Feto-placental vascularization: A multifaceted approach.

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Review: Feto-placental vascularization: A multifaceted approach

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Abstract

Doppler Ultrasound allows the *in vivo* study of feto-placental hemodynamics. Doppler flow velocity waveforms (FVW's) obtained from the umbilical arteries reflect downstream blood flow impedance, thus giving indirect evidence of vascular villous tree characteristics. Pulsatility Index, which quantifies FVW's, decreases throughout normal pregnancy, indicating decreasing impedance and is often higher in cases of fetal growth restriction (FGR). Different approaches (morphometrical, morphological, mathematical, immunohistochemical and molecular) have contributed to elucidation of which anomalies of the vascular villous tree underlie Doppler findings. 3D ultrasound may be useful in the study of feto-placental perfusion. However, the unsolved question is why developmental villous tree anomalies occur. Crucial to the success of future research is definition of the population studied based on the uniform and correct definition of FGR.

Keywords: Placenta; Doppler; Morphometry; Fetal growth restriction

1. Introduction

The introduction of Doppler ultrasound technology allowed for the first time the *in vivo* study of normal and abnormal human feto-placental hemodynamics, which is essential for both understanding and monitoring fetal conditions. At first, efforts were made to obtain blood flow measurements in the umbilical vein; however, *in vitro* and *in vivo* studies showed that quantitative measurements were biased by unacceptable uncertainty, due to errors on the measurement of both cross sectional area of fetal vessels and blood velocity. Therefore, there was a switch toward "qualitative" measurements that are angle independent. Typically, flow velocity waveforms (FVW's) obtained by the analysis of Doppler signal derived from pulsating vessels display changes in flow velocity over the cardiac cycle.

FVW's characteristics depend on the following variables: 1) heart rate, 2) distance of the sampling site from the heart, 3) vessel elastic properties, 4) input pressure and 5) downstream impedance to flow that strongly affects diastolic velocity. Notably, since changes in systolic velocities are negligible, measurement of the ratio between peak systolic and end diastolic velocities during the cardiac cycle yields information about downstream impedance. Hence high end diastolic velocities reflect low downstream impedance and low diastolic velocities reflect high downstream impedance. Pulsatility index (PI) is used to describe this ratio (Fig. 1).

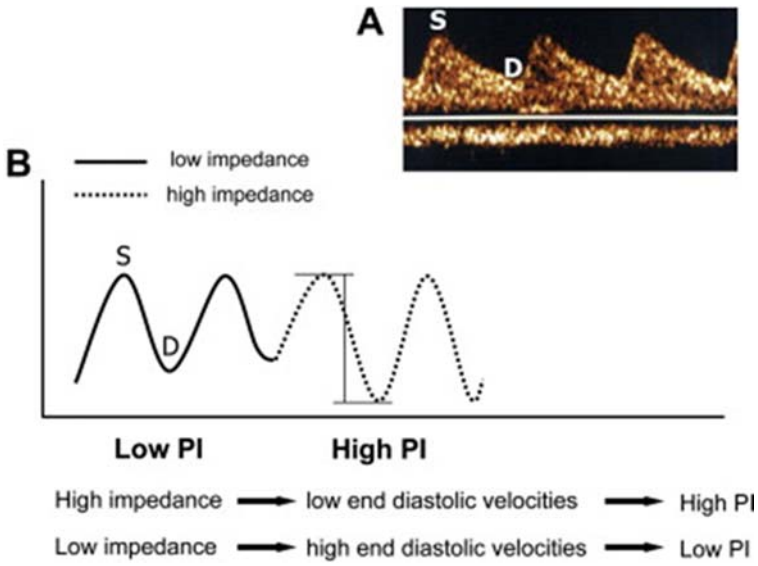

Fig. 1. A) Normal flow velocity waveforms obtained from the umbilical artery. B) Schematic drawing of waveforms with different end diastolic velocities indicating low and high impedance to flow. S: systole; D: diastole; PI: pulsatility index.

