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Assessment of Placental Vascularization by Three-dimensional Power Doppler "Vascular Biopsy" in Normal Pregnancies

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Aim To describe the evolution of placental vascularization during a normal course of gestation and the blood flow 3D power Doppler indices obtained by "placental vascular biopsy."

Methods A prospective study was carried out on 99 normal singleton pregnancies from 14 to 40 weeks. Placental vascularization was evaluated by 3D power Doppler ("placental biopsy"). The spherical volume acquired was analyzed using the VOCAL™ imaging program (Virtual Organ Computer-aided AnaLysis). Three vascular indices, vascularization index (VI), flow index (FI), and vascularization-flow index (VFI), were calculated. Equations and regression coefficients for placental volume and vascular indices (VI, FI, VFI) of the placental biopsy were calculated according to gestational age. Relationships between 3D placental flow indices and fetal growth parameters: biparietal diameter, head circumference, abdominal circumference, femur length, estimated fetal body weight, maximum systolic velocity (US), and resistance index in the umbilical artery (URI) were evaluated by calculating their correlation coefficients.

Results All 3D Doppler indices had a significant relationship with gestational age. The most significant relationship was observed for FI, and the least significant for VI ($r=0.58$, $r=0.29$, respectively; $P<0.01$ for both). The FI increased linearly with gestation, whereas the VI showed a dispersion of values with a plateau from the 30th week onwards and a decrease from 37th week to the end of pregnancy. The VFI behaved as a combination of both VI and FI indices from which it was derived. All 3D Doppler indices were significantly related to fetal biometric parameters, except VI and fetal weight. A significant correlation was observed between 3D Doppler indices and maximum systolic velocity and URI.

Conclusions 3D power Doppler technique of placental vascular "biopsy" is an appropriate tool for routine evaluation of the human placental vascular tree during gestation. 3D Doppler indices change as pregnancy progresses and are significantly related with fetal biometry and umbilical artery Doppler velocimetry.

The recent advent of three-dimensional (3D) power Doppler has allowed a structural study of the vascular net and blood flow calculation in different organs and body regions. Until now, vascularization and blood flow of the ovary (1-4), ovarian follicles (5), endometrium (6,7), and ovarian tumors (8-10) have been evaluated.

Using 3D power Doppler, Hafner et al (11) studied the chorionic vascular tree during the first trimester of pregnancy, although they did not distinguish between placental and non-placental vascularization. Pretorius et al (12) and Matijević and Kurjak (13) investigated intraplacental vascular morphology in the third trimester and confirmed that this method showed a high sensitivity for visualization of the second and third order villous branches (13). Recently, Yu et al (14) used this technique to show that the fractional blood volume through the placenta correlates with gestational age and fetal growth indices.

Except during the first trimester of pregnancy (11), 3D power Doppler cannot explore the placental vascular tree as a whole (12-14). To avoid this difficulty, we have developed a method called "placental vascular biopsy," and confirmed its clinical reproducibility (15,16).

Developing a technique that allows placental vascularization study is of great interest for two reasons. First, it would allow visualization of an abnormal and deficient development of the placental vascular tree, which is strongly associated to fetal growth alterations (17,18). Second, intraplacental blood flow reduction could appear earlier than umbilical resistance increase (19,20) to detect this condition.

The aim of the present study was to describe the normal evolution of the vascularization during gestation and the blood flow 3D power Doppler indices obtained by "placental vascular biopsy." This initial step is mandatory for the later clinical application of this method in diagnosing and management of abnormal pregnancies.

Material and Methods

Patients

Ninety nine normal pregnancies have been prospectively evaluated from January to December 2003: 31 primiparae (31%) and 68 multiparae (69%), aged 23 to 44 years. Examinations were performed from 14 weeks and 4 days to 40 weeks and 2 days of pregnancy. None of the patients had any gynecologic or obstetric complications in their medical history and they all had a normal course of pregnancy. None of them had previously had illnesses, such as hypertension, diabetes mellitus, cardiovascular problems, or any other. Thirteen patients were excluded from the study for the following reasons: a spontaneous miscarriage (1 case); bilateral hydronephrosis of the fetus (1 case); pregnancy-associated hypertension (2 cases); growth restriction confirmed postnatally (3 fetuses); too many artefacts in the color Doppler signal by the placental volume, unsuitable for the study (6 cases).

