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Umbilical venous volume flow and placental morphometry in cases of the syndrome of fetus retardation of growth

Abstract: Objective to determine the reproducibility of measurement of umbilical venous volume flow components and to calculate umbilical venous volume flow in normal and of the syndrome of fetus retardation of growth (SFRG) in a cross-sectional study. Morphometric analysis was performed on placental samples from 58 pregnancies with abnormal Doppler waveforms in the uterine, placental and umbilical circulations at 32-34 weeks, and 10 pregnancies with normal waveforms.

Results: The volume of placental villi reduced from 350.5 cm^3 in controls to 286.4 cm^3 *(P* < 0.05) in the severest cases. The volume of the fetal capillaries reduced from 59.7 cm^3 to 20.5 cm^3 *(P* < 0.05). These reductions were associated with increased placental infarction. The myometrial segments of the spiral arteries were severely constricted, demonstrating failure of physiological conversion secondary to deficient trophoblast invasion. Umbilical venous volume flow measurements demonstrate an acceptable reproducibility. Umbilical venous volume flow is reduced in the SFRG but, when related to fetal weight, both normal and reduced values were obtained.

Conclusion: The placental vascular bed is greatly reduced in cases of chronic fetal hypoxia. We propose impaired placental perfusion causes oxidative stress and regression of the fetal vasculature, leading to fetal growth retardation. Measurements of umbilical venous vessel area and time-averaged velocity resulted in acceptable reproducibility of volume flow calculations, which show a seven-fold increase at 32-36 weeks of gestation. In SFRG volume flow is significantly reduced

Keywords: Umbilical volume, Doppler ultrasonography, Morphometry, syndrome of fetus retardation of growth.

1. Introduction.

Doppler ultrasonography has become a non-invasive method for monitoring the functioning of the utero-placental circulations in vivo during human pregnancy. From analysis of the umbilical waveform it is possible to assess the impedance to placental bloodflow, and to accurately predict fetal hypoxia [1-3]. Various attempts have been made to correlate the Doppler abnormalities with Umbilical venous volume flow and placental morphometry structural changes in order to provide a mechanistic explanation for their origin [4-8]. The results have been varied, ranging from claims of

a reduction in the number of arteries within the supporting stem villi to a reduction in the capillary vascular bed within the terminal villi, the principal site of gaseous exchange. The underlying cause of the placental lesions is not known, although the fact that Doppler changes in the umbilical circulation are invariably seen subsequent to similar changes in the uterine arteries strongly suggests they are a secondary phenomenon. Recently, it has been proposed that the placenta is hyperoxic, rather than hypoxic as commonly assumed, in cases of the syndrome of fetus retardation of growth (SFRG) [9].

2. Materials and methods.

2.1. Clinical details. Patients were selected from women attending the supraregional obstetric referral perinatal centre Delivery in Kharkov, Ukraine with the approval of the local ethics committee. A total of 58 cases of SFRG and umbilical venous maximum flow velocity (mm/s) was measured using Doppler system (Toshiba SSA 140 A, Toshiba Corp. Medical Systems Division, Tokyo, Japan) with a transabdominal probe between 32 and 34 weeks of pregnancy. For each case the maximal rates of systolic (S) and diastolic (D) blood flow were measured. From these two indices were calculated: the systolic-diastolic ratio (SDR) = S/D, and the index of resistance (IR) = (S - D)/S. The cases were classified into three groups of increasing severity (Table 1): group1(n=29)- the women with SFRG 1 degree, group 2 (n=18)- the women with SFRG 2 degree, group 3 (n=11) - the women with SFRG 3 degree. The commonest cause of CHF was preeclampsia during the second half pregnancy, and this accounted for 78% of the cases in Group 3.

These were matched to a control group of 10 patients in which Doppler ultrasonography was within the normal range. All the pregnancies delivered a single live infant between 38 and 40 weeks, and all women gave their informed written consent to participate in the study.