Importantly, PI values obtained from the umbilical arteries (UA) also reflect development of the villous vasculature [1] and [2]. Taken together with uterine artery FVW's, umbilical Doppler is a powerful tool in clinical practice, particularly in the diagnosis and management of small for gestational age (SGA) fetuses. Once a fetus has been diagnosed as SGA, normal uterine and umbilical FVW's suggest that it is small, but otherwise normal, while abnormal uterine/umbilical FVW's suggest that the growth disorder is due to an underlying defect in placental development, which is diagnostic of fetal growth restriction (FGR). FGR is defined as a fetus unable to reach its genetically determined growth potential [3]. FGR fetuses are the ones that take advantage of timed delivery, which to date is the only tool available in clinical practice. Nowadays, monitoring fetal condition is based on UA Doppler and fetuses with the highest PI values have the worst outcome [4]. Generally, three broad categories of abnormal PI of increasing severity are considered: PED (positive end diastole), AED (absent end diastole) and RED (reverse end diastole) (Fig. 2). PED suggests close

monitoring, while fetuses with AED and RED patterns require prompt delivery, provided that an acceptable gestational age has been reached.

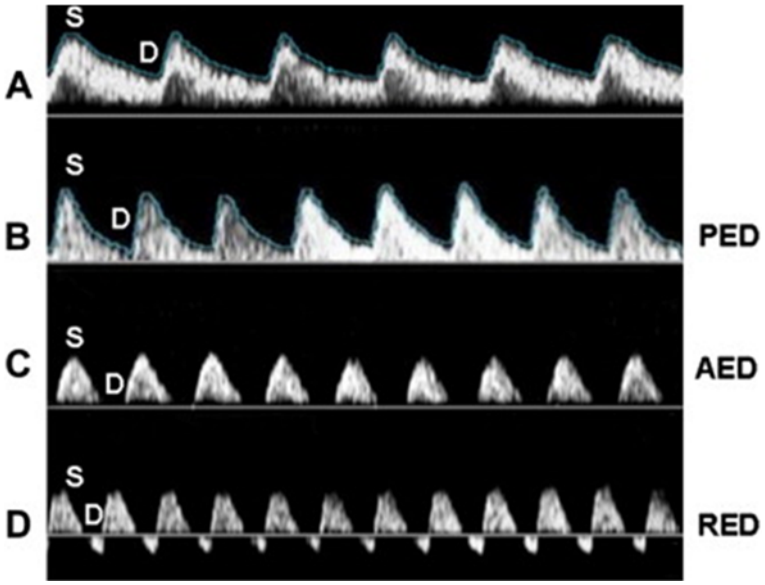

Fig. 2. Umbilical artery FVW tracings obtained in normal and abnormal third trimester pregnancies. A) Normal: end diastolic velocities are high. B) End diastolic velocities are present, but clearly reduced (compare to A). This pattern is defined as PED. C) End diastolic velocities are absent. This pattern is defined as AED. D) During diastole flow velocities appear in the lower channel, indicating flow in the opposite direction relative to the systolic phase. This pattern is defined as RED. FVW's: Flow Velocity Waveforms; PED: Positive End Diastole; AED: Absent End Diastole; RED: Reverse End Diastole; S: Systole; D: Diastole.

Besides its obvious use in clinical practice, Doppler ultrasound can directly contribute to research on the placenta and has stimulated translational research. In this paper we will discuss studies published in the Doppler era with the goal of understanding 1) *which* anomalies of the villous vascular tree determine different patterns of FVW's and 2) *why* these changes occur.

2. Which vascular villous tree abnormalities lead to pathological umbilical FVW's?

In normal pregnancy, UA PI values are high during first and early second trimesters. End diastolic velocities are absent until 13–15 weeks of gestation, then they steadily increase while PI values decrease. Based on previous knowledge the decrease in PI values was related to the development of the vascular villous tree [5]. Villous vascularization during first and second trimesters is largely dependent upon sprouting and branching angiogenesis, which leads to the formation of immature

villous trees whose vascular core is composed by a growing network of fetal capillaries [6]. At the end of second trimester, there is a switch to non-branching angiogenesis, resulting in the formation of terminal capillary loops which, as the third trimester progresses, form a complex vascular network within the mature intermediate and terminal villi, responsible for nutrient and gas exchange between mother and fetus [7].

The correlation between PI values and villous development was illuminated by mathematical modeling based on simple assumptions about feto-placental physiology [8] and [9]. Using information derived from human and animal studies, we developed a mathematical model that considered the progressive dichotomous branching of stem villous vessels, the increase of perfusion pressure and UA cross sectional area, fetal heart rate and blood viscosity. Through this model we were able to predict the mean values of umbilical blood flow and PI throughout normal pregnancy and to verify that PI values decrease in a way comparable to that observed *in vivo* [10]. Similar results were reported in another study applying a different mathematical approach [11].

Mathematical models are useful to validate hypotheses and to simulate different conditions by changing parameters according to available data. Based on histological data, we simulated abnormalities of the vascular villous tree that could affect FVW's [12]. Using a variety of experimental approaches, we found that different vascular abnormalities were the culprit in altered FVW's. When the villous tree stops growing, PI values stop decreasing. When various degrees of obliteration of the villous tree were simulated, either through an occlusive process or reduction of stem villous vessel lumen, PI increased. In addition, we and others [11] and [12] showed that a large decrease in feto-placental blood flow (50–60%) must occur before PI becomes severely abnormal (AED). Van den Wijngaard et al. [13] reported that increased arterial stiffness increases PI in the entire feto-placental circulation while blood viscosity and peripheral bed compliance have limited influence on the flow profile. Although taken together these studies underscore the key role of the vascular villous tree in the establishment of UA FVW's, one limitation is that they take into account only stem villi, disregarding the peripheral ones.

Histological, morphometrical and stereological studies also enter the picture [14] and [15]. Kuzmina et al. found significant reduction of terminal and stem villi capillary volume in pregnancies with severe abnormalities of UA FVW's, even though only 40% of these cases were documented FGR [14]. Mayhew and colleagues investigated a well selected population of FGR pregnancies and found a significant decrease in villous and capillary volume, surface and length [15]. Our studies using placentae from pregnancies complicated by FGR with PED or AED/RED demonstrated a significantly lower percentage of villous tissue in AED/RED compared to control and PED placentae. [16]. We also found that peripheral villi of FGR-AED placentae contain slender, unbranched and uncoiled capillary loops, corroborating previous observations [17]. This pattern would suggest that the impedance determining the abnormal UA FVW's occurs mainly at the level of peripheral villi. Unlike AED, PED placentae exhibited a significantly higher percentage of peripheral villi, with a

netlike arrangement of capillaries, forming multiple branched terminal villi. These changes resemble those observed in placentae derived from pregnancies complicated by preeclampsia at term [6], high altitude [18], maternal anemia [19], which could represent an adaptive response to hypoxia. If impedance were determined at the level of peripheral villous vessels, a decrease of impedance, rather than an increase, would be expected in these cases. Hence stem villous vessels must be the main players in establishing FVW patterns.

Previous studies, relating abnormal UA Doppler to stem villous vessel characteristics in FGR fetuses reported conflicting results. Fok [20] found thick-walled vessels, causing partial occlusion of the smaller arteries, while Jackson [21] reported thinner vessel walls. A possible explanation for these conflicting results is that the FGR groups were heterogeneous, including cases of both FGR and normal SGA. When FGR cases were selected according to rigorous criteria, AED placentae consistently showed increased stem villous vessel wall thickness [22], [23] and [24]. In support of the latter finding, we reported differences in the expression of α-SMA, a molecule involved in vascular contractility, consisting in its redistribution between stem and peripheral villi, indicating that the vasculature of both villous types contributes to the umbilical Doppler AED pattern [25]. In contrast, PED placentae have smaller and thinner-walled villous vessels, which could account for the slight increase in impedance to blood flow [24].