Weight increase, blood pressure, serial biometrics, and monthly ultrasonography-Doppler scanning showed normal results in all 86 women. Also, the selected women did not have any medical complications, such as pregnancy induced hypertension, gestational diabetes, abnormal placentation, or bleeding. All of them took a prophylactic multivitamin and dietary iron supplements. All of them gave their informed consent to participate in the study, without congenital defects or neonatal complications.

Equipment and Volume Acquisition

A single observer (LTM) performed all B-mode and 3D-mode ultrasound and Doppler scans using a Voluson 730 (Kretztechnik Ibérica, Madrid, Spain) and an abdominal multifrequency probe (2.2-6.5 MHz). All patients were scanned in a semi-recumbent position. An initial 2D conventional study provided data about fetal position and presentation, body movements and fetal heart rate, placental location and umbilical cord insertion. It was followed by a fetal biometry to measure biparietal diameter, head circumference, abdominal circumference, and femur length, demonstrating normal fetal growth according to our own tables. Fetal weight was calculated by the formula of Hadlock et al (21). A morphological study did not show any structural abnormalities of the head, thorax, heart, abdomen, spine, and extremities in any of the fetuses. A conventional pulsed Doppler study of the umbilical artery was also performed too, and the umbilical resistance index (URI) calculated according to the formula URI = US–UD / US, where US is the maximum systolic velocity and UD the minimum tele-diastolic velocity.

3D power Doppler was used to obtain a representative volume of the placental vascular tree according to the technique previously described (15,16). In summary, power Doppler window (pulse repetition frequency at 600 Hz and a wall filter of 50 Hz) was placed over the placenta including the entire vascular tree from basal to chorionic plates. The 3D volume box was placed over the highest villous vascular density zone at a fixed 35° angle. Volume acquisition was made during a time interval varying from 10 to 15 s in the absence of fetal movements and with the mother being as still as possible. Each complete U/S examination lasted between 10 and 15 minutes. The "biopsies" were acquired from the central part of the placenta if it was located anteriorly, lateraly, or at the fundus (73 cases; 86%); and from the most accessible lateral part if it was posteriorly inserted (12 cases; 14%).

Calculation of Power Doppler Indices

The stored volumes were further analyzed using the VOCAL (Virtual Organ Computer-aided Analysis) program included in the computer software. Each volume was recovered in succession from the hard disk and processed using the multiplanar system. The three planes of placental volume obtained were explored to localize the zone where the power Doppler found the highest vascular density. Using plane A as a work pattern, the limits of a virtual reference axis were placed between basal and chorionic plates, excluding both of them, and then the volume of a sphere was obtained and measured automatically by rotating around that fixed axis. If the placental thickness was correctly enclosed in that sphere, the sample was considered adequate and volume and vascularization values were calculated using the computer program. If the thickness of the placenta was not selected correctly or any of the blood vessels from the basal or chorionic plates were included, the limits of the reference axis were relocated to obtain a new measurement (Fig. 1).

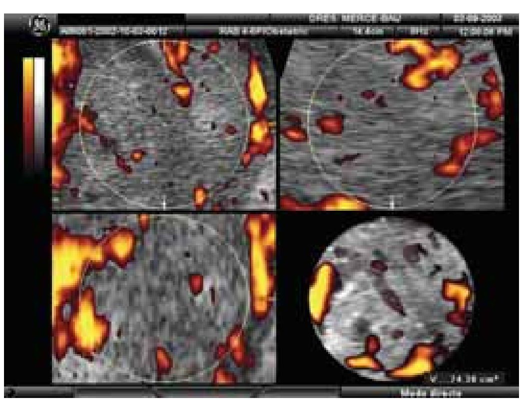

Figure 1. "Placental biopsy" technique by 3D power Doppler. A placental tissue sphere is obtained between basal and chorionic plates.

