# MORPHOLOGY AND PATHOMORPHOLOGY

# Vascular Endothelial Growth Factor and Its Receptors in the Placenta of Women with Type 1 Diabetes Mellitus

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We performed a morphological study of placentas from women with type 1 diabetes mellitus receiving insulin therapy (insulin pump). Expression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1, VEGFR-2, VEGFR-3) was demonstrated by immunohistochemical methods. Processes of branched angiogenesis predominated in the placentas from women with type 1 diabetes mellitus. Immunohistochemical study revealed more intensive reaction of VEGF and its receptors in syncytiotrophoblast and capillary endothelium of terminal villi.

**Key Words:** *villus; diabetes mellitus; placenta; vascular endothelial growth factor*

Placenta plays a central metabolic role and apart from the synthesis of various hormones it regulates transport of nutrients from the mother to the fetus and promoted metabolic adaptation of the maternal organism to different periods of pregnancy. Pregnancy against the background of diabetes mellitus is associated with high incidence of fetal and maternal pathologies; some of them are determined by placental dysfunction [2].

Type 1 diabetes mellitus (DM1) in pregnant women is associated with disturbances in glucose and oxygen metabolism, which affects to the development and function of the placental villous tree [6]. Under these conditions, the main changes occur in cells contacting with maternal blood (syncytiotrophoblast) and fetus (endothelium of villous vessels) [5]. In turn, adequate growth and remodeling of fetoplacental vessels depends on various factors located on both sides of the placental barrier. Pathological changes in maternal hemodynamics and chemical composition of the blood (hypoxia, hyperglycemia) changes in the level of growth factors, cytokines, and inflammatory mediators can directly affect the growth, development, and function of fetoplacental vessels and, hence, the fetus.

The leading role in angiogenesis processes in the placenta is played by vascular endothelial growth factor (VEGF) regulating the development of new blood vessels via interaction with the corresponding tyrosine kinase receptors [7,9].

Here we performed immunohistochemical analysis of VEGF and its receptors (VEGFR-1, VEGFR-2, VEGFR-3) in the placenta of women with DM1.

## MATERIALS AND METHODS

We performed morphological analysis of 18 placentas obtained after term delivery (39-40 weeks). The main

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group is presented by 12 pregnant women (age 26-39 years) with medium-severe DM1 receiving intensified scheme of insulin therapy (ultrashort acting insulin Aspart) according to the basis bolus principle via insulin pump. The control group included 6 women (age 23-37 years) with normal body weight and physiological pregnancy.

After macroscopic examination of the placentas, specimens taken from the central zone were fixed in 10% neutral formalin. Histological study was performed on paraffin sections stained with hematoxylin and eosin. Immunohistochemical analysis was performed routinely using ready-to-use rabbit polyclonal antibodies to VEGF, VEGFR-1 (Flt-1), VEGFR-2 (Flk-1), VEGFR-3 (Flk-4) and a polymer detection system (Spring Bioscience). The samples were boiled in citrate buffer (pH 6.0) for primary demasking of the antigen and incubated for 15 min with 0.3% $H_2O_2$ for blockade of endogenous peroxidase. Hematoxylin was used for background staining. Quantitative analysis of immunohistochemical expression of VEGF and its receptors was performed in syncytiotrophoblast, vascular endothelial cells, and mesenchymal cells of terminal and mature intermediate villi using an image analysis system on the basis of Axio Imager M1 microscope and AxioVision software (Carl Zeiss). The data were processed using Statistica 6.0 software.

## RESULTS

Histological study of control preparations of the placenta stained with hematoxylin and eosin showed that the villous tree was primarily presented by capillarized terminal villi, while mature intermediate villi were less numerous. The structure of the placentas generally corresponded to the pregnancy term. The compensatory and adaptive processes (syncytial knots and syncytiocapillary membranes) were moderately expressed. Fibrinoid depositions (primarily around the truncal villi) and small calcinosis foci were seen near the basal plate.

In DM1, the maturity of placental villi corresponded to gestation term in 4 cases (33.3%), while in 8 placentas (66.7%) maturation of the villous tree was delayed by 2-4 weeks. The villous tree was primarily presented by medium-size and small rounded villi with abundant capillaries and syncytial knots. It should be noted that capillaries were located not only at the periphery of the villus, but the equally distributed in it. In 3 cases, changes of the villi (chorangiosis) were detected, and small chorangioma (1×1×1 cm) was found in one placenta. Thickening of syncytiotrophoblast indicated increased epithelium-capillary distance. In some villi, more or less pronounced edema and increased number of stromal macrophages (Hofbauer cells) were observed. The relative contribution of intervillous space decreased under these conditions.

Immunohistochemical analysis of control placenta preparations showed cytoplasmic localization of VEGF in all cells of the villi. The maximum expression was found in syncