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Umbilical vein vasomotion detected *in vivo* by serial three-dimensional pixelwise spatially angle-corrected volume flow measurements

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KEYWORDS: 3D-fetal perfusion measurement; PixelFlux; umbilical vein; vasomotion

ABSTRACT

Objective *To explore changes in volume flow in the umbilical vein in healthy second-trimester fetuses.*

Methods *This was a prospective observational pilot study performed at Stavanger University Hospital, Norway, between May and October 2013. Serial three-dimensional ultrasound recordings from the umbilical vein were acquired every 30 s in a 5-min period in 43 fetuses at 17–20 weeks' gestation. The recordings were analyzed with pixelwise spatially angle-corrected volume flow measurements.*

Results *We observed variation in the umbilical vein volume flow in all fetuses, ranging from a mean minimum of 1.01 mL/s to a mean maximum of 2.60 mL/s. The minimum of all measurements was 57% compared with the mean value and the maximum was 148% of the mean value. The individual flow volume measurements ranged between 0.11 and 4.14 mL/s (mean, 1.76 mL/s). Within this range, an undulating course of all perfusion parameters was observed, with a full period of 4–5 min duration.*

Conclusion *Healthy second-trimester fetuses show cyclical variation in blood flow in the umbilical vein.*

INTRODUCTION

Sufficient fetal perfusion is a prerequisite for normal development of the fetus. Placental insufficiency is associated with compromised blood flow to the fetus. Measurement of blood flow is therefore crucial in fetal surveillance. During daily clinical practice, perfusion is assessed using velocities and indices (resistance index (RI) and pulsatility index (PI)) and not by calculating the blood flow volume. More relevant than arterial flow velocity changes is the total amount of blood transported through the umbilical vein towards the fetus. The PixelFlux technique allows such three-dimensional (3D) perfusion volume measurement in the umbilical vein[1]. The first report described its use to differentiate normal fetuses from those with intrauterine growth restriction (IUGR)[2]. The aim of this study was to investigate changes in umbilical vein flow volume during a 5-min observation period in healthy second-trimester fetuses.

METHODS

We examined 43 low-risk singleton pregnancies undergoing routine second-trimester ultrasound examination at Stavanger University Hospital in Norway from May to October 2013 (Table 1). Gestational age, determined according to the ultrasound measurements of biparietal diameter and femur length, was between 17 and 20 weeks. The fetuses did not show any signs of fetal distress or malformations, and all women were from a low-risk population with no known risk factor for compromised pregnancy. Maternal diseases were ruled out by medical history, clinical examination and appropriate laboratory investigations at the discretion of the responsible birth provider.

Perfusion measurements were carried out with the commercially available PixelFlux software (Chameleon Software, Munster, Germany; www.chameleon-software.de). 3D datasets were recorded on a Voluson E6 ultrasound scanner (GE Medical Systems, Zipf, Austria) using a 3.5–7.5-MHz curved multifrequency transabdominal transducer. The preset of the machine was kept constant in order to achieve comparable results. 3D recordings of the umbilical cord were obtained at 30-s intervals over a 5-min period. This interval was chosen in order to collect enough data for a vasomotion interval of about 4 min,

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Table 1 Maternal and delivery characteristics in study group of 43 singleton pregnancies

Characteristic	*Value*
Age (years)	29 (21–38)
Nulliparous	9 (21)
BMI (kg/m^2)	24 (16–31)
Smoking in pregnancy	3 (7)
GA at delivery (weeks)	40 (36–42)
Cesarean section	4 (9)
Operative vaginal delivery	4 (9)
Breech presentation	1 (2)
Intrauterine fetal death	1 (2)
5-min Apgar score	10 (0–10)
Birth weight (g)	3590 (2595–4625)

Data are given as median (range) or *n* (%). BMI, body mass index; GA, gestational age.

as was expected from preliminary observational studies (Figure 1).

These 3D datasets were then reviewed with the 4DView software (GE Medical Systems). Straight segments of the umbilical cord with a narrow angle towards the ultrasound propagation line were looked for, at which horizontal sections of the cord were made. The aim was to depict a section of the umbilical vein free of aliasing with a sharp outline. This image was then utilized for 3D perfusion measurements using the PixelFlux software. The images were calibrated by the software for distance and flow velocity, displayed as the color bar within the image. The umbilical vein was encircled and the 3D perfusion measurement was made within 1 s by the software.

