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Fetoplacental vascular alterations associated with fetal growth restriction

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ABSTRACT

Introduction: Placental functional impairment in pregnancies with fetal growth restriction (FGR) can arise from fetoplacental vascular abnormalities. We aimed to compare the micro and macrovasculature of placentas from normal pregnancies with those showing late onset FGR.
Methods: Placental arterial casts ($n = 12$ normal, 6 FGR) were prepared. Chorionic arterial number and inter-branch length were examined. Microvascular features were quantified in CD34-stained tissue sections obtained by systematic ($n = 12$ normal, 12 FGR) and targeted ($n = 6$ normal, 6 FGR) sampling from the placental periphery and centre.
Results: Adjusted for the weight of the placenta or the surface area of the chorionic plate, the number of chorionic arteries was similar in normal and FGR arterial casts. Inter-branch length per unit placental weight was greater in the first generation of arterial branches in FGR ($p < 0.05$). Villi in FGR placentas were more poorly vascularised, particularly at the periphery and in grossly visible hypovascular regions. Intermediate and terminal FGR villi in these areas exhibited reduced vessel lumens, loss of CD34, and infilling with CD34-negative cells of what appeared to be previously existing vascular spaces.
Conclusion: Differences in chorionic arterial branching patterns between normal and FGR placentas arise from differences in placental size. FGR placentas show microvascular regression and extreme hypovascularity in peripheral areas. These features may well limit the ability of the placenta to meet fetal nutrient requirements late in gestation. Targeted sampling is more effective than systematic random sampling in revealing vascular defects.

1. Introduction

Fetal growth restriction (FGR), characterised by failure of a fetus to achieve its genetically endowed growth potential, is a heterogeneous condition of global significance affecting up to 8% of pregnancies [1]. It is associated with increased perinatal morbidity and mortality [2,3] and serious adverse health outcomes in childhood [4,5] and later life [6,7]. Maternal, fetal or environmental factors may impair fetal growth [8,9], but underlying placental insufficiency is associated with the majority [10]. The two anatomically distinct but functionally continuous vascular systems in the placenta – the uteroplacental and the fetoplacental circulations – perform crucial synergistic roles to ensure normal blood flow and efficient trans-placental transfer of gas and nutrients between the mother and fetus [11]. Structural and/or functional abnormalities in either or both systems may compromise fetal growth.

In humans, the fetoplacental vasculature may be divided into two broad categories – the macrovasculature, comprising the umbilical and chorionic plate vessels, and the microvasculature, comprising intravillous vessels and capillaries. The majority of studies of the fetoplacental vasculature in FGR concentrated on the microvasculature where reduced number, elongation and sparse arrangement of terminal villous capillaries [12,13] were shown. The concentration on microvascular angioarchitecture is probably the result of the villi being the main functional unit of the placenta. However, functional alterations such as increased vascular reactivity to vasoactive agents were also evident in larger vessels on the chorionic plate in FGR [14]. The purpose of this study was to examine the morphology of both the micro and macrovasculature of the fetal placenta and determine whether villous vascularity is inhomogeneous spatially throughout the volume of the placenta in

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FGR. We hypothesised that structural alterations are found at all vascular levels in the FGR placenta. To test this hypothesis we examined branching patterns and quantified chorionic plate vessels in FGR and normal placentas, and examined vascular densities in central and peripheral areas of the placentas.

2. Materials and methods

All placenta tissues were obtained from women who delivered at St. Mary's Hospital, Manchester under Biobank ethical approval (REC 08/H1010/55). Informed consent was obtained prior to delivery and normal ($n = 33$) and FGR ($n = 24$) placentas were obtained within 30 min of delivery. FGR was defined as individualised birth ratio (IBR) $\leq$5th centile.

2.1. Quantifying the fetoplacental macrovasculature

Preliminary experiments were done using three techniques to identify a sensitive technique to identify and quantify as many chorionic plate vessel branches up to the smallest accessible branches. Techniques compared were photography, angiography and corrosion casting.

2.1.1. Photography and vessel tracing

The fetal and maternal sides of normal placentas ($n = 3$) were photographed with a scale bar in the background. Placental trace outline was created in Powerpoint. Placental disc outline was coloured red, arterial outlines green and the cord insertion blue (Fig. 1A) using RGB colour model 255.

2.1.2. Angiography

The cord was clamped immediately after delivery to ensure the vessels were kept dilated by congested blood. One umbilical artery was cannulated within the cord (ahead of the Hyrtl's anastomosis, about 5 cm before cord insertion) using a 20G cannula held in place with a suture. The two umbilical arteries are connected by the Hyrtl's anastomosis near the cord insertion in most human placentas [15,16]. Therefore, cannulating one of the arteries ahead of the anastomosis permitted flow of perfusate into both arteries. About 20 ml of 5000 i.u./L heparin sulphate in phosphate buffered saline (PBS) was infused to prevent intravascular coagulation. About 20 mls of iohexol radio-opaque dye (OmnipaqueTM 300, GE Healthcare, Norway) was injected manually through the cannula to fill the arteries. Radiographs of the fetal side of the placenta were taken simultaneously (Fig. 1B). In the initial experiment, the dye outlined the vessels as they filled up but disappeared quickly into the placenta tissue due to its low viscosity. Therefore we substituted Omnipaque with barium sulphate (20–40 ml depending on placenta/vessel size) (Bacco U.K Limited), diluted to 0.2 g/ml of water. This filled the arterial system down to the villous capillaries, gave a better outline and stayed longer in the vessels. Angiograms of the placental arteries were then taken with the umbilical cord in different positions. Angiography was performed on 3 normal placentas.