Vessel area (mm^2) and Doppler-derived time-averaged flow velocity (mm/s) were multiplied to calculate volume flow (mL/min) including flow per kg fetal weight. The coefficient of variation (CV) for vessel area and flow velocity scans and tracings were determined. From umbilical venous cross-sectional vessel area and time-averaged velocity data, the CV for umbilical venous volume flow was determined.

2.2. Placental samples. After delivery each placenta was weighed, and then three blocks 2 cm x 2 cm x 2 cm were removed, one from the margin of the disc, one from under the cord insertion and one equidistant between the other two. The samples were fixed in 10% formolsaline, embedded in paraffin wax and sections were stained with haematoxylin and eosin. Objective to determine the reproducibility of measurement of umbilical venous volume flow components and to calculate umbilical venous volume flow in normal and SFRG 1, 2 and 3 degree in a cross-sectional study.

2.3. Myometrial samples. In order to study the maternal spiral arteries small samples of the myometrium were excised at the time of caesarean section. In cases of vaginal delivery curettage of the placental bed was performed immediately after delivery. Between two and three biopsy samples per patient were fixed in 10% formol saline, embedded in paraffin wax and sections were stained with haematoxylin and eosin. Physiological conversion of individual spiral arteries was classified as

'complete' or 'incomplete' according to the histological criteria of Brosens and Renaer. No in order to confirm the interpretation of the arterial changes sections from three biopsy samples of the Control group and nine samples of Groups 1-3 were stained immunohisto-chemically for cytokeratin 7. Sections (7 mm) were prepared by dewaxing, rehydration, and incubation for 15 min in 3% hydrogen peroxide (H_2O_2). Antigen retrieval was preformed by microwaving in citric acid buffer pH 6.0 for 1.5 min. After blocking for 1 h in 5% horse serum, mouse anti-human cytokeratin monoclonal antibody (Dako, Ely, UK) was applied at a 1:100 dilution in 2.5% horse serum overnight at 4C. Sections were washed in Tris-buffered saline with 0.1% Triton X-100, Tween 20 (TBS-TT), and incubated with biotinylated anti-mouse secondary antibody (Vector, Peterborough, UK) diluted 1:200 for 1 h at room temperature. After washing in TBS-TT, Vectra-stain Elite ABC reagent (Vector, Peterborough, UK) was applied for 45 min at room temperature. Slides were developed in Tris-maleate buffer, pH 7.4 with 0.5 mg/ml DAB and H_2O_2 as substrates. Sections were lightly counterstained in Gill 2 hematoxylin.

2.4. Morphometric analysis.

All estimates were made at the light microscope level by a combination of point and intersect counting using the VIDS IV system (Synoptics Ltd., Cambridge, UK). Fields of view were selected in a systematic random fashion by scanning the sections stepwise in the x- y directions, using one corner of the coverslip as a random start point. Approximately 10 fields of view were analysed per section, and three blocks were examined per placenta.

Table 1

Doppler flow velocimetry data (mean ± S.D.) for the systolic-diastolic ratio (SDR) and the index of resistance (IR) at the different degree of SFRG

Parameter	Control (n = 10)		Group 1 (n =29)		Group 2 (n =18)		Group 3 (n = 11)	
	SDR	IR	SDR	IR	SDR	IR	SDR	IR
Uterine arteries	1.69±0.10	0.45 ± 0.07	1.96 ± 0.06*	0.49 ± 0.01*	2.27±0.07*	0.53±0.01*	2.83 ± 0.12*	0.68 ± .02*
Spiral arteries	1.53 ±0.09	0.35 ± 0.07	1.70 ± 0.06*	0.40 ± 0.02*	1.76±0.05*	0.44±0.01*	1.92 ±0.05*	0.47 ± .02*
Umbilical arteries	1.88±0.10	0.47 ± 0.03	2.56 ±0.10*	0.61 ± 0.01*	2.61±0.13*	0.65±0.04*	3.07 ± 0.09*	0.68 ± .02*
Stem villi arteries	2.52 ±0.15	0.58 ± 0.05	2.68 ± 0.08	0.67 ± 0.02*	3.37±0.17*	0.67± 0.02*	4.03 ± 0.20*	0.74 ± .02*

Significant difference to control at *P* compared < 0.05.