The same site of measurement was chosen throughout the entire series whenever possible. If the fetus moved, a new examination site was chosen in a segment of the cord running as straight as possible towards the transducer. In some cases, the fetus turned upside down and the flow direction changed with respect to the transducer, showing a switch of coloration of the umbilical vein, from blue to red or vice versa. Minor deviations from the 30-s interval were assigned to a total of 11 examination time slots by separating the slots at 15 s after each prescribed 30-s interval. This means a measurement obtained between 15 and 45 s after the preceding exam was assigned to the next time slot, a measurement earlier than 15 s after the prior measurement was assigned to the same time slot and the mean value was calculated and used for timeline calculations of maximum and minimum flow.

Measurements of the vessel's horizontal area and the flow velocities were corrected for the angle of the vein relative to the ultrasound beam to obtain the mean flow volume. Measurement of the angle α of the umbilical vein with respect to the horizontal plane is therefore important in this method. A horizontal section of the vein determines the vessel's cross-sectional area stretched by the factor cosine^{-1} α. Multiplication of this area by each pixel's flow velocity depicted in the same area, which is equal to the flow velocity along the longitudinal axis of each pixel multiplied by the cosine α, allows the pixelwise calculation of the true flow volume.

All flow volumes were then expressed as percentages of the calculated mean flow volume (100%). Timelines were established to determine the number of minima and maxima during the 5-min period of examination.

Each fetus was measured over a period of 5 min. The time courses of all fetal measurements were arranged so that all minima of the individual time course were allocated to the central time slot. The overall observation for the entire population spanned 10 min, since some fetuses showed minimal flow in the first time slot and some in the last time slot. To establish a period, at least two maxima separated by one minimum were required. This comprised two turning points of the curve, at which the first derivation of the curve changes from a positive to a negative value (defining a maximum). If a full period was not captured under these premises, the period could be extrapolated if two turning points were identified, one in which the first derivative of the curve changes from a positive to a negative value (a maximum) and one in which the first derivative of the curve changes from a negative to a positive value (a minimum). The time interval from the maximum to the minimum was half of a full period. In this manner, longer periods than 5 min could be extrapolated.

Statistical analysis

A time course of all patients' data was established, separated for each patient, the 30-s time slot of the individual measurement, the flow volume and the umbilical venous trans-sectional area. The minimum value of the individual perfusion volume was determined. Then all of the time courses of all patients were arranged in a manner so that all minima were assigned to the central time slot (Figures 2 and 3). Thus an arrangement of perfusion data was achieved, from 5 min before to 5 min after the central time slot, defined by the individual minima in the central time slot. Subsequently the mean values for perfusion volumes and umbilical vein areas of all fetuses were calculated for each time slot and diagrams with trend lines showing the changes from one time slot to its neighboring one were calculated. The trend was calculated using a sixth-grade polynomial equation. The mean interval for the changes of all perfusion parameters was reported as a full period, measured from one maximum to the next maximum.

RESULTS

In total, 76% of all 465 measurements were recorded within an interval of ± 3 s from the prescribed 30-s interval (Figure 4). Twelve fetuses moved during the examination. We observed variation in umbilical vein volume flow in all fetuses, ranging from a mean minimum of 1.01 mL/s to a mean maximum of 2.60 mL/s. The minimum value of all measurements was 57% of the mean value and the maximum value was 148% of the mean value (mean, 1.76 mL/s). A wide variation in flow volume was found among all fetuses (range,

