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MORPHOMETRIC ANALYSIS OF THE VASCULARIZATION OF THE TERMINAL VILLI IN NORMAL AND DIABETIC PLACENTA

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ABSTRACT

Samples from 5 placentas of healthy mothers and from 5 placentas of mothers with insulin-dependent diabetes mellitus were assessed quantitatively with respect to the vascularization of the terminal villi. Tissue blocks were taken from the central parabasal region of the placenta. The fixed and plastic resin embedded samples were cut into 2-4 μm thick sections. After staining the sections were submitted for evaluation to an image analyzer. On 250 transverse sections of the terminal villi from both the normal and diabetic placentas the following ratios were calculated: total area of capillaries / total area of villus (Ac/Av). The values of the Ac/Av ratio were found significantly higher in healthy mothers (0.243 ± 0.102) as compared to diabetic mothers (0.212 ± 0.105). The levels of mean Ac/Av ratios multiplied with the weight of individual placentas were not significantly different in the control (183.20 ± 20.84) and the diabetic group (179.14 ± 27.90). The smaller development of the capillary bed in the terminal placental villi under diabetic conditions is probably compensated with the increased formation of new villi manifested in higher placental weight.

Key words: diabetes mellitus, morphometry, placenta, vascularization.

INTRODUCTION

The placental capillaries are branched in the terminal placental villi. Their surface layer (trophoblast) and the capillary wall form the transport barrier in the human placenta. The well developed and functionally efficient capillary bed is necessary for the optimal foetomaternal transport. The diabetic status of the pregnant woman influences the development of the placental capillary bed as was described in microscopic studies e.g. by Asmussen (1982), Björk (1982),Semmler et al. (1982), Semmler & Emmrich (1989). Their findings among others were hypovascularization, normal vascularization and also hypervascularization of the terminal villi. In view of these discrepant results we followed the vascularization of the terminal placental villi under normal and diabetic conditions using a stereologic method.

MATERIAL

Samples from 5 placentas of healthy mothers (control group) and from 5 placentas of mothers with type I diabetes mellitus (insulin-dependent diabetes mellitus), at the end of gestation, were used. All the diabetic mothers had diabetes lasting at least 12 years (range 12 - 19 years) with signs of diabetic retinopathy. No other complications (e.g. hypertension, pre-eclampsia) were discovered. All the patients had moderate or good diabetic control throughout their pregnancies. Samples were always taken from the central parabasal region of the placenta. The material was cut into small pieces, fixed in aldehyde mixture (Karnovsky, 1965), postfixed with osmium tetroxide and embedded into Epon 812. From each placenta fifteen blocks were obtained. Ten randomly chosen blocks were cut into 2-4 μm thick sections and stained with toluidine blue. One section per block was used and on each section five terminal villi were evaluated.

METHOD

The sections were submitted for evaluation to an image analyzer Olympus CUE - 2. A 40x objective was used. On 250 transverse sections of the terminal villi from both the normal and diabetic placentas the total area of foetal capillaries and the area of villus were measured (Fig. 1) and the following ratios were calculated:

$$\frac{\text{total area of foetal capillaries } [\mu m^2]}{\text{total area of villus } [\mu m^2]} \quad (Ac/Av) \qquad (1)$$

Ac/Av is a ratio which can be difficult to interpret (Braendgaard & Gundersen, 1986), therefore we multiplied it with the weight of non-trimmed placenta in order to get a simple expression of vascularization in the whole placenta. The data were compared using Student's t-test.

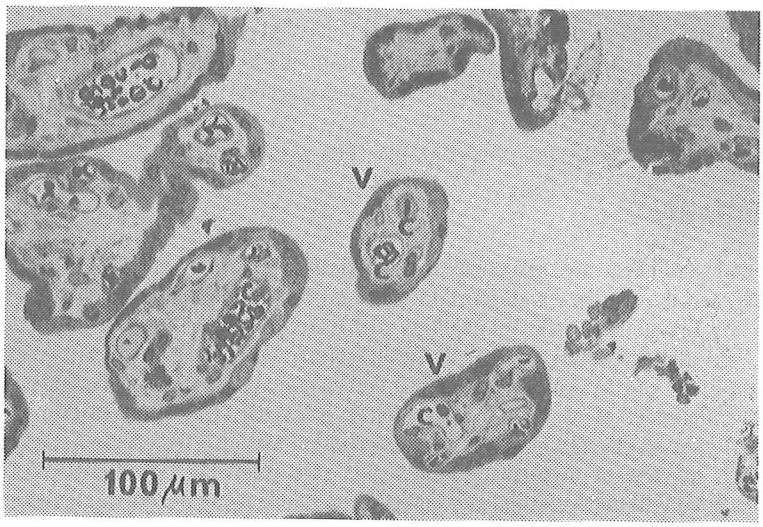

Fig. 1: Placental terminal villi at the end of gestation (c= capillary, v=villus)

RESULTS

The values measured on the individual placentas reflect not only the natural variability in both the control and the diabetic group but also the marked difference of the Ac/Av ratios between both groups of placentas. The average value ± SD of the Ac/Av ratio was higher in the control group than that in the diabetic group ($2p < 0.05$) (Table 1). The lowest values of Ac/Av ratio were more numerous in the placentas of diabetic mothers than those in the placentas of healthy mothers and the highest values (range 0.50 - 0.59) were found only in placentas of healthy mothers. The comparison of values of Ac/Av ratios multiplied with the weight of placenta demonstrated that the difference between the control and the diabetic group is not significant ($2p > 0.05$).

Table 1: Summary of results

	No.	Ac/Av [mean ± SD]	placental weight [g]	mean Ac/Av x placental weight [g]
control group	1	0.243 ± 0.106	800	194.40
	2	0.262 ± 0.109	780	204.36
	3	0.232 ± 0.106	665	154.28
	4	0.266 ± 0.091	730	194.18
	5	0.211 ± 0.091	800	168.80
	total [mean ± SD]	0.243 ± 0.102		183.20 ± 20.84
diabetic group	1	0.225 ± 0.135	970	218.25
	2	0.164 ± 0.083	1115	182.86
	3	0.225 ± 0.096	650	146.25
	4	0.189 ± 0.078	1000	189.00
	5	0.257 ± 0.103	620	159.34
	total [mean ± SD]	0.212 ± 0.105		179.14 ± 27.90

DISCUSSION

In patients with diabetes mellitus several related lesions are caused by the alteration of vascularization. Considering that the placental circulation is not (because of its foetal origin) a part of the maternal circulatory system the diabetic status of the mother can influence the placental capillary bed indirectly. On the other hand the placental circulation constitutes an anatomical and functional unit with the foetus. Therefore the placental development probably depends on the foetal requirements. The described findings of morphological manifestations of the maternal diabetes are different. Asmussen (1982) found numerous newly formed small capillaries penetrating into the trophoblast. Nevertheless the de novo formation of the capillaries takes place throughout the whole normal gestation (Kosanke et al., 1991; Scheffen et al., 1991). Two microscopic studies made by Semmler et al. (1982) and Semmler & Emmrich (1989) show that the consistent normoglycemic metabolic control throughout pregnancy reduces placental structural disorders and that the hypovascular villi are symptomatic for the more severe forms of maternal diabetes. Using the angiographic method Björk (1982) found the hypovascular villi in the placentas of diabetic mothers as well.

The greater weight of the diabetic placentas is connected with the more intensive development of new terminal villi, with their increased branching and with the increased villous surface area (Teasdale, 1983; Björk and Persson, 1984; Boyd et al., 1986). The capillaries in the newly developed villi have thinner basement membranes. Their higher proportion explains the lower average thickness of the capillary basement membrane in the placentas of mothers with more severe forms of diabetes (Jirkovská, 1991).

On the basis of that information we tried to estimate the proportion of the capillaries in terminal placental villi using the stereologic method. We are aware that the 2-D measurements of Ac\Av ratio are not possible to be interpreted in view of the 3-D organization of the capillary bed. Nevertheless the significantly smaller vascularization of terminal villi demonstrates a negative effect of maternal diabetes mellitus upon the formation of foetal placental capillaries. The differences of the values of Ac\Av ratios multiplied with the placental weights were found not significant. We judge that the underdeveloped capillary bed in the terminal villi of diabetic placentas is compensated with the more intensive formation of the new villi in heavier diabetic placentas (see Table 1).

From the greater weight of the placenta follow not only the increase of the resorptive area of syncytiotrophoblast but also the decrease of the volume of intervillous space (Nylund et al., 1982). Also the developed diabetic vasculopathy of spiral arteries in placentas of mothers with severe diabetes can cause the reduction of blood flow in the intervillous space (Björk et al., 1984). The foetus of the diabetic mother suffers from hypoxia and acidosis due to more glycosylated and thus functionally inferior maternal haemoglobin. In addition the higher supply of glucose in the maternal blood can have serious consequences for the biological functions of proteins involved in the transport barrier. The non-enzymatic glycation alters the functional efficiency of the important molecules in the basement membranes (e.g. type IV collagen) (van Boekel, 1991). Such conditions are suspected to cause (after the exhaustion of the functional reserves of the transport barrier) an extensive enlargement of the resorptive surface without the corresponding development of capillary bed.

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