The VOCAL program calculates automatically grey-scale and color values from the acquired volume, a sphere in this case (15,16). Three-dimensional volume is constituted of smallest units of volume – "voxels." Voxels contain all the information about grey-scale and color, according to an intensity scale ranging from 0 to 100. According to these values, this measure system obtains three power Doppler indices to evaluate vessels and blood flow. The vascularization index (VI) measures the number of color voxels in the studied volume, which represent the blood vessels within the tissues, and expresses it as a percentage. The flow index (FI) is the average color value of all the color voxels, and it shows the average blood flow intensity. The vascularization flow index (VFI) is the average color value of all the grey and color voxels of the sphere and represents both blood flow and vascularization.

Statistical Analysis

Statistical analysis was done by the SPSS for Windows, version 9.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to verify the normal distribution of data. Pearson correlation coefficient was used to establish the linear association between 3D placental flow indices, fetal growth, and umbilical Doppler parameters. Linear or quadratic regression was evaluated to model the relationship between placental volume (PV), vascularization (VI), flow (FI), and vascularization-flow (VFI) indices, and gestational age considered as independent variables. The regression model was chosen according to its better fit on the scatterplot. The $P<0.05$ value was considered statistically significant.

Results

A total of 86 normal pregnancies between 15 weeks and 4 days and 40 weeks and 2 days were evaluated in this study (Table 1). "Placental biopsy" showed that placental volume (PV) increased progressively and significantly with the gestational age ($r=0.46$; $P<0.001$) (Fig. 2). All 3D Doppler indices had a significant relationship with gestational age, with FI having the most significant relationship ($r=0.58$; $P<0.001$) (Fig. 3), and VI the least significant relationship ($r=0.29$; $P=0.025$) (Fig. 4). The FI increased linearly with gestation, whereas the VI showed a clear dispersion of values with a plateau from the 30th week onwards and a decrease from the 37th week to the end of pregnancy. The VFI behaved as a combina-

Table 1. Characteristics of 86 pregnancies in a study of placental vascularization by 3D power Doppler ultrasound

Parameter*	Value
Age (years, mean±SD)	33.5±3.9
No. (%) of primiparae	28 (33)
Gestational age at delivery (days, mean±SD)	275±7
No. (%) of cesarean deliveries	18 (21)
No. (%) of male newborns	53 (62)
Newborn weight (g, mean±SD)	3,360±425
Newborn height (cm, mean±SD)	50.8±2.3
5' Apgar score (mean±SD)	9.6±0.5

*SD – standard deviation.

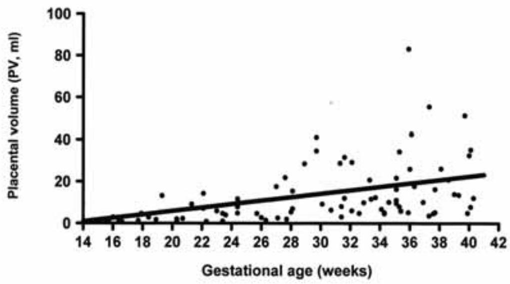

Figure 2. Individual values of 3D-obtained placental volume (PV) and its relationship to gestational age (y=-13.375+0.893x; r=0.46; *P*<0.001).

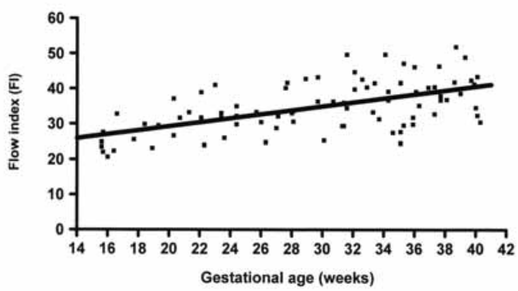

Figure 3. Individual values of flow index (FI) related to gestational age (y=17.930+0.565x; r=0.58; *P*<0.001).

tion of both VI and FI indices from which it was derived (r=0.32; *P*=0.013) (Fig. 5).