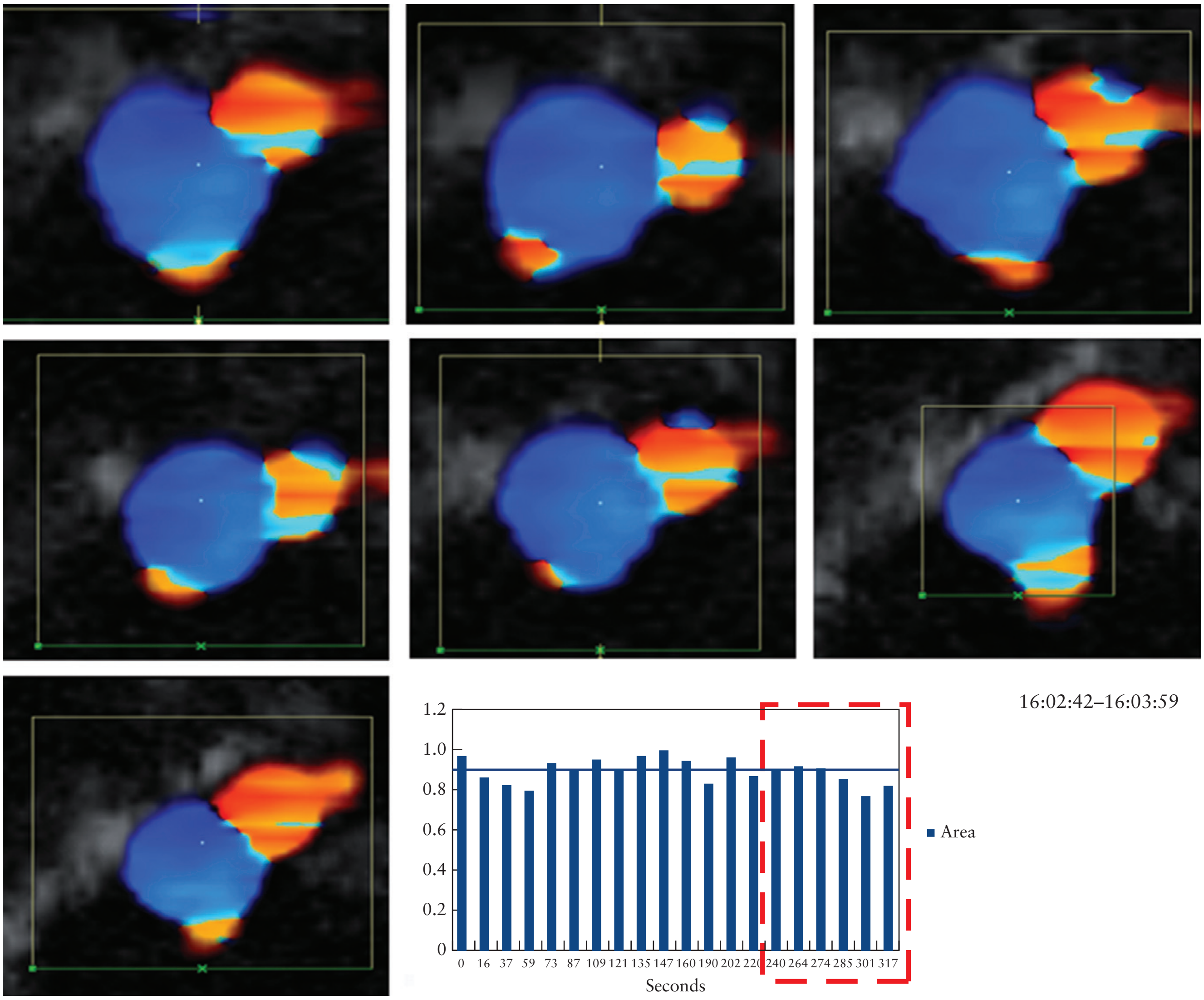

Figure 1 Examples of vasomotor changes to area of umbilical vein, in horizontal section, during an examination of 1 min 17 s. This period is highlighted within a longer measurement by the red frame in the graph (area in cm^2).

0.11–4.14 mL/s). In a single fetus, the ratio of maximum flow to median flow was 1.48 and the ratio of median flow to minimum flow was 1.74 (all ratios given as median of all fetuses). No correlation was found between the duration of the individual vasomotion period and the basic fetal and maternal clinical parameters at delivery (Table 2).

An undulating course of all perfusion parameters was observed within a full period, measured from first minimum value to the next minimum value, of 4.5 min duration (Figures 2 and 3). The correlation between flow volume and umbilical vein area was highly significant throughout the vasomotion period ($P < 0.001$ throughout). Changes in vessel area, as well as its flow velocity, contributed to the changing flow volume (Figure 3). The changes in vessel area were more pronounced than were velocity changes. Altogether they resulted in a clearly undulating time course of the flow volume (Figure 2).

