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QUANTITATIVE CHARACTERIZATION OF THE BRANCHING OF PLACENTAL VILLOUS CAPILLARIES

Marie Jirkovská[1], Lucie Kubínová[2], Jiří Janáček[2], Josef Bendl[3]

[1]Institute of Histology and Embryology, 1st Faculty of Medicine, Charles University
[2]Institute of Physiology, Academy of Sciences of Czech Republic
[3]Department of Obstetrics and Gynecology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

ABSTRACT

The topological properties and spatial arrangement of the capillary bed in terminal villi of normal human placentas were studied using confocal microscopy. The Euler number of capillaries was estimated in villi sampled by the optical disector principle. The corresponding topological reconstructions were made and surface renderings of selected villi and their capillary bed were performed. The capillary bed with no redundant connections was found in 80 per cent of villi, those with one connection in 15 per cent and those with two connections in 4 per cent of cases. It is concluded that in the terminal villi of normal placentas the longitudinal growth of capillaries appears to predominate over their branching.

Key words: capillaries, Euler number, placental terminal villus, surface rendering, topological reconstruction.

INTRODUCTION

Although the capillary bed of terminal villi plays a key role in human placental transport, little is known about its organization. It is difficult to study the variability of topological properties of the villous capillary bed by conventional microscopical techniques providing only two-dimensional images (Arts, 1961; Boe, 1969). Due to technical reasons, neither corrosion casts (Kaufmann et al., 1985; Akiba et al., 1987; Burton 1987), nor the manual construction of three-dimensional models from serial physical sections (Kaufmann et al., 1985) allow extensive and systematic study of the three-dimensional (3-D) arrangement of capillaries in terminal placental villi. On the other hand, confocal microscopy proved to be useful in studies of the villous capillary bed arrangement (Kubínová et al., 1996; Jirkovská et al., 1998) because it enables thorough analysis of series of thin optical sections encompassing entire terminal villi. Such series of confocal images were thus exploited also in the presented study.

As no systematic study of the spatial arrangement of placental capillary bed has been reported till now, our aim was to perform a pilot study with terminal villi sampled uniformly from normal human placentas at the end of gestation. The topology of the villous capillary bed

was quantified by its Euler number. The capillary branching pattern was described in more detail by the corresponding topological reconstruction. For visualization of the villous capillary bed, a computer-based 3-D reconstruction of the villus and its capillaries was made.

MATERIALS AND METHODS

Five human placentas at the end of normal pregnancies (39th - 41st week of gestation) were collected after the termination of pregnancy by a Caesarian section for breech lie, orthopaedic, or ophthalmological reasons. Immediately after placental delivery, seven full-depth tissue samples were taken following systematic random sampling. Each sample was cut into three blocks corresponding to parabasal, middle and parachorial zones and fixed for 24 hours. The fixative solution contained 2.62 g dried sodium phosphate, 8 mL glutaraldehyde 25 %, 20 mL formaldehyde 36 %, 170 mL water, adjusted at pH 7.3 (Jauniaux et al., 1991). In order to stain the samples *in toto,* 1 g eosin was finally added to 200 mL of fixative. Specimens were then dehydrated and embedded in paraffin. From each tissue block a 120 μm thick section was cut by a microtome HM 340 E (MICROM Laborgeräte, Walldorf, Germany), deparaffinated, mounted and analyzed by a confocal microscope BioRad MRC 600 using the inverted microscope Nikon Diaphot (planapochromat oil immersion objective x 40 , N.A. = 1.30). Fifteen different fields were sampled systematically from each section. Individual well developed terminal villi (i.e. those having a narrowed neck) lying approximately parallel to the section plane, i.e. completely inside thick physical sections, were sampled for further analysis by the optical disector principle (Gundersen, 1986). The thickness of the optical disector was 20 μm.

In sampled villi, the topological properties and the spatial arrangement of the villous capillaries were studied:

a) Topological properties

The Euler number of the capillaries (represented by the capillary lumina) was measured by the method of the sweeping plane (De Hoff, 1968) and the topological reconstruction of the capillary bed was made. For these measurements it was usually sufficient to focus through the studied villus; in the case of villi with a more complicated course of capillaries, confocal images from a series of optical sections (1 μm apart) were captured, stored and analysed by carefully comparing subsequent sections (see Kubínová et al., 1996; Jirkovská et al., 1998).

b) Spatial arrangement

The surface renderings of several selected villi and their capillary beds were made: First, series of optical sections of the villus (1 μm apart) were printed. Then, in each section, contours of capillary and villus profiles were traced manually, using a digitization tablet Genius1812D (Kye Systems, Taiwan) and a custom-made programme CutView (developed by Z.Tomori and R.Hlinka, Institute of Experimental Physics, Košice, Slovak Republic). The obtained digital data were further processed in the IRIS Explorer graphical programming environment run on an INDY (SGI, USA) graphical workstation by using custom-made modules (e.g. 3-D smoothing by the 3-D Gaussian filtration). Finally, the surfaces of the villus and its capillary bed were detected and visualized by IRIS Explorer modules.