Images were overlain with a quadratic test lattice. Where the horizontal and vertical lines of the test grid met constituted a test point. The number of points falling on stem villi, intermediate and terminal villi, fetal capillaries, intervillous space, intervillous fibrin and placental infarcts were counted and expressed as a fraction of the total number of points falling on the sections.

The number of intersections the test lines made with the villous surface and with the capillary luminal margins were also counted, and so their respective surface densities could be estimated. Finally, the numbers of villous and capillary profiles were recorded, with two

sides of the test lattice acting as forbidden lines, and villous and capillary length densities calculated.

2.5. Statistical analysis. Data groups were compared by an unpaired t-test using the Statgraphics statistical programme (STSC, Rockville, Maryland, USA). Results were considered significant at $P < 0.05$.

3. Results.

All pregnancies in the control group were delivered vaginally of a normal healthy fetus with an APGAR score of 9 or above (Table 2). By contrast, as the severity of the utero - placental vascular pathology increased, a greater number of pregnancies were delivered by caesarean section for fetal distress. Mean birthweight decreased across the groups, although there was greater variability in birthweight amongst Groups 2 and 3. These babies also had lower APGAR scores immediately after birth.

Within the placenta the volume of the intermediate and terminal villi was significantly reduced in the vascularly compromised pregnancies, although their surface area and length increased. This suggests a change in the topology of the villous tree, with increased branching. By contrast, the volume, surface area and length of the supporting stem villi increased (Table 3). The total volume of the fetal capillaries within the intermediate and terminal villi was significantly reduced in Groups 1-3 compared to the controls, along with the mean capillary diameter (Table 4) (Fig. 1). Total capillary length showed no significant differences across the groups, whereas capillary surface area actually increased in Group 3 (Table 4). Despite the increase in volume of the stem villi, the total volume of their capillaries was also reduced across the groups, along with their length and surface area. Within the intervillous space fibrin deposition and placental infarction was significantly increased in the pathological pregnancies compared to the controls (Table 5).

In the normal pregnancies the majority (60%) of spiral arteries were of large diameter, and their walls were formed largely by fibrin, with a thin endothelial lining (Fig. 2A). Numerous invading extravillous trophoblast cells were identified within the endometrium and myometrium, and some were observed within the walls of converted vessels (Fig. 2A) The incidence of full conversion was greater in the centre of the placental bed than towards the periphery. In the samples associated with abnormal Doppler waveforms only 25% of the vessels were fully converted. The majority were constricted, with several layers of smooth muscle within their walls (Fig. 2B), and often fibrin was deposited in their lumens. Invading extravillous trophoblast cells were scarce within the endometrium, and were never observed within the myometrium (Fig. 2B).

Table 2

Clinical data (mean ± S.D.) relating to the patient groups

Group	Number caesarean deliveries	Number of forceps deliveries	Birth weight (g)	Placental weight (g)	APGAR score				
					9-10	8	6-7	<6	Still-born
Control (n= 10)	0	0	3.552 ± 98	518 ±19	8	2	0	0	0
1 (n = 29)	1	0	2.700 ± 69*	430 ± 37*	8	9	11	1	0
2 (n = 18)	3	2	2.400 ± 99*	390 ± 33*	4	7	5	2	0
3(n = 11)	5	0	2.250 ± 75*	340 ±26*	1	3	2	4	1

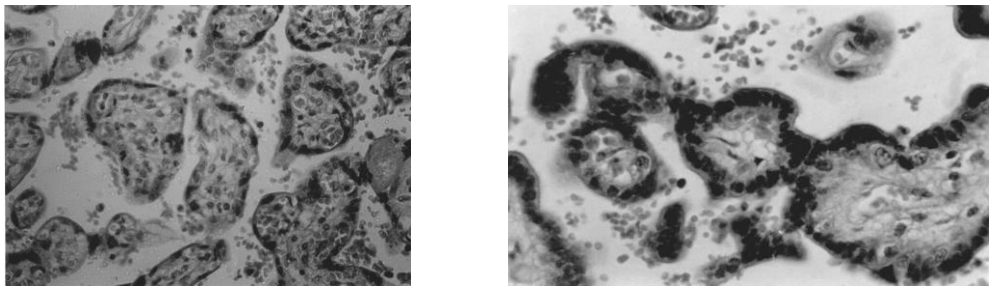
*Significant difference compared to control at $P < 0.05$.

