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DOPPLER STUDY OF THE PERIPHERAL FLOWS IN EARLY GESTATION

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The aim of this study was to determine the first trimester human peripheral arterial and venous blood flow between 5 - 10 weeks of gestation. Two hundred twenty four women with singleton, uncomplicated pregnancies were prospectively studied with transvaginal ultrasound. Ductus venosus, umbilical artery waveforms and pulsatility indexes (PI) were assessed as well as the waveform of the umbilical vein and the mean velocity (V_{mean}) of the umbilical artery flow. The heart rate was also obtained and analyzed. The fetal heart rate showed a positive correlation with increasing gestational age $R=0.76$ ($p<0.000001$). Recordings from the umbilical artery, umbilical vein and ductus venosus were obtained starting from 7 weeks of gestation. The signal from the ductus venosus presented always as antegrade flow during atrial contractions. The pulsatility index (PI) of DV as well as PI of the umbilical artery remained unchanged during the study (statistically non-significant). The umbilical artery, using Doppler tracing was investigated and an absent diastolic flow was documented in every case. Umbilical artery V_{mean} increased from 3.8 ± 0.32 cm/s to 9.0 ± 0.21 cm/s from 7 to 10 weeks of gestation ($p < 0.005$). Recordings from the umbilical vein showed the pulsation during atrial contractions. Ductus venosus blood velocity and waveform patterns did not change significantly during the study period. Pulsation in the umbilical vein is a typical Doppler finding at the embryonic time. Placental volume blood flow increased significantly with no change in the placental vascular impedance.

Key words: *embryo, first trimester, peripheral flows, hemodynamics, umbilical artery flow*

INTRODUCTION

In human embryos during the 4th and 5th week, there are three important circulations: the intraembryonic, yolk sac and the umbilical (embryo-chorionic) circulation. The umbilical circulation supplies embryonic blood to the chorionic villi by way of two umbilical arteries located in the connecting stalk. Umbilical arteries connect the descending aorta with the capillary nets of the chorionic villi. The blood coming from the chorion, which contains oxygen and nutritive substances that have diffused, or been reabsorbed from the maternal blood through trophoblast and endothelium, returns by way of the two umbilical veins. After the asymmetric development of the liver and heart, the right umbilical vein regresses. The left umbilical vein becomes the definitive umbilical vein. The vein forms a large anastomosis with the portal vein and runs as a large canal, venous duct and it opens into the inferior vena cava. Recent technological improvements in Doppler ultrasonography allow us to evaluate the physiological changes in the fetoplacental blood circulation during the time of organogenesis (1-8).

The aim of this longitudinal study was to determine the first trimester human peripheral arterial and venous blood flow and fetal heart rate between 5 - 10 weeks of gestation. Furthermore, we attempted to determine retrospectively any abnormal Doppler signals in pregnancies that later miscarried.

MATERIALS AND METHODS

Two hundred twenty four women with singleton, uncomplicated pregnancies were prospectively studied with transvaginal ultrasound. Maternal age ranged from 18 - 40 years, with median age of 26 years. Participation in the study was voluntary. The institutional review board approved the study. All pregnant women were asked to sign an informed consent prior to the examination, and two examiners performed all the examinations. Inclusion criteria: Women between the years of 18-40 with a positive urine pregnancy test and a single pregnancy at the 5th-10th week of gestation assessed by using the last menstrual period (LMP) were included. Exclusion criteria: Women with any known medical or surgical illness, previous spontaneous abortions, stillbirths or first trimester complications like vaginal bleeding were excluded from the study. All ultrasound examinations were performed at the Ultrasound Laboratory of the hospital in Ruda Slaska in Department of Obstetrics and Gynecology. Crown-rump length was recorded to determine the timing of the pregnancy as accurately as possible. Ultrasound examinations on study subjects were performed at the time of entering the study and every two weeks thereafter up to a maximum of three examinations or 10 weeks of gestation, whichever was earlier. Equipment: Ultrasound examination was performed using two-dimensional and color-coded Doppler to direct pulsed Doppler using a Voluson Expert 730. A 5.0 MHz transvaginal probe was used. The mechanical and thermal indexes were displayed continuously on the screen and kept at or below <0.7 and <0.5, respectively. The Ductus venosus and umbilical artery waveforms and pulsatility indexes (PI) were assessed as well as the waveform of the umbilical vein and V_{mean} of the umbilical artery flow. The heart rate was also obtained and analyzed. The subsequent control obstetric USG examinations were established for all survivors at the gestational age of 11-14