DISCUSSION

We observed a variation in the venous umbilical blood flow volume over a 5-min time period, fluctuating from 57% to 148% of the mean value in 17–20-week fetuses. The range given is adapted to the absolute changes in each singleton fetus to make the data more meaningful. The wide overall variation in fetal measurements, from 0.11 to 4.14 mL/s (mean, 1.76 mL/s), may reflect actual states of metabolism and/or physical and mental activity. All fetuses, whether resting or moving, showed clear vasomotion of the umbilical vein. The vasomotion period in resting and moving fetuses differed by only 0.1 min. Interestingly, these changes did not occur stochastically, but occurred in intervals that lasted an average of the normalized time curves, 4.5 min. This points to an autochthonous, active process that probably has its origin in the umbilical vein itself.

Its significance is likely to be twofold: (1) in a preliminary study[2], we detected significantly reduced

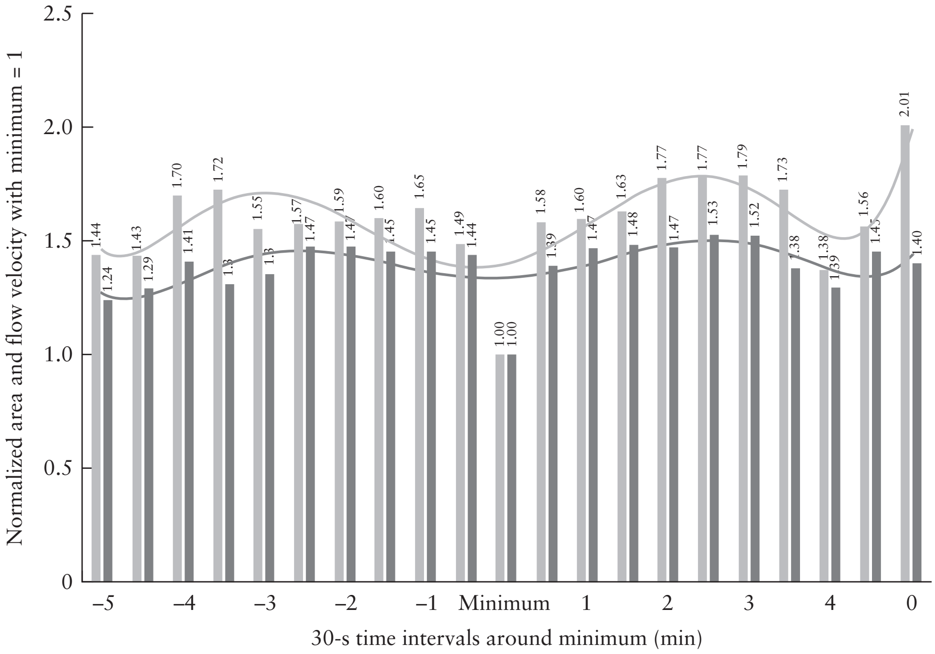

Figure 2 Time course of changes in umbilical venous area and flow velocity in 30-s intervals in 43 healthy second-trimester fetuses. All data refer to the individual minimum values during the examination and sixth-grade polynomial trends are given. Light gray bars and curve indicate area and polynomial (area), respectively; dark gray bars and curve indicate flow velocity and polynomial (flow velocity), respectively.