In general, 3D Doppler indices were significantly related to fetal biometric parameters and fetal weight. Vascularization index was correlated significantly with DBP (r=0.28; *P*=0.015), HC (r=0.27; *P*=0.028), AC (r=0.25; *P*=0.031), FL (r=0.27; *P*=0.020), but the correlation coefficient was not significant for fetal weight (r=0.20; *P*=0.083). VFI also significantly correlated with DBP (r=0.33; *P*=0.004), HC (r=0.31; *P*=0.010), AC (r=0.31; *P*=0.007), FL (r=0.32; *P*=0.005), and fetal weight (r=0.27; *P*=0.019). FI showed the highest correlation with DBP (r=0.64; *P*<0.001), HC (r=0.62; *P*<0.001), AC (r=0.64; *P*<0.001), FL (r=0.64; *P*<0.001), and fetal weight (r=0.60; *P*<0.001).

Vascularization index correlated positively with US (r=0.29; *P*=0.013) and negatively with URI (r=-0.24; *P*=0.043). The higher positive correlation coefficient was found between FI and US (r=0.44; *P*<0.001) and the negative one with URI (r=-0.48; *P*<0.001). Lastly, VFI also signifi-

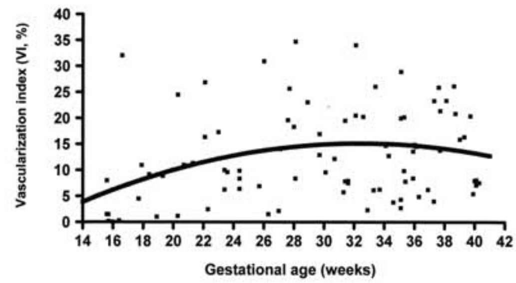

Figure 4. Individual values of vascularization index (VI) related to gestational age ($y=-19.668+2.144x-0.033x^2$; r=0.29; *P*=0.025).

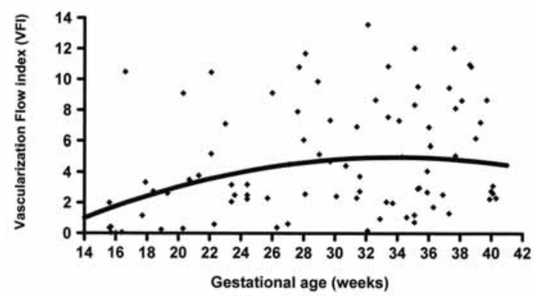

Figure 5. Individual values of vascularization flow index (VFI) related to gestational age ($y=-6.530+0.678x-0.010x^2$; r=0.32; *P*=0.013).

cantly correlated with US (r=0.28; *P*=0.015) and URI (r=-0.27; *P*=0.021).

Discussion

The "placental biopsy" technique with the 3D power Doppler was designed to obtain a representative sample of placental vascularization, which allowed for the evaluation of the placental vascular tree as a whole (15,16). Power Doppler shows a high sensibility to depict a vascular tree because it is based on Doppler signal amplitude instead of mean frequency values (22,23). Moreover, it does not show aliasing effect and the color map is independent of the insonation angle (22,23). Nevertheless, artefacts can appear due to maternal or fetal movements. This is the reason why we excluded the measurements of placental volume in 6 women. Some authors recommend a fast low resolution acquisition to avoid this kind of artefacts (4,5). According to our experience, this method would not eliminate artefacts either. It could only be done by increasing the power

Doppler pulse repetition frequency to 900 Hz. Nevertheless, this technical modification is not recommended because of the possibility of losing some color signal.