A new approach to the *in vivo* study of the placenta is based on three dimensional (3D) Power Doppler technology that simultaneously allows volumetric calculations and evaluation of tissue perfusion. On the basis of Color Doppler and Power Doppler signals detected from each selected placental volume, several dimensionless parameters ranging from 0 to 100 are evaluated. Vascularization index (VI) describes the overall perfusion, i.e. the percentage of flow in a given tissue. Vascularization flow index (VFI) combines the information of vessel presence (perfusion) and amount of transported blood cells (blood flow), and is assumed to assess blood velocity in the same sample volume. Flow index (FI) provides information on how many blood cells are being transported at the time of 3D sweep. It therefore evaluates the overall blood flow in the sample volume. Since it is not possible to insonate the entire placenta beyond the first trimester of pregnancy, the Doppler signal is obtained from different sampling sites, considered representative of the whole (Fig. 3). Some studies showed that VI, VFI and FI do not change with gestational age in normal pregnancies indicating that the increase in vascularization (number of vessels and volume flow) is proportional to the increase in placental volume [26] and [27]. The results of other studies suggested that VI and VFI (but not FI) or FI (but not VI and VFI) increase throughout pregnancy [28] and [29]. Recently, Noguchi et al. [30] found lower values of VI, FI and VFI in FGR placentae; however, UA FVW's were abnormal in only 3 of the FGR cases. We recently studied 3D indices in well defined groups of FGR placentae [26]. All three indices were significantly lower in AED placentae, while only VI and VFI were lower in PED placentae compared to controls. Thus, similarly to UA FVW's, 3D Doppler detects different degrees of placental vascular abnormality in pregnancies complicated by FGR. Inconsistency of results among studies, both in normal and pathological pregnancies, may reflect yet unsolved methodological

problems. Also the interpretation of results may be flawed by incomplete understanding of the factors affecting 3D Doppler indices [31], [32] and [33].