weeks, 20 weeks and 30 weeks. All Doppler studies and real-time images were recorded on DVDs and hard disk for further analysis. The time of patients entering into the study was divided into 5 groups: 6 weeks collecting patients from 5.1-6.0 weeks, 7 weeks (6.1-7.0), 8 weeks (7.1-8.0), 9 weeks (8.1-9.0), and 10 weeks (9.1-10.0). Data from the neonatal period including: the mode and time of delivery, neonatal weight and Apgar score were collected and analyzed. The possible risks that may be due to Doppler are directly related to the acoustic intensity used. According to current consensus in the scientific community, the principle of As Low As Reasonably Achievable (ALARA) should be applied to the use of Doppler for research in the first trimester of pregnancy and we adhered to this practice (8-12).

Statistical analysis

For study analysis we used the statistical tests as follows; ANOVA test with "post hoc" Tukey's test, Spearman's Rank Correlation Test. Accuracy of adjustment of the parabolic regression to study results was determined with the use of a regression factor. A level of $p < 0.05$ was considered significant. All obtained values are given as means, standard deviations (SD) and medians.

RESULTS

A total of 358 examinations were performed on 224 pregnancies. Seventeen (7.5%) pregnancies miscarried - thirteen during the time of organogenesis, and four after finishing this period. Data from the miscarried embryos were evaluated separately. 85% of studies were successful and the Doppler signal was adequate at the first examination. In all cases entering the study at 6 weeks, we could not obtain the diagnostic Doppler signal from the fetoplacental vessels at that time but they were examined successfully one week later. We were able to measure the fetal heart rate using the M-mode technique at 6 weeks and to use the Doppler technique at 7 weeks, placing the Doppler gate in the intraembryonic circulation but not in the embryochorionic circulation (it was not seen in the 2D view). Finally, 224 pregnancies within 7-10 weeks of gestation with complete Doppler data were qualified for subsequent analysis. The fetal heart rate showed a positive correlation with increasing gestational age - $R=0.76$ ($p < 0.000001$). Mean fetal heart rate was 131 ± 16 beats/min at 7 weeks and 167 ± 7 beats/min at 10 weeks of gestation ($p < 0.00001$). Recordings from the umbilical artery, vein and ductus venosus were obtained starting from 7 weeks of gestation. The first signal from the ductus venosus was obtained at 7 weeks of gestation and it presented always as antegrade flow during atrial contractions in all cases. The pulsatility index (PI) of the DV remained unchanged during the study and varied from 0.9 ± 0.22 at the 7th week to 0.87 ± 0.14 at the 10th (*Table 1, Fig. 1*). The umbilical artery Doppler tracing was investigated during the study and absent diastolic flow was documented in every case. The pulsatility index of the umbilical artery (PI) remained unchanged and varied from 1.38 ± 0.28 at 7 weeks to 1.55 ± 0.53 at 10 weeks (statistically non-significant) (*Table 1, Fig. 2*). Umbilical

Table 1. Results of examined parameters and correlation with gestational age in weeks [obtained values are given as mean and standard deviation]

Parameter	Gestational weeks				Statistical test Test ANOVA
	7	8	9	10	
CRL mm	7.60±2.01	12.96±2.36	19.50±2.42	26.77±2.66	p<0.000001
FHR - bpm	131±16	156±14	169±8	172±6	p<0.000001
UMBA (PI)	1.38±0.28	1.37±0.37	1.39±0.29	1.55±0.53	NS (p=0.42)
UMB Vmean	6.0±3.4	8.5±3.3	9.4±1.4	9.0±1.7	P<0.005
DV (PI)	0.907±0.22	0.935±0.29	0.876±0.22	0.870±0.14	NS (p=0.72)