Table 3

Morphometric data (mean ± S.D.) relating to development of the villous tree

Parameter	Control (n = 10)	Group 1 (n =29)	Group 2 (n =18)	Group 3 (n = 11)
Terminal villi:				
Volume (cm^3)	350.5 ± 20.9	370.1 ± 14.3	320.7 ± 18.7	286.4 ± 19.9*
Surface area (m)	12.26 ±1.35	13.91 ± 0.39*	14.12 ± 0.49*	15.97 ± 0.39*
Length (km)	132.8 ±4.5	159.8 ±4.8*	165,6 ± 5.9*	187.3 ±6.3*
Stem villi arteries:				
Volume (cm^3)	105.1 ± 3.4	123.6 ±10.1*	144.6± 27.4*	143.1 ± 27.4*
Surface area (m)	0.57 ± 0.08	0.91 ±0.12*	0.77 ± 0.02*	0.56 ± 0.07
Length (km)	1.93 ±0.36	2.36 ±0.19*	2.66 ±0.11*	2.93 ± 0.15*

*Significant difference compared to control at $P < 0.05$.



A B

Fig. 1. Terminal villi from (A) a normal placenta displaying dilated capillaries (arrowed), compared to (B) a Group 3 placenta demonstrating the reduction in fetal capillary volume in the latter. Scale bars = 50 mm.

Table 4

Morphometric data (mean ± S.D.) pertaining to the vascularisation of the villous tree

Parameter	Control (n = 10)	Group 1 (n=29) =29)	Group 2 (n =18)	Group 3 (n = 11)
Terminal villi:				
Capillary volume	59.7 ± 4.2	36.9 ± 2.9*	29.8 ± 1.9*	20.5 ± 1.2*
Capillary surface area (m^2)	11.90±0.87	10.69 ±1.04	11.59 ±0.94	13.20 ± 1.27*
Capillary length (km)	557.4 ± 0.9	463.4 ± 0.9	421.2 ± 1.0	463.3 ± 1.2
Capillary diameter (mm)	12.36 ± 0.28	12.20 ±0.16	11.76 ±0.08	10.12 ±0.24*
Stem villi:				
Capillary volume (cm)	18.6 ±2.4	16.7 ± 2.2	15.3 ± 1.9*	12.8 ± 1.7*
Capillary surface area (m^2)	2.46 ± 0.27	2.05 ± 0.15*	1.51 ±0.09*	1.04 ±0.14*
Capillary length (km)	66.8 ± 8.3	54.4 ± 8.3	43.2 ± 4.4*	35.2 ±3.1*
Capillary diameter (mm)	23.39 ± 0.12	22.33 ± 0.36	21.98 ±0.13	20.27 ± 0.21

*Significant difference compared to control at $P < 0.05$.

Table 5.

Morphometric data (mean ± S.D.) pertaining to the intervillous space

Parameter	Control (n = 10)	Group 1 (n =29)	Group 2 (n =18)	Group 3 (n = 11)
Volume of intervillous space (cm^3)	152.7 ± 14.3	151.8±15.3	149.2 ± 11.3	146.4 ± 10.9
	4.36 ± 0.01	16.31 ± 0.4*	19.42 ± 0.3*	23.89 ± 0.3*
Volume of fibrin (cm^3)	2.87 ±0.13	3.02 ± 0.06*	3.29 ±0.11*	4.49 ± 0.32*

*Significant difference compared to control at $P < 0.05$.

The results of this study are consistent with previous findings that increasing severity of abnormal Doppler waveforms in the uterine and umbilical circulations is associated with SFRG [11].

However, more recently structural analyses of the placental villous tree in cases of severe intrauterine growth retardation have indicated that not all the histological changes seen can be explained on this basis.

