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

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ABSTRACT

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

Methods *A prospective observational pilot study was performed at Stavanger University Hospital, Norway between May and October 2013. Serial three-dimensional ultrasound acquisitions from the umbilical vein every 30 seconds in 43 fetuses in 17th to 20th gestational week over a five minutes interval were analyzed with pixelwise spatially angle corrected volume flow measurements*

Results *We observed variation in flow in the umbilical vein volume 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 58% compared to the mean value, and the maximum was 145%. The individual flow volume measurements ranged between 0.11 ml/s and 4.14 ml/s (mean 1.76 ml/s). Within this range a swinging course of all perfusion parameters was observed with a full period of 4 to 5 minutes duration.*

Conclusion *In all fetuses we observed a cyclic variation in blood flow in the umbilical vein.*

INTRODUCTION

A sufficient fetal perfusion is the prerequisite for a normal development of the fetus. Placental insufficiency is associated with a compromised blood flow to the fetus. The measurement of the 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 would be the total amount of blood transported through the umbilical vein towards the fetus. The PixelFlux-technique allows such a three-dimensional perfusion volume measurement in the umbilical vein [1]. A first report described its use to differentiate normal from intrauterine growth restricted (IUGR) fetuses [2]. The aim of this study was to investigate eventual changes in umbilical vein flow volumes during a five minutes observation period in healthy second trimester fetuses.

METHODS

We examined 43 low risk single fetuses attending their routine second trimester ultrasound examination at Stavanger University Hospital in Norway from May to October 2013 (Table 1). The gestational age was between pregnancy week 17 and 20 according to the ultrasound measurement of biparietal diameter and femur length. The fetuses were without any signs of fetal distress or malformations, and all women were from a low risk population without any known risk factors for compromised pregnancies. Maternal diseases were ruled out by anamnesis, clinical exams 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, Germany: www.chameleon-software.de). 3D Datasets were recorded with a Voluson E6 ultrasound scanner (GE, Zipf, Austria), with a 3.5-7.5 MHz curved multifrequency transabdominal transducer. The preset of the machine was never changed in order to achieve comparable results. 3D recordings of the cord were taken in 30 second intervals over a five minutes period. This interval was chosen to collect enough data for a vasomotion interval of about four minutes as was expected from preliminary observational studies (Figure 1).

These 3D-data sets were then reviewed with the 4D View software (General Electric Company). Straight running segments of the umbilical cord with a narrow angle towards the ultrasound propagation line were searched for and here horizontal cuts of the cord were made. The aim was to depict a cut of the umbilical vein free of aliasing with a sharp outline. This image was then used for 3D perfusion measurements with the PixelFlux software. The images were calibrated by the software for distances and the flow velocities displayed by the color bar within the image. The umbilical vein was encircled and the 3D-perfusion measurement was made within one second by the software.

The same site of measurement was chosen throughout the entire series whenever possible. If the fetus moved around a new examination site was chosen in a segment of the cord running as straight towards the transducer as possible. In some cases the fetus turned upside down and the flow direction changed with respect to the transducer with a switch of the coloration of the umbilical vein from blue to red or vice versa. Minor deviations from the 30s interval were assigned to altogether 11 examination time slots by separating the slots at 15s after each prescribed 30s interval. This means, a measurement that took place 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.

All measurements of the vessel's area and the flow velocities were corrected according to the spatial angle of the vein with the ultrasound beam and the individual's mean flow volume was determined. The angle α of the umbilical vein towards the horizontal plane is important to this method. The images of the vein are analyzed in the horizontal plane. The venous flow is directed along the longitudinal axis of the winding umbilical vein.

A rectangular cut of the vein would determine the vessels area. The multiplication of this area by the flow velocity along the longitudinal axis would allow the calculation of the true flow volume. But in utero the angle α is unknown. Any angle α with the horizontal plane reduces the flow velocity by the factor of the cosine α and stretches the area of each pixel by the same factor. The PixelFlux-software calculates from images in the horizontal plane the true flow volumes, taking into account these mutually extinguishing factor cosine α.

All flow volumes were then referred to the calculated mean flow volume (100%) and expressed as percentages of this value. Time lines were established to determine the number of minima and maxima during the 5 minute-period of examination.