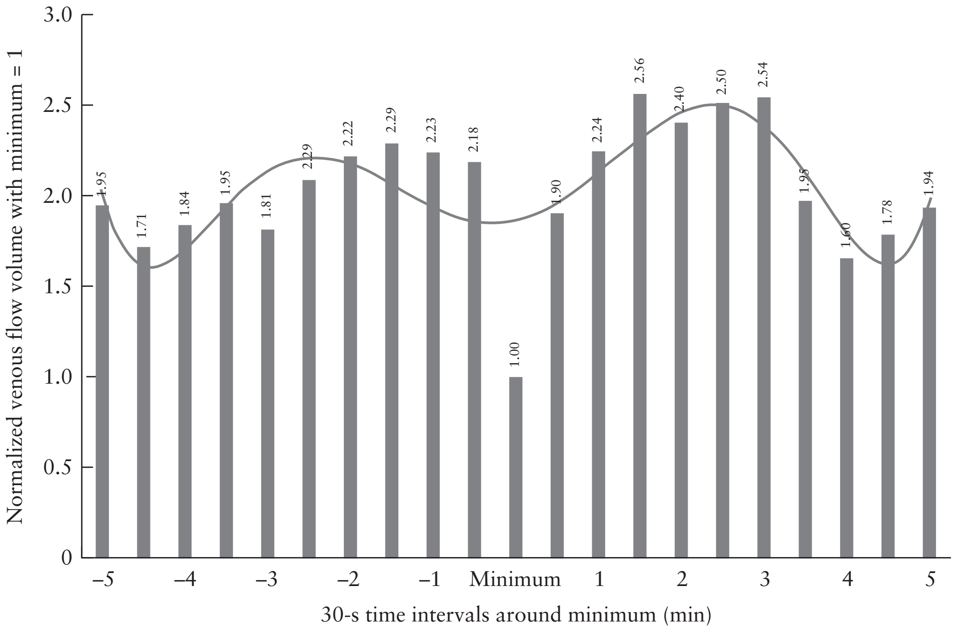

Figure 3 Time course of changes in umbilical venous flow volume in 30-s intervals in 43 healthy second-trimester fetuses. All data refer to the individual minimum values during the examination and a sixth-grade polynomial trend is given. The mean duration is 4.5 min. Bars indicate flow volume; curve indicates polynomial (flow volume).

global fetal perfusion per kg body weight in IUGR fetuses (141 mL/kg/min) compared with SGA fetuses (253 mL/kg/min) and normal weight fetuses (226 mL/kg/min); (2) it is important to show that measurement differences in a certain fetus at different time points may be a reflection of vasomotion and thus subject to change. The vasomotoric flow volume ranged from 0.11 to 4.14 mL/s, which corresponds to a range of 6.6–248.4 mL/min. This range spans values from a minimum flow to more than a 37-fold increase, in fetuses of 17–20 weeks' gestation, and thus may underscore the need to measure the fetal flow volume. In the singleton fetus, the range of flow volumes was distinctively less (57–148% of the individual mean). In a fetus of 17 weeks, the expected weight is about 0.3 kg. So far, only limited data from our own preliminary study[2] are available to estimate the actual fetal flow volume with respect to growth restriction but not to

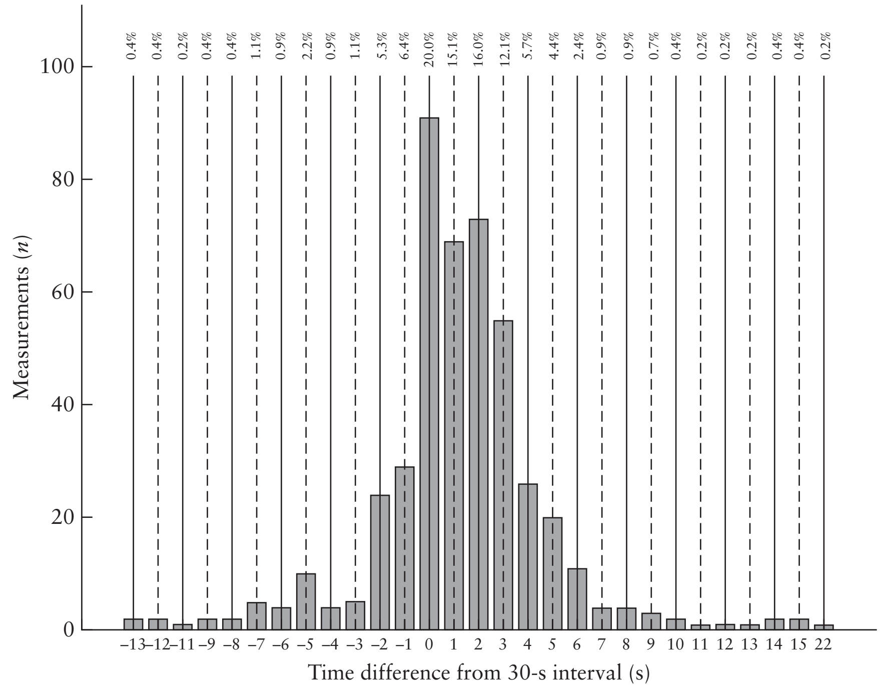

Figure 4 Distribution of measurement intervals, with respect to the prescribed 30-s interval, in 43 healthy second-trimester fetuses.