Table 1: Proportion of villous capillaries with no connection (i.e. $\chi = 1$), one connection (i.e. $\chi = 0$), two connections (i.e. $\chi = -1$), and three connections (i.e. $\chi = -2$).

Placenta No.	Total number of villi	Ratio of villi with no connection [%]	Ratio of villi with 1 connection [%]	Ratio of villi with 2 connections [%]	Ratio of villi with 3 connections [%]
1	63	77.8	19.0	1.6	1.6
2	70	85.7	8.6	4.3	1.4
3	74	67.6	23	9.5	0
4	80	87.5	8.8	3.8	0
5	89	83.1	14.6	2.2	0
Mean	**75**	**80.3**	**14.8**	**4.3**	**0.6**

RESULTS

The capillary bed with no redundant connections (corresponding to the Euler number χ equal to 1) was found most frequently (Table 1). More than half of villous capillaries were formed by a single loop (Fig.1,2a) while the rest of them consisted most often of three concurrent branches. The capillary bed with one connection (i.e. with $\chi = 0$) was found on average in 15 per cent of cases (Fig.1,2b); there were about 4 per cent of those having two connections. From 376 examined villi, only two had three connections while more than three redundant connections were not found in any of the villi.

DISCUSSION

The present study confirmed that the above methods are feasible to study a uniform and sufficiently large sample of terminal villi and thus obtain objective information on the arrangement of their capillary beds. The staining of specimens *in toto* by eosin applied simultaneously with fixation followed by eosin differentiation during the dehydration process results in a good and uniform staining of tissues within the thick slice, suitable for observations by a confocal microscope. The application of the optical disector principle, used for unbiased sampling of villi, was simple and straightforward as well as the measurement of the Euler number and the topographical reconstruction of capillary bed when in most cases it was sufficient to focus through the studied villus without the need to save the series of villus sections. As a result, the measurement of one villus usually took only several minutes. The 3-D reconstruction of the capillary bed was much more time-consuming because it involved not only the capture of confocal images but also the manual tracing of contours of capillary and villus profiles as the automatic segmentation of capillaries was not feasible. On the other hand, the 3-D representation of the spatial arrangement of capillaries and their relationship to the other tissue components of the villus gives a comprehensive information.

The development of the villous capillary bed is a complex process ensuring the fetal supply. Our findings in the normal placenta show that the capillaries in the terminal villi often have a tortuous course but their main direction is usually approximately parallel to the longitudinal axis of the villus. Taking into account the rare occurence of redundant connections, it can be concluded that the longitudinal growth of the capillaries predominates

over their branching. On the other hand, the manifestations of intravillous capillary proliferation are commonly present in the form of blindly ending buds arising from the capillary wall (see Fig.1 and also Jirkovská et al., 1998).

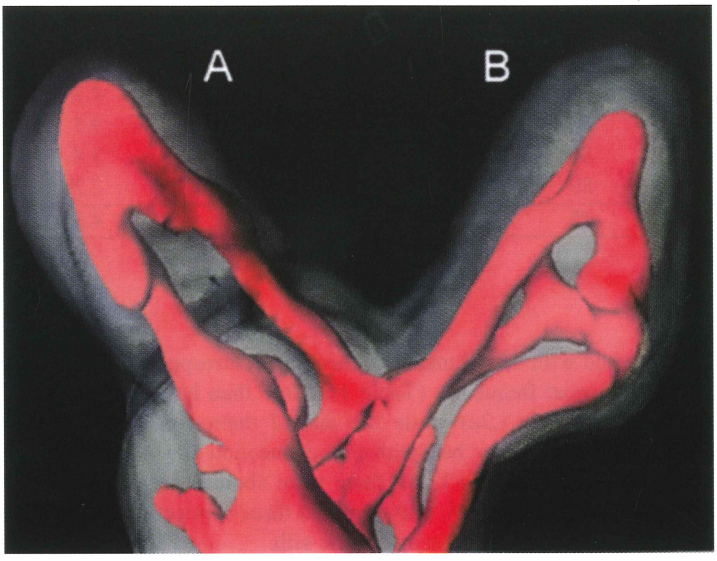

Fig.1. Surface rendering of two terminal villi (grey) with their capillary bed (red). The capillaries of the left villus (A) are forming a single loop while the capillary bed of the right villus (B) has one redundant connection.