Each fetus was measured over five minutes. Then the time-courses of all fetal measurements were arranged in a way that all minima of the individual time-course were put in a central time slot. So the overall observation for the entire population span 10 min, since some fetuses hat their minimal flow in the first time slot and some had it in their last time slot. To establish a period, at least two maxima and one minimum, lying in between, are necessary. This requires two turning points of the curve, where the first derivation of the curves changes its sign from plus to minus – defining a maximum. If not a full period was captured under these premises the period could be extrapolated if two turning points were identified, one where the first derivative of the curve changes sign from plus to minus (a maximum) and one where the first derivative of the curve changes sign from minus to plus (a minimum). The time interval from the maximum to the minimum was half the full period. This way longer periods than five minutes could be extrapolated.

Statistical analyses

A time course of all patients' data was established separated for each patient, the 30s-time slot of the individual measurement, the flow volume and the umbilical venous transsectional 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 that all minima were assigned to the central time slot (fig. 2 and 3). Thus an arrangement of perfusion data was achieved reaching from 5 minutes before to 5 minutes after the central time slot, defined by the individual minima in the central time slot. Then the mean values for perfusion volumes and umbilical vein areas of all fetuses were calculated for each time slot and diagrams with trend lines for the changes from one timeslot to its neighboring one were calculated. The trend was calculated with a 6th-grade polynomial equation. The mean interval for the changes of all perfusion parameters was read out as a full period from one maximum to the next.

RESULTS

In all, 75.9% of all 465 measurements were within an interval of ± 3 s from the prescribed 30s interval (Figure 2). 12 fetuses moved during the exam. We observed variation in flow in the umbilical vein volume 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 58% compared to the mean value, and the maximum was 145%. (Mean 1.76 ml/s). A wide variation was found among all fetuses (0.11 to 4.14 ml/s). In a single fetus the ratio of maximum flow to median flow was 1.45 and the ratio of median to minimum flow was 1.72 (all ratios given as median of all fetuses). No correlation of the duration of the individual vasomotoric period with basic fetal and maternal clinical parameters could be found (Table 2).

Within this range a swinging course of all perfusion parameters was observed with a full period from minimum to the next minimum of 4.5 minute duration (Figure 3 and 4). The correlation between flow volumes and umbilical vein areas was highly significant throughout (p always < 0,001). Changes of the vessel's area as well as its flow velocity contributed to the changing flow volumes (fig. 3). The changes of the area were more pronounced than the velocity changes. Altogether they resulted in a clearly swinging time course of the flow volumes (Figure 4).

DISCUSSION

We observed a variation in the venous umbilical blood flow volume over a five minutes time period swinging from 58 to 145 % of the mean value in fetuses from 17^{th} to 20^{th} gestational week. The range given is adapted to the absolute changes in each single fetus to make the data more meaningful. The wide overall variations in all fetal measurements from 0.11 ml/s to 4.14 ml/s (mean 1.76 ml/s) may reflect actual states of metabolism and / or physical and mental activity. All fetuses, resting and moving ones, showed a clear vasomotion of the umbilical vein. The vasomotion period in resting and moving fetuses differed by only 0.1 min.

Interestingly, these changes occurred not stochastically, but in intervals that lasted in the average of the normalized time-curves 4.5 minutes. This points to an autochthonous, active process; which eventually has its origin in the umbilical vein itself.

Its significance might be twofold:

1. In a preliminary study [2] we detected a significantly reduced global fetal perfusion per gram body weight in IUGR fetuses (141 ml/kg*min) compared to SGA fetuses (253 ml/kg*min) and normal weighted 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. This vasomotoric volume flow change ranged from 0.11 to 4.14 ml/s = 6.6 – 248 ml/min. This range spans values from a minimum flow to its more than 37-fold increase in fetuses from the 17th to the 20th week and thus may underscore the need to measure the fetal volume flow. In the single fetus the range of flow volumes was distinctively less (58 to 145 % of the individual mean). In a fetus of 17 weeks the expected fetal weight is about 0.3 kg. So far, only limited data from an own preliminary study[2] are available to estimate the actual fetal volume flow with respect to growth restriction but not to fetal age. A normally developing fetus thus has an average normal perfusion of 226 ml/min*kg which would refer to about 68 ml/min in a fetus of 300 g, if such an extrapolation would be ventured. Vasomotion might change this mean flow volume in a range of about 1 to 3 (58 to 145 % 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 parameters (histological changes in rejection[12], fibrosis[7] and inflammation[16] as well as diabetic changes of microperfusion[10]) , also in the female genital tract[6, 17]. The three-dimensional 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 volume flow measurements reflect the fetal supply with 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 of fetal volume perfusion in healthy fetuses. We found changing flow volumes in all 43 fetuses with a mean frequency in the entire group of 4.5 minutes. Individually the patterns of contractions 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 in these contractions became less frequent (3.3 min after 6-8 hours). The rapidly decreasing numbers of umbilical rings investigated over time biased this observation[24]. Within 9 minutes in average 2 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 who found a spontaneous in vitro vasomotion frequency of about 1.5/ min[24]. The meaning of this vasomotion for the fetal well-being 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 is studied here for the first time in human fetuses in vivo. 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 intrauterine growth restriction. IUGR might not only be a consequence of general low flow volumes 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 a maternal signal. Thus, changes of the umbilical vein flow volume caused by autonomous venous contractions could be a forefront reaction to changing fetal needs and not coupled directly to a change of the 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 placenta blood pool on its fetal side. The PixelFlux-method is the first technique to calculate true fetal volume flows and thus might eventually open up new perspectives for the understanding of fetal phenomena.

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Conflict of Interest: Thomas Scholbach co-developed the PixelFlux-software and is shareholder of Chamaleon-Software GmbH

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Table 1: Maternal and labour characteristics

Maternal characteristics	Median (range) or n
Maternal age (years)	29 (21-38)
Nulliparous (%)	21
BMI	24 (16-31)
Gestational age at delivery (weeks)	40 (36-42)
Smoking in pregnancy	3
Labour characteristics	
Caesarean section	4
Operative vaginal delivery	4
Breech	1
Intrauterine fetal death	1
Apgar score after 5 minutes	10 (0-10)
Birth weight (grams)	3590 (2595-4625)

Table 2: No significant correlation was found between vasomotion period and maternal or fetal features at delivery

	parity	maternal age [years]	BMI	Birth weight [g]	Umbilical artery ph	Duration of pregnancy [d]	Mean Agpgar score 10 min	Apgar score 1. minute
Spearman rank correlation coefficient of the visually determined individual vasomotor period [min]	,241	-,060	,077	-,021	-,033	,153	,171	,164
Significance (2-sided)	,120	,701	,623	,895	,872	,328	,274	,294
N	43	43	43	43	27	43	43	43

Legends

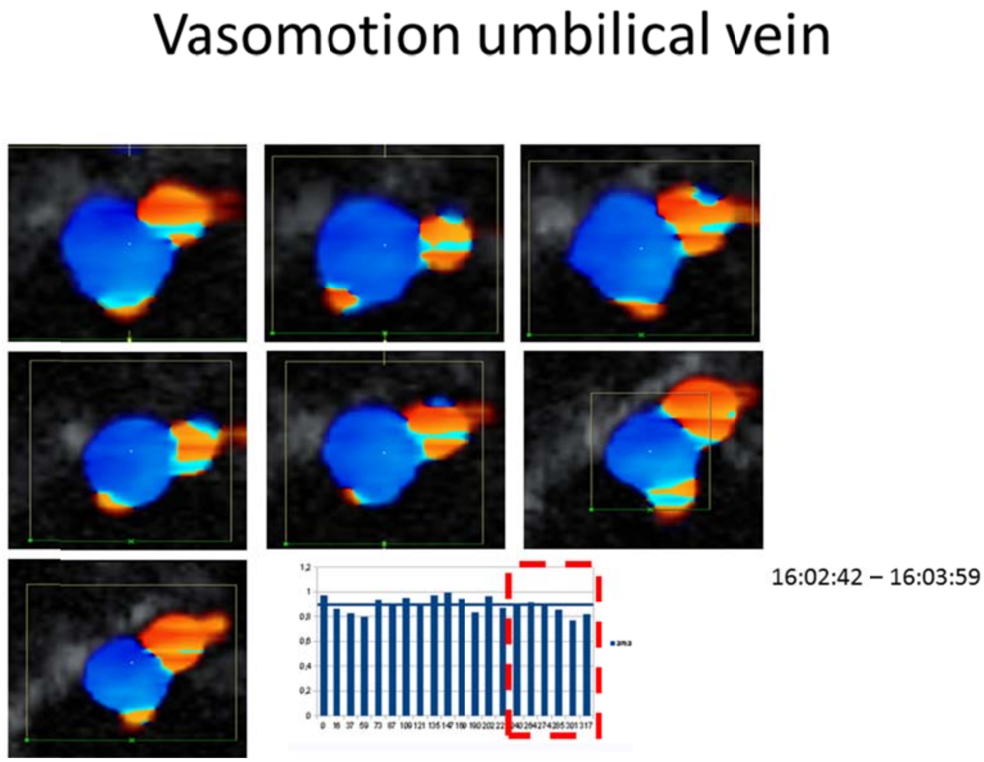

Figure 1: Example of the vasomotoric changes of the area of the umbilical vein in horizontal cuts during an examination time of 1:17 min. This period is highlighted within a longer measurement by a red frame in the diagram below (area in cm^2).

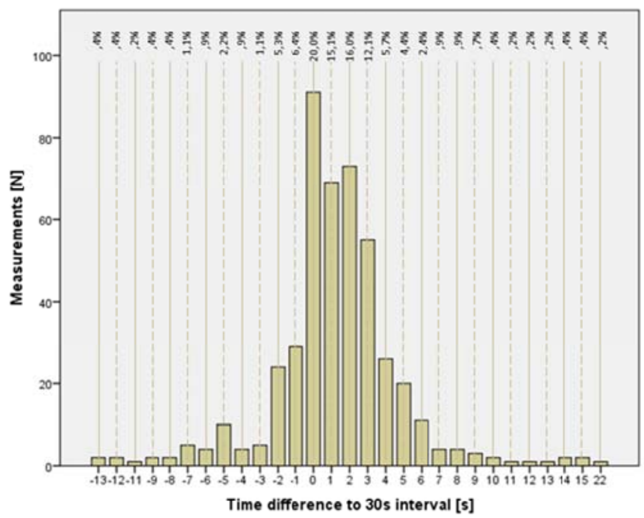

Figure 2: Distribution of measurement intervals with respect to the prescribed 30s interval

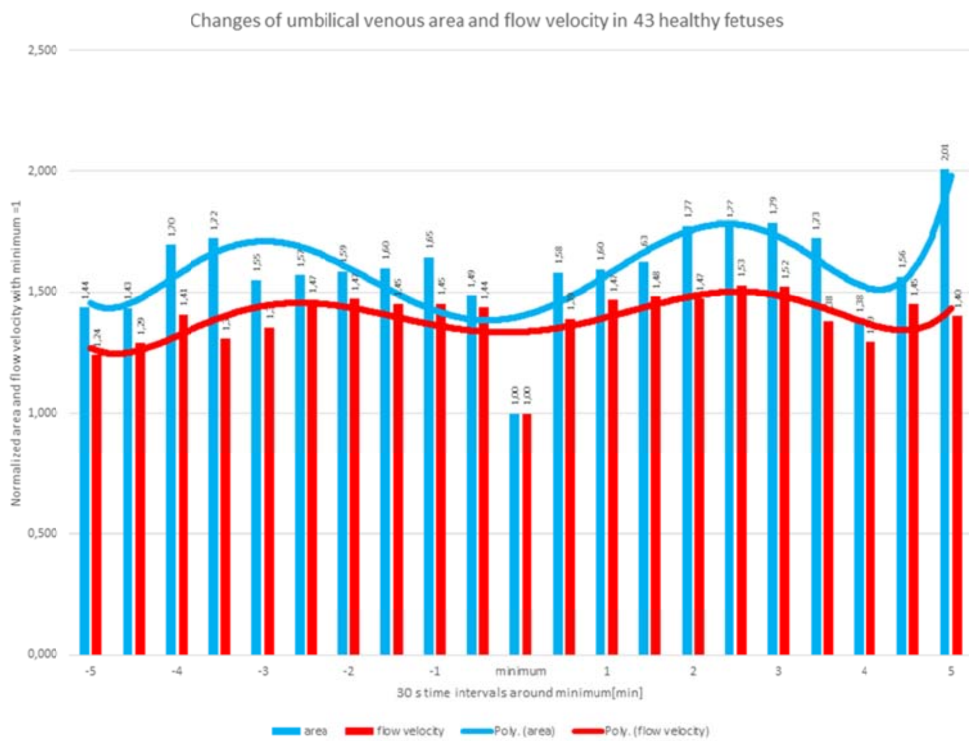

Figure 3: Time course of changes in umbilical venous area and flow velocity in 43 fetuses in 30-seconds-intervals. All data are referred to the individual minimum values during the examination, the 6th-grade polynomical trends are given.

Figure 4: Time course of changes in umbilical venous flow volume in 43 fetuses in 30-seconds-intervals. All data are referred to the individual minimum values during the examination, the 6th-grade polynomical trend is given. The mean period's duration is 4.5 minutes.