypertension would certainly be a focus of future research with the PixelFlux technique since preeclamptic hypertension is a major concern for fetal well-being.

The technique would require simplification and automation if it is to be used for routine screening. The next logical step should therefore be to stratify placentas according to gestational age and use these to develop a time course of placental perfusion. After defining normal perfusion intensities at various gestational ages in a larger group of pregnancies, the detection of fetuses at risk due to local or general perfusion derangements would become a realistic aim once the technique has been simplified and automated.

References

1 *Benirschke K, Kaufmann P, Baergen R.* Pathology of the human placenta. 5th edn. New York.: Springer 2006

2 *Kingdom JC, Kaufmann P.* Oxygen and placental vascular development. Adv Exp Med Biol 1999; 474: 259 – 275

3 *Spencer K et al.* First-trimester maternal serum PP-13, PAPP-A and second-trimester uterine artery Doppler pulsatility index as markers of pre-eclampsia. Ultrasound Obstet Gynecol 2007; 29: 128 – 134

4 *Papageorghiou AT et al.* Multicenter screening for pre-eclampsia and fetal growth restriction by transvaginal uterine artery Doppler at 23 weeks of gestation. Ultrasound Obstet Gynecol 2001; 18: 441 – 449

5 *Martin AM et al.* Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. Ultrasound Obstet Gynecol 2001; 18: 583 – 586

6 *Gomez O et al.* Uterine artery Doppler at 11-14 weeks of gestation to screen for hypertensive disorders and associated complications in an unselected population. Ultrasound Obstet Gynecol 2005; 26: 490 – 494

7 *Konje JC et al.* 3-dimensional colour power angiography for staging human placental development. Lancet 2003; 362: 1199 – 1201

8 *Bower SJ et al.* Prediction of pre-eclampsia by abnormal uterine Doppler ultrasound and modification by aspirin. Br J Obstet Gynaecol 1996; 103: 625 – 629

9 *Hartung J, Kalache KD, Chaoui R.* Three-dimensional power Doppler ultrasonography (3D-PDU) in fetal diagnosis. Ultraschall in Med 2004; 25: 200 – 205

10 *Dubiel M et al.* Fetal and placental power Doppler imaging in normal and high-risk pregnancy. Eur J Ultrasound 1999; 9: 223 – 230

11 *Menigault E et al.* Feto-maternal circulation: mathematical model and comparison with Doppler measurements. Eur J Ultrasound 1998; 7: 129 – 143

12 *Scholbach TM, Stolle J, Scholbach J.* Three-Dimensional Volumetric Spatially Angle-Corrected Pixelwise Fetal Flow Volume Measurement. Ultraschall in Med 2011; 32: E122 – E128

13 *Scholbach T.* Dynamic Tissue Perfusion Measurement – Basics and Applications. Sonography (ed. by Kerry Thoirs at InTech – Open Access Publisher) 2012, ISBN 978-953-307-947-949, 293 – 324

14 *Schulten-Wijman MJ et al.* Evaluation of volume vascularization index and flow index: a phantom study. Ultrasound Obstet Gynecol 2008; 32: 560 – 564

15 *Jones NW et al.* In vitro dual perfusion of human placental lobules as a flow phantom to investigate the relationship between fetoplacental flow and quantitative 3D power doppler angiography. Placenta 2009; 30: 130 – 135

16 *Merce LT, Barco MJ, Bau S.* Reproducibility of the study of placental vascularization by three-dimensional power Doppler. J Perinat Med 2004; 32: 228 – 233

17 *Rizzo G et al.* Placental vascularization measured by three-dimensional power Doppler ultrasound at 11 to 13 + 6 weeks' gestation in normal and aneuploid fetuses. Ultrasound Obstet Gynecol 2007; 30: 259 – 262

18 *de Paula CF et al.* Quantitative analysis of placental vasculature by three-dimensional power Doppler ultrasonography in normal pregnancies from 12 to 40 weeks of gestation. Placenta 2009; 30: 142 – 148

19 *Noguchi J et al.* Placental vascular sonobiopsy using three-dimensional power Doppler ultrasound in normal and growth restricted fetuses. Placenta 2009; 30: 391 – 397

20 *Rizzo G et al.* Effects of maternal cigarette smoking on placental volume and vascularization measured by 3-dimensional power Doppler ultrasonography at 11+0 to 13+6 weeks of gestation. Am J Obstet Gynecol 2009; 200: 415e1 – 415e5

21 *Merce LT et al.* Intervillous and uteroplacental circulation in normal early pregnancy and early pregnancy loss assessed by 3-dimensional power Doppler angiography. Am J Obstet Gynecol 2009; 200: 315e1 – 315e8

22 *Zalud I, Shaha S.* Evaluation of the utero-placental circulation by three-dimensional Doppler ultrasound in the second trimester of normal pregnancy. J Matern Fetal Neonatal Med 2007; 20: 299 – 305

23 *Zalud I, Shaha S.* Three-dimensional sonography of the placental and uterine spiral vasculature: influence of maternal age and parity. J Clin Ultrasound 2008; 36: 391 – 396

24 *Lax A et al.* The value of specific MRI features in the evaluation of suspected placental invasion. Magn Reson Imaging 2007; 25: 87 – 93
25 *Duncan KR*. Fetal and placental volumetric and functional analysis using echo-planar imaging. Top Magn Reson Imaging 2001; 12: 52 – 66
26 *Francis ST et al.* Non-invasive mapping of placental perfusion. Lancet 1998; 351: 1397 – 1399
27 *Gowland PA et al.* In vivo perfusion measurements in the human placenta using echo planar imaging at 0.5 T. Magn Reson Med 1998; 40: 467 – 473
28 *Merce LT et al.* Assessment of placental vascularization by three-dimensional power Doppler "vascular biopsy" in normal pregnancies. Croat Med J 2005; 46: 765 – 771
29 *Scholbach T et al.* New method of dynamic color doppler signal quantification in metastatic lymph nodes compared to direct polarographic measurements of tissue oxygenation. Int J Cancer 2005; 114: 957 – 962
30 *Castellucci M et al.* Villous sprouting: fundamental mechanisms of human placental development. Hum Reprod Update 2000; 6: 485 – 494
31 *Kingdom J et al.* Development of the placental villous tree and its consequences for fetal growth. Eur J Obstet Gynecol Reprod Biol 2000; 92: 35 – 43