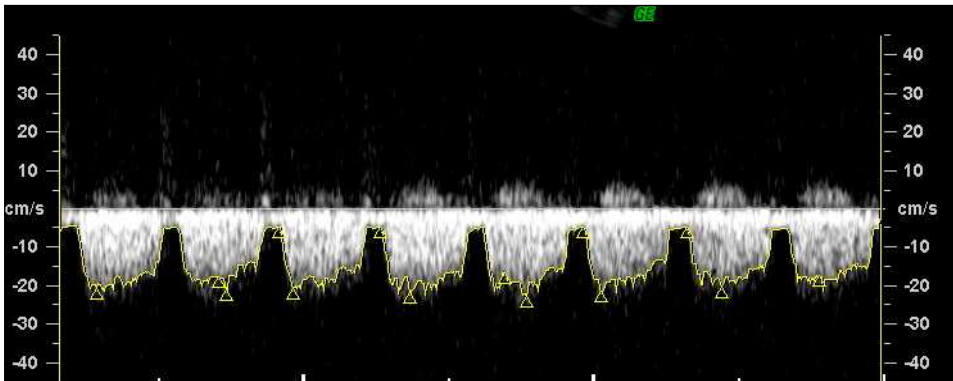


Fig. 1. The Ductus venosus Doppler tracing and measurement of pulsatility index (PI).

artery V_{mean} increased from $3.8 + 0.32$ cm/s to $9.0 + 0.21$ cm/s from 7 to 10 weeks of gestation ($p < 0.005$). Recording from the umbilical vein was obtained from 7 weeks and showed pulsations during atrial contractions that continued to be present at 10.

In the group of nonsurvivors the fetal heart rate was abnormal in 9 cases (53%). Significant bradycardia occurred in 7 cases and tachycardia in 2. In nonsurvivors that were miscarried prior to 7th week (13 cases - 76%) we could not analyze the peripheral flows, in 4 miscarried cases that were above 7th week (4 cases - 24%) we didn't notice any changes in flows when compared to the survivors at the same gestational weeks. Due to the small number of miscarried cases at different weeks of gestation we could not use any statistical tests to show the significance of that observation.

We checked the fetal anatomy during the obstetric ultrasound examination at 11-14, 20 and 30 weeks of gestation. Doppler assessment of the peripheral circulation was performed in all cases (DV, umbilical vein and umbilical artery). Fetal echocardiography examinations were performed routinely at 20 weeks of

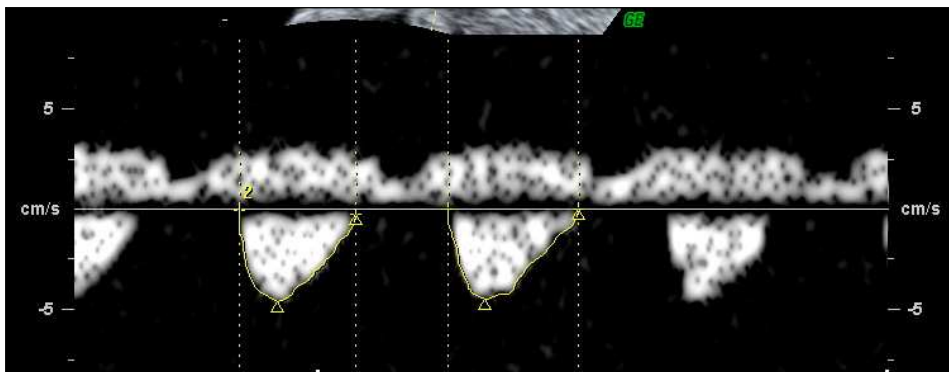


Fig. 2. The umbilical artery Doppler tracing and measurement of pulsatility index (PI).

gestation and at this period we found fetal anomalies in 5 cases: heart defect (mild aortic valvular stenosis) in one case, gastroschisis in one case, megacystis in one case, renal agenesis with ahydramnion in one case and IUGR with abnormal Dopplers and features of circulatory centralization in one case, which was found at 20 weeks, and the peripheral flows at 8 and 13 weeks was normal. In 65% of cases, neonates were delivered vaginally, and in 35% cesarean sections were performed. The mean neonatal weight was $3287\text{g} + 322\text{g}$ and the median Apgar score at 5 minute was 9.

DISCUSSION

The results of the present study demonstrated the characteristics of investigation of the embryochorionic circulation early in the first trimester in low risk pregnancies. The detection rate of the blood flow in the umbilical vessels and ductus venosus increased above 7 weeks of gestation. Prior to this time, circulatory flow was not possible to be assessed. According to earlier observations on embryonic anatomy, the yolk sac, which is present until the end of the first trimester is covered by a vascular network leading to paired vitelline arteries and veins (1-3). Before the vitelline veins enter the sinus venosus of the embryo, they form hepatic sinusoids and, ultimately, the hepatocardiac portion of the inferior vena cava. Vitelline arteries, on the other hand, are connected to the dorsal aorta and they ultimately form the celiac, superior mesenteric and inferior mesenteric arteries. The connecting stalk also includes umbilical vessels at that time. Initially, the umbilical veins pass on each side of the liver, with some becoming connected to the hepatic sinusoids. From the 8th to the 12th weeks of pregnancy, the proximal parts of both umbilical veins and the remainder of the right umbilical vein disappears, so that the left umbilical vein is the only one to carry blood from the placenta to the liver. With the increase in placental

circulation, a direct communication is formed between the left umbilical vein and the right hepatocardiac channel, the ductus venosus (3-5).

According to the studies of Makkikallio *et al.* (14) it appears that the blood circulation in the yolk sac gradually stops after the proper functioning of the ductus venosus begins. Makkikallio *et al.* (16) showed in their work, the total disappearance of the arterial yolk sac blood circulation by 10+ weeks of gestation and a simultaneous increase in the umbilicoplacental blood flow. Our study supports these earlier embryological studies. However, yolk sac blood circulation was not a point of interest in our study (11-13). An absence of diastolic flow in the umbilical artery was seen throughout the study period, as in previous studies (5, 11-13). The maximum velocity (V_{max}) in the umbilical artery increased in our study from 7 to 10 weeks, as well as the V_{mean} values. The rise in V_{max} and V_{mean} suggests that the volume of blood flow in the umbilical artery increases. In contrast, however, the PI values remained unchanged. These findings demonstrate that, between 7 and 10 weeks of gestation, the volume of blood flow in the umbilicoplacental circulation increases significantly, probably reflecting an increased fetal cardiac output with no changes in the placental vascular impedance. Similar observations were presented by Makkikallio *et al.* (14, 16).

Ductus venosus blood velocity waveforms already had an antegrade flow during atrial contraction during the first trimester of pregnancy. This finding is in agreement with earlier studies that have shown that the blood velocity pattern is reversed or absent during atrial contraction in only 1-2% of the normal human fetuses in early pregnancies. Ductus venosus PIs in this study were comparable with those reported by Antolin *et al.* and others (14-17, 21). Fetal heart rate was possible to obtain from the 6th week, using intracardiac measurements but we analyzed only cases that were included in the study starting from the 7th week. The fetal heart rate showed the positive correlation with increasing gestational age, a finding that is similar to observations in other studies (11-13).

In the group of non-survivors miscarried after finishing the 7th week, we didn't find any abnormal Doppler flows in the peripheral circulation. Otherwise the fetal heart rate was abnormal in 53% of cases in this group. That observation was also described by Benson (22).

Our study shows the difference in placental physiology between the early first and second trimester. Normal findings were noted in the embryonic period such as pulsation in the umbilical vein, or absent end diastolic flow in the umbilical artery being an abnormal indicator in the second trimester.