We have previously demonstrated a good reproducibility and intra-observer correlation of 3D Doppler indices (15,16). Intra-class correlation coefficient is higher than 0.85 for every index, showing the best intra-observer agreement and low variation coefficient for the flow and vascularization-flow indices (15,16). A good intra- and inter-observer agreement for VI, FI, and VFI has been also demonstrated for the evaluation of adnexal masses, taking a cube as a sample volume (8). Recently, acceptable variability of 3D Doppler indices has been reported in the study of ovarian vascularization (5). The authors concluded that it could be applied in clinical practice, as well as in research studies.

3D power Doppler not only allows a complete and adequate study of the placental vascular tree through the identification of the different branches of villous vessels (12,13), but produces a quantitative evaluation of the number of vessels by means of the vascularization index and the blood flow by the flow index and vascularization-flow index application (14). This method depicts villous vessels of the first, second, and third order (12,13), achieving a visualization percentage superior to bidimensional Doppler (13). A recent bidimensional power Doppler study (24) reports the villous arteries detection to the fourth order in normal gestations, with a characteristic percentage detection increase between the 28th and 38th week of pregnancy for the third and fourth order villous arteries.

In the present study, we demonstrated that 3D Doppler indices correlated significantly with gestational age, although each of them exhibited different behavior. Whereas FI (placental flow) increased in a linear and progressive manner along gestation, the VI (number of placental vessels) increased up to the 30th week, maintaining a plateau up to the 37th week and decreasing onwards. We also observed a great dispersion of the index values, explaining a greater variation coefficient (15) and a lesser reproducibility (16). The vascularization-flow index reflects the behavior of both indices, VI and FI, from which it derives and is calculated. Yu et al (14) demonstrated that VI, FI, and VFI intraplacental indices increase with gestational age in normal pregnancies, but do not describe the volume applied and the place selected to obtain a representative sample of placental vascularization. We have observed that placental volume obtained by "3D biopsy" increases progressively and is significantly related to gestational age.

Similar to Yu et al (14), we found that 3D Doppler indices and biometric parameters of fetal growth showed significant correlation, although this correlation was the strongest for the flow index. The maximum systolic velocity and umbilical resistance index were also significantly related to 3D Doppler indices. Nevertheless, we believe this finding needs further investigation because intraplacental vascularization must reflect and be related to umbilical venous flow and even uterine vessels blood flow. Therefore, we have started a new study to fully evaluate these functional vascular relationships.

Unfortunately, we know now that umbilical Doppler alterations express pathological vascular changes in the villous tree only in its late stages, giving them a low sensitivity as a diagnostic tool (19). Umbilical artery resistance is increased only when a 70% of placental vessels become affected (25-28). Therefore, it can be assumed that the blood flow in the intra-placental vascular tree could be affected in some cases with normal umbilical Doppler (24). It has been reported that an intra-placental vascular resistance greater than the umbilical resistance is associated to a higher pregnancy complications rate (29) and intra-placental vascular resistance registered by multigate spectral Doppler is more sensitive and precedes umbilical Doppler alterations in the detection of fetal growth restriction (19). Mu et al (24) demonstrated that the number of first, second, and third order villous arteries is significantly diminished and no fourth order vessel can be detected in fetal growth restricted pregnancies. The 3D power Doppler placental vascular "biopsy" method and the results of the present study about vascularization, flow, and vascularization-flow indices evolution in normal pregnancies seem essential in the investigation of this hypothesis.

In conclusion, our study confirmed that 3D power Doppler technique of placental vascular "biopsy" is an appropriate tool for routine evaluation of the human placental vascular tree during gestation. 3D Doppler indices change as pregnancy progresses and are significantly related with fetal biometry and umbilical artery Doppler velocimetry. Whereas intra-placental flow index shows a linear and progressive increase during whole pregnancy period, the vascularization index shows a plateau during the third trimester and decreases at gestational term. These results could be of great importance for the predictive and diagnostic evaluation of fetal growth restriction presenting with placental vascular tree alterations.

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