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Placental morphometry and Doppler flow velocimetry in cases of chronic human fetal hypoxia

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Abstract

Objective: To investigate the structural basis of abnormal Doppler waveforms in the utero-placental circulations in cases of chronic fetal hypoxia.
Study design: 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.
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 and distress.

Keywords: Placenta; Hypoxia; Doppler ultrasonography; Morphometry; Oxidative stress

1. Introduction

Doppler ultrasonography has become a routine 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 placental 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 severe intrauterine growth retardation [9]. This theory may explain the basis for many of the morphological changes observed, but does not account for how the hyperoxia is initiated. Here we report morphometric data demonstrating a substantial reduction in the villous capillary bed in placentas associated with severe Doppler abnormalities at 32–34 weeks of pregnancy. We propose that incomplete invasion of the endometrial spiral arteries in early pregnancy leads to poor maternal perfusion of the placenta. Periods of vasoconstriction may result in fluctuating oxygen tensions within the organ, which have been shown in vitro to generate oxidative stress within the placental vessels [10].

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Subsequent regression of the capillaries would increase placental vascular resistance and also impair placental transfer, resulting in growth retardation and reduced oxygen extraction from the maternal blood. Consequently, the venous side of the placenta would become hyperoxic.

2. Materials and methods

2.1. Clinical details

Patients were selected from women attending the supraregional obstetric referral centre Delivery Unit Number 5 in Kharkov, Ukraine with the approval of the local ethics committee. A total of 58 cases of chronic hypoxia of the fetus (CHF) were identified by colour Doppler ultrasonography using a 24 MHz Prizma scanner (Diasonics International, Les Ulis Cedex B, France) 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). The abnormal haemodynamics in the uterine and feto-placental circulations were the result of extra-uterine pathologies, for example idiopathic hypertension, anaemia and chronic pyelonephritis, and obstetric pathologies, such as preeclampsia and threatened miscarriage. The commonest cause of CHF was preeclampsia during the second half pregnancy, and this accounted for 78% of the cases in Group 3. CHF was sometimes associated with a small-for-dates fetus, and this was most common in Group 3 were it occurred in 40.6% of cases.

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.

2.2. Placental samples

After delivery each placenta was weighed, and then three blocks 2 cm × 2 cm × 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% formol saline, embedded in paraffin wax and sections were stained with haematoxylin and eosin.

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 [11].

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 immunohistochemically for cytokeratin 7. Sections (7 μm) 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 sites

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 ± 0.02*
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 ± 0.02*
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 ± 0.02*
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 ± 0.02*

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