2.1.3. Corrosion casting

One umbilical artery was cannulated ahead of the Hyrtl's anastomosis and heparinised as described for angiography technique. Freshly prepared casting material – Batsons no 17 Anatomical Corrosion Kit (Polysciences Inc, Europe), was manually injected through the cannula as in published protocols [17,18] until back pressure prevented further injection. The cord was then clamped below the point of cannulation, to prevent leakage of the polymer. The placenta was left overnight on a polythene sheet placed on ice to lower the temperature and thus allow polymerisation to occur at a more uniform rate. The following day, the whole casted placenta was immersed in 500 ml of 20% w/v potassium hydroxide (KOH; Fisher Scientific, Lutterworth, UK) within a gasket sealed tub, in a water bath at 40 °C. KOH solution was changed 6 hourly until the tissue was completely corroded. The solution was then replaced with distilled water for another 6 h to rinse off the KOH. The rinsed cast was photographed and air dried. Corrosion casting was performed on 12 normal and 6 FGR placentas.

2.1.4. Data analyses

A numbering scheme, devised by modification of a model previously described by Gordon et al. [19], was used to identify and quantify the chorionic arterial branches in the casts. The numbering scheme was applied for the three techniques – photography, angiography and corrosion casting. A schematic description of this numbering scheme is shown in Fig. 1A. In brief, the arterial network from each umbilical artery was analysed separately. The umbilical artery with the larger

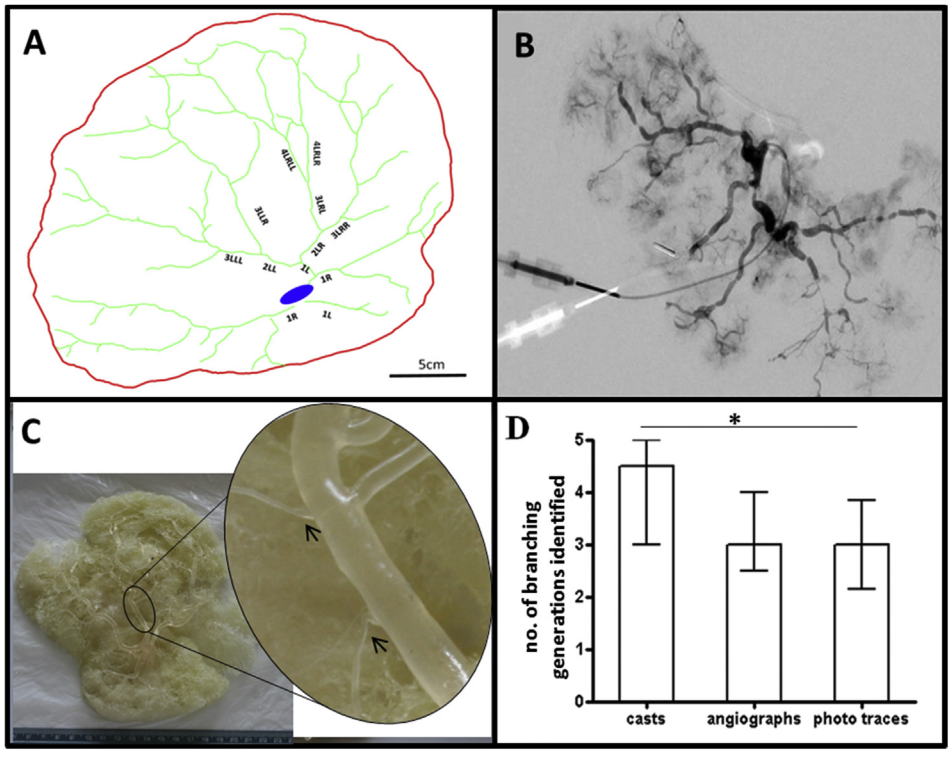

Fig. 1. Comparison of three different methods of fetal arterial vascular analysis in normal placentas: **A** – Photo tracing showing the numbering scheme devised as model for identifying and quantifying the arterial segments. Chorionic arterial segments were classified from umbilical artery insertion on the chorionic plate (as shown in A). With the placenta border outlined in red, the arteries in green and the cord insertion point marked with blue oval, the number of branches measurable was noted. On the chorionic plate, each umbilical artery bifurcates to give rise to generations of right and left divisions. The artery with the larger network of branches was labelled umbilical artery 1(UA1) and the other artery UA2. For example, the arterial segment labelled "3LRL" in image A is the left branch of the right second branch of the first left branch of umbilical artery 1. **B** – Angiography, **C** – Corrosion casting, image showing chorionic arterial segments and intraplacental arteries (arrows in inset) of a normal placenta. **D** – Graph of $n = 3$ (normal placentas) comparisons using the three different methods (medians $\pm$ interquartile range shown) demonstrating a trend to more branches using corrosion casting. Significance refers to comparison of casts with photo traces. *: $p = 0.0302$; Kruskal-Wallis with Dunn's multiple comparison test.