Peripheral Doppler flows are a part of our cardiovascular profile score, known as a useful, practical scale for the assessment of the fetal circulation (23-25) in the second and third trimesters. Qualitative analysis of the blood velocity profile in the second trimester may indicate fetal compromise. For example, the appearance of pulsations in the UV flow is known to be associated with circulatory compromise. This cannot be extrapolated to the umbilical venous

circulation in the first trimester, because of the different haemodynamics at this time. For example, the physical distance between the heart and the umbilical vein is markedly shorter. Similarly, reduced, absent or reversed diastolic flow in the UA is associated with increased placental vascular impedance in the second and third trimester circulation (19, 20). Absent diastolic flow is a consistently normal feature of the UA waveform for much of the first trimester. Atrial venous pulsations are transmitted backwards along the central veins acting as transmission lines and the pressure changes transmitted along the central veins reflects the pressure amplitude of the atrial contraction. Under physiological conditions during the second half of pregnancy, these pulsations do not reach the UV to cause visible velocity variation due to the reflections at the DV junction and damping in the wide reservoir-like UV. Thus the flow in the UV is continuous. Pulsations are seen in early pregnancy, which is ascribed, in part, to the small venous diameter and reduced compliance promoting the transformation of the pressure wave to the velocity pulsation (20-22). This is the major event separating the first and second trimesters is the trophoblast invasion in the placenta. The resulting lowering of the relative placental resistance appears to dramatically alter the loading conditions of the fetal ventricles. This combined with the improvement in the diastolic function of the fetal ventricles results in the biphasic inflow and improved early ventricular filling (12). How these cardiac changes induce the changes in the peripheral circulation is not well understood currently. Nonetheless, the differences in the blood pressure between week 7 and week 20 (1 versus 20 mmHg), the difference in hematocrit, viscosity, arterial and venous compliance and many other factors dictate the need for a flexible physiological approach to the first trimester circulation.

In summary, ductus venosus blood velocity and waveform patterns did not change significantly during the study period. Atrial pulsations in the umbilical vein were typical Doppler finding at this early embryonic period. Placental volume blood flow increased significantly with no change in the placental vascular impedance. Abnormal heart rhythm was a frequent sign of poor prognosis in embryonic period.

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Conflict of interest statement: None declared.

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Doppler ultrasound evaluation of the fetoplacental circulation is not indicated in low-risk pregnancies 7. Maternal conditions. diabetes mellitus. The spectral Doppler indices measured at the fetal end, the free loop, and the placental end of the umbilical cord are different with the impedance highest at the fetal end. The changes in the indices are likely to be seen at the fetal end first. Ideally, the measurements should be made in the free cord, however, for consistency of recording in cases being followed up, a fixed site would be more appropriate, i.e. fetal end, placental end, or intra-abdominal portion. Due to difficulty with measuring the cord at the fetal end in many growth-restricted fetuses, measurement in a free loop is accepted. As Doppler techniques enable one to study flow velocity waveforms in a non-invasive way, it became one of the most ideal methods to analyze the maternal and fetal circulations and in particular that of the uterine and umbilical arteries. Although Satomura [86] described the feasibility of Doppler ultrasound to determine flow velocity in a peripheral artery, it took almost 20 years until the next important development in this field, when Fitzgerald and Drumm [34] used Doppler ultrasonography to investigate the human fetal circulation. Recently, a one-stage screening for pregnancy complications by color Doppler assessment at 23 weeks' gestation was introduced [4]. A mean PI of more than 1.45 was considered increased. Bilateral uterine artery notches were also noted. study of the reproductive system hemodynamics in large animals. This technic is based in the Doppler Effect Principle that. proposes the change in frequency of a wave for an observer (red blood cells) moving relative to the source of the respective. to weak blood flow and independent of the Doppler angle, improving the evaluation of vessels with small diameters and slow. blood flow. Conclusion: Doppler ultrasonography principles, methods of evaluation and reproductive system anatomy have been described. Uterine vascular perfusion and spectral-Doppler measurements during early gestation in mares: New concepts of evaluation. *Animal Reproduction Science*. 121(S): 281-283.