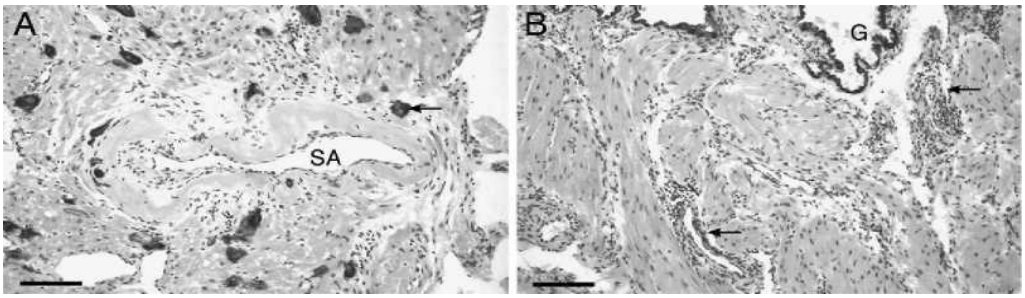


Fig. 2. Myometrial sections of spiral arteries from (A) a normal pregnancy, and (B) a pathological pregnancy immunostained for cytokeratin 7. In (A) numerous extravillous trophoblast cells (arrowed) can be seen within the myometrium, and even within the wall of a fully converted spiral artery (SA). In (B) the epithelium of the uterine glands (G) reacts positively for cytokeratin 7, but no extravillous trophoblast cells are present in either the endometrium or myometrium. As a result, the spiral arteries (arrowed) retain the smooth muscle within their walls, and remain of small calibre. Scale bars = 100 mm.

As Doppler abnormalities of the umbilical circulation are rarely seen in the absence of uterine arterial abnormalities it is most likely that they are a secondary phenomenon. We propose therefore the following model for the aetiology of the feto-placental abnormalities. Deficient trophoblast invasion for immunological or other reasons during early pregnancy leads to incomplete conversion of the spiral arteries.

The intermediate and terminal villi are the principal sites of gaseous exchange, and decreased vascularisation will inevitably impair placental exchange. This will lead to fetal hypoxia and growth retardation, but also reduced oxygen extraction from the intervillous space and so hyperoxia on the venous side of the placenta as a tertiary event (Fig. 3.).

No significant difference was found between umbilical venous cross-sectional areas measured at three different locations of the umbilical cord. Data were therefore grouped together for further analysis. Total CV was 9.1% for umbilical venous cross-sectional area (recordings, 5.4%; measurements, 7.3%) and 12% for time-averaged velocity (recordings, 7.3%; measurements, 10.5%), resulting in a coefficient of variation for umbilical venous volume flow of 8.1 % between recordings and 11.9% between measurements, respectively.

Umbilical venous volume flow is reduced in the SFRG but, when related to fetal weight, both normal and reduced values were obtained. A statistically significant difference for mean umbilical artery pulsatility index (PI) was established between the SFRG subsets i.e. those with normal and those with reduced umbilical venous volume flow per kg fetal weight.

Volume flow measurement in the umbilical vein requires that the angle of vessel interrogation and vessel size are meticulously established. Small errors in the volume flow components will result in larger errors in the volume flow calculation. In this study, the maximum frequency method was used to determine the mean umbilical venous velocity. However, this method assumes a parabolic flow profile.