Table 2 Correlation between duration of visually determined individual vasomotion period (in minutes) of 43 healthy second-trimester fetuses and maternal or fetal characteristics at the time of delivery

Characteristic	r_s	P*	n
Parity	0.241	0.12	43
Maternal age in years	−0.060	0.701	43
Body mass index	0.077	0.623	43
Birth weight in g	−0.021	0.895	43
Umbilical artery pH	−0.033	0.872	27
Duration of pregnancy in days	0.153	0.328	43
Apgar score at 10 min	0.171	0.274	43
Apgar score at 1 min	0.164	0.294	43

*Two-sided. r_s, Spearman's rank correlation coefficient.

fetal age. Therefore a normally developing fetus has an average perfusion of 226 mL/kg/min which equates to about 68 mL/min in a fetus of 300 g. Vasomotion might change the mean flow volume by about one- to three-fold (57–148% of the individual mean) and might have importance for the actual and timely regulation of fetal blood supply, according to the metabolic needs, which might be changing rapidly due to movements and brain activity and, more gradually, due to growth.

The pixelwise evaluation of blood flow in a variety of tissues[3–7] and organs[8–13], in man and animals[14], has demonstrated highly significant correlation to relevant physiological (oxygenation[15]) and pathophysiological (histological changes in rejection[12], fibrosis[7] and inflammation[16] as well as diabetic changes of microperfusion[10]) parameters, and perfusion changes in the female genital tract[6,17]. 3D fetal perfusion volume measurements have the potential to describe fetal perfusion very precisely[1]. In contrast to traditional PI, RI and flow velocity measurements, the novel flow volume measurements reflect the fetal supply of blood more precisely since a certain quantity of blood is necessary to transport certain amounts of oxygen and nutrients. Spontaneous contractions observed *in vitro*[18,19] can be modified by pharmaceuticals or intrinsically produced substances[20–23]. In order to understand these mechanisms and to find an approach to their meaning *in vivo*, we conducted a study to monitor spontaneous changes in fetal volume perfusion in healthy fetuses. We found changing flow volumes in all 43 fetuses, with a mean period in the entire group of 4.5 min. Individually, the patterns of contraction varied and individual vasomotive periods ranged from 1.7 to 10 min. This wide range of vasomotion periods might be influenced by the measurement protocol. Refined results might be achieved in studies with longer observation times, narrower observation intervals and a larger group of fetuses. *In-vitro* observations of excised umbilical veins showed spontaneous contractions with an initial frequency of 1/min. Later on, these contractions became less frequent (every 3.3 min after 6–8 h). The rapidly decreasing numbers of umbilical rings investigated over time biased this observation[24]. Within 9 min, an average of two contractions could be observed, which corresponds to a contraction period of 270 s (4.5 min) or a contraction frequency of 0.22/min. This contrasts to the observations from Garcia-Huidobro *et al.* who found a spontaneous *in-vitro* vasomotion frequency of about 1.5/min[24]. The meaning of this vasomotion for fetal wellbeing needs to be elucidated by further research. It seems possible that this phenomenon is a mechanism to react vividly to changing metabolic needs of the fetus.

This hypothesis is supported by our findings. Nothing is known so far about the phenomenon, which we have studied *in vivo* for the first time in human fetuses. It is conceivable that the ability of the umbilical vein to contract within certain limits is a prerequisite for the normal adaptation of the growing fetus. Its disturbance might result in IUGR. IUGR might be a consequence not only of general low flow volume but also of a restricted amplitude of vasomotion, which cuts perfusion peaks in times of need. Fetal signal molecules may circulate within the arterial–placental–umbilical venous passage. A signal molecule emitted by the fetus may not cross the placental barriers and may thus lead to a more prompt reaction in the umbilical vein than would a maternal signal. Thus, changes of the umbilical vein flow volume caused by autonomous venous contractions could be an early reaction to changing fetal needs and not coupled directly to a change in maternal perfusion. Hypothetically, this could be imagined as an early warning system that might react faster than a more general response of the uterine perfusion. It could be initiated by local mediators and could mobilize the placental blood pool on the fetal side.

The PixelFlux method is the first technique to calculate true fetal flow volumes and thus might eventually provide new perspectives for understanding fetal adaptation to intrinsic and extrinsic influences on fetal growth.

DISCLOSURE

T.S. codeveloped the PixelFlux software and is a shareholder in Chamaleon Software GmbH.

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