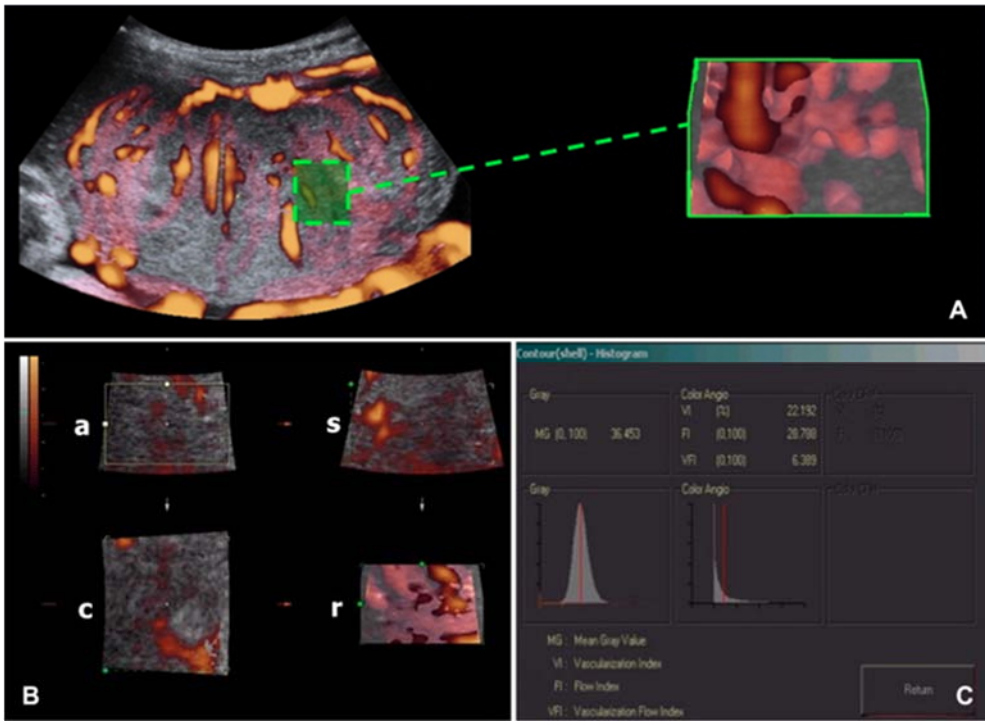

Fig. 3. Sampling of placental volumes by 3D sonography: A) Left panel: Two dimensional ultrasound and Power Doppler imaging of the placental vessels. Right panel: "virtual" placental biopsy obtained by 3D ultrasound; B) the acquired volume is displayed as three orthogonal perpendicular planes: axial (a), sagittal (s) and coronal (c); 3D volume rendering (r) is also represented; C) the software VOCAL (Virtual Organ Computer-aided Analysis) automatically calculates Vascularization Index (VI), Flow Index (FI) and Vascularization Flow Index (VFI). These indices are dimensionless.

3. Why do changes occur?

Understanding the abnormalities underlying UA FVW's is useful, but does not explain why these anomalies occur and how they are responsible for the development of FGR. The study of known placental angiogenic and anti-angiogenic factors in well defined populations of FGR may help to answer the question. FGR villous explants exposed to hypoxia have been shown to over-express soluble fms-like tyrosine kinase 1 (sFlt-1) [34]. We investigated sFlt-1 expression in FGR, normal SGA, term and preterm control placentae, and in twin pregnancies complicated by selective FGR [35]. The expression of sFlt-1 was comparable in SGA placentae relative to controls, while it was significantly increased in AED-FGR placenta. Previous studies on sFlt-1 in FGR placentae reported contrasting data attributable to the heterogeneity of FGR definition [36] and [37]. Our results may in

part explain the abnormal angio-architecture found in FGR placentae. Elevated placental sFlt-1 could sequester locally produced VEGF and PlGF, thus impairing their binding to VEGF receptors and their consequent biological function. This would result in an anti-angiogenic balance in FGR placentae, affecting intermediate and terminal capillary loop differentiation. Importantly, the high expression of sFlt-1 is likely to be triggered by low placental oxygen tension typical of the FGR placenta via hypoxia-inducible transcription factor-1 (HIF-1).

Another possible explanation for altered villous vascularization and function stems from our study on transforming growth factor (TGF) β1. TGF-β1, secreted by trophoblastic cells, acts on smooth muscle cells of villous vessel walls by increasing their contractile force and this associates with increased expression of α-SMA. While TGF-β1 expression was significantly higher in FGR-AED placentae, no differences in α-SMA levels were found compared to controls. However, changes in α-SMA spatial distribution were reported, i.e. an increase in medium and small stem villi and a decrease in peripheral villi, The α-SMA increase in stem villi may explain the thicker stem villi vessel walls in AED placentae [20], [22], [23], [24] and [26].

4. Conclusions

The vascular villous tree abnormalities underlying umbilical Doppler FVW's have been elucidated. The PED pattern is mainly determined at the level of stem villi, while abnormalities of both stem and peripheral villi contribute to AED. Some preliminary data from 3D ultrasound show a well defined difference in blood flow between PED and AED placentae. However, these results must be interpreted with caution since the actual meaning of the 3D derived indices is controversial [33] and [38]. The main question, still unanswered, is why and when the abnormalities develop. Hypoxia and molecules regulated by the hypoxic status certainly play an important role, but much further investigation is needed. Crucial to the success of future research is the classification of the population studied based on the uniform and correct definition of FGR.

Conflict of interest

The authors declare that they have no actual or potential conflict of interest.

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