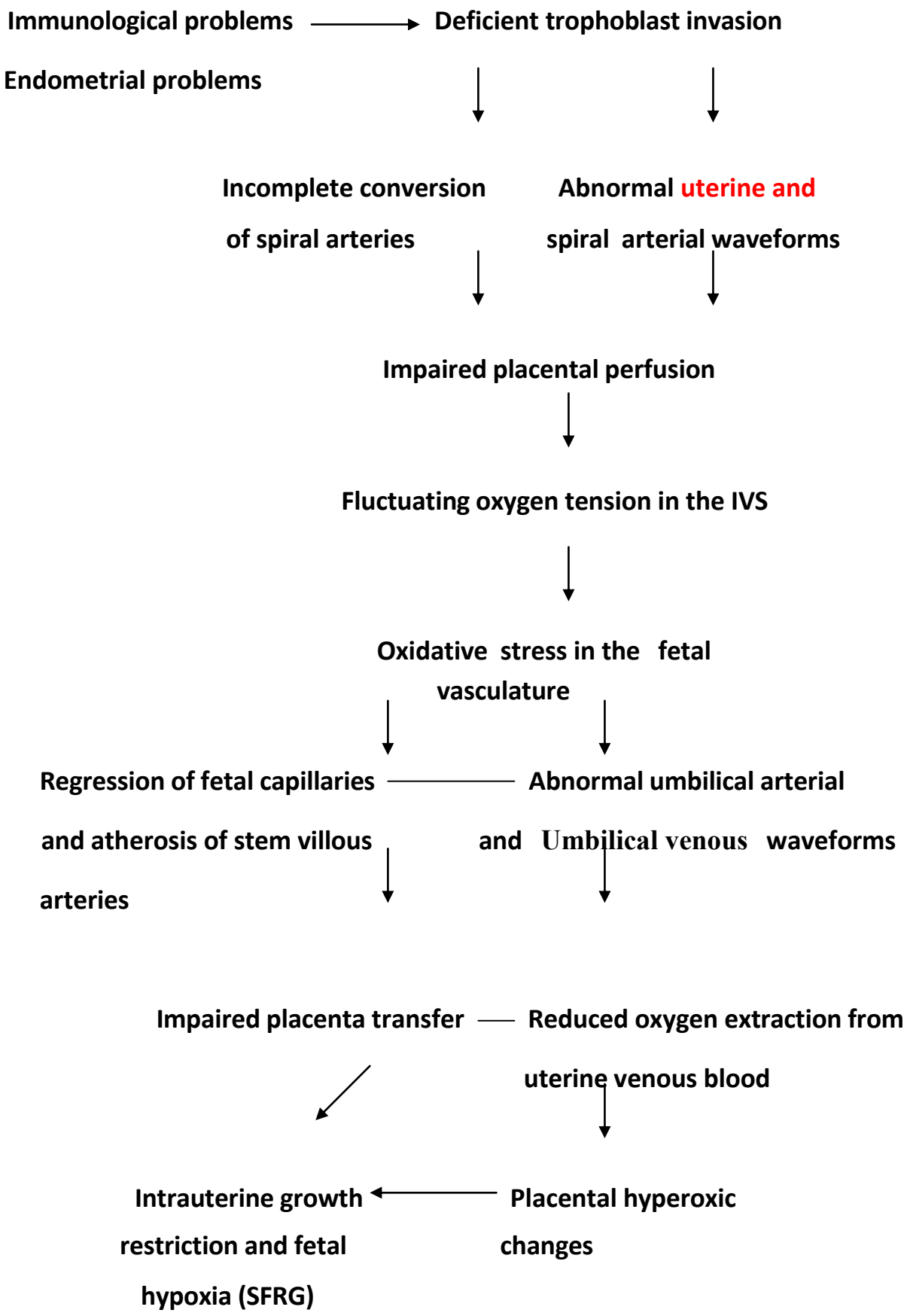


Fig. 3. Theoretical pathogenesis of placental changes in intrauterine growth retardation associated with abnormal Doppler waveforms in the uterine and umbilical circulations.

This model is compatible with the increased amount of placental infarction and fibrin deposition observed in Groups 2 and 3, which are not features of placentation under conditions of hypobaric hypoxia but are associated with ischaemia-reperfusion in other systems.

5. Conclusion. Human placental villous and vascular development is impaired in cases of chronic fetal hypoxia and SFRG. The placental vascular bed is greatly reduced in cases of SFRG. We propose impaired placental perfusion causes oxidative stress and regression of the fetal vasculature, leading to fetal growth retardation. Measurements of umbilical venous vessel area and time-averaged velocity resulted in acceptable reproducibility of volume flow calculations, which show a seven-fold increase at 32-36 weeks of gestation. In SFRG umbilical venous volume flow is significantly reduced.

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