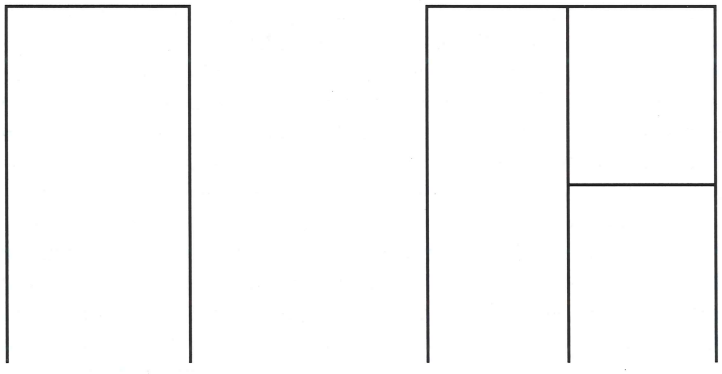

Fig.2. Topological reconstructions of the capillary bed shown in Fig.1. (a) Topological reconstruction of a single loop (A). (b) Topological reconstruction of the capillary bed (B) having one redundant connection.

The presented results somewhat revise models of the villous vascularization based on previous studies (Arts, 1961; Boe, 1969; Kaufmann et al., 1985). The high frequency of capillary bed consisting of a single loop or three concurrent branches with predominantly longitudinal course shows that these arrangements can be considered as optimal in the normal pregnancy. However, the situation can be different in various clinical disorders of pregnancy, as the changed structural picture of the villous vascularization has been found e.g. in the case of terminal villi deficiency, maternal diabetes mellitus or intrauterine growth retardation (Benirshke and Kaufmann, 1995). In our preliminary studies of diabetic placentas, villous capillary bed with a higher number of redundant connections was frequently observed (Jirkovská et al., 1998).

Many gestational disorders are presumably caused by different types of hypoxia and these questions have been recently extensively discussed (Kingdom and Kaufmann, 1997; Burton, 1997; Dancis, 1997). As one common organ reaction to hypoxia is the restructuring of the blood supply (Guillemin and Krasnow, 1997), the presented methods of analysis of the villous vascular bed can contribute to the elucidation of some placental pathologies.

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REFERENCES

Akiba K, Kuwabara Y, Mizuno M, Fukuda S. Semiquantitative study of the vascularization of human term placenta using scanning electron microscopy. J Clin Electron Microsc 1987; 20: 5-6.

Arts NFT. Investigations on the vascular system of the placenta. Am J Obstet Gynecol 1961; 82: 147-166

Benirschke K, Kaufmann P. Pathology of the human placenta, 3rd edn. Springer, Berlin Heidelberg New York,1995.

Boe F. Studies of the human placenta. III. Vascularization of the young fetal placenta. A. Vascularization of the chorionic villus. Acta Obstet Gynecol Scand 1969; 48: 159-166.

Burton GJ. The fine structure of the human placental villus as revealed by scanning electron microscopy. Scann Microsc 1987; 1: 1811-1828.

Burton GJ. On 'Oxygen and Placental Villous Development: Origins of Fetal Hypoxia'. Placenta 1997; 18: 625-626.

Dancis J. On 'Oxygen and Placental Villous Development: Origins of Fetal Hypoxia'. Placenta 1997; 18: 623-624.

De Hoff RT. Curvature and topological properties of interconnected phases. In: De Hoff RT, Rhines FN, eds. Quantitative microscopy. Mc Graww Hill, New York, 1968: 291-325.

Guillemin K, Krasnow V. The hypoxic response: Huffing and HIFing. Cell 1997; 89: 9-12.

Gundersen HJG. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. J Microsc 1986; 143: 3-45.

Jauniaux E, Moscoso G, Vanesse M, Campbell S, Driver M. Perfusion fixation for placental morphologic investigation. Hum Pathol 1991; 22/5: 442-449.

Jirkovská M, Kubínová L, Krekule I, Hach P. Spatial arrangement of fetal placental capillaries in terminal villi: a study using confocal microscopy. Anat Embryol 1998 - in press.

Kaufmann P, Bruns U, Leiser R, Luckhardt M, Winterhager E. The fetal vascularisation of term human placental villi. II. Intermediate and terminal villi. Anat Embryol 1985; 173: 203-214.

Kingdom JCP, Kaufmann P. Oxygen and placental villous development: origins of fetal hypoxia. Placenta 1997; 18: 613-621.

Kubínová L, Jirkovská M, Hach P. Stereology and confocal microscopy: Application to the study of placental terminal villus. Acta Stereol 1996; 15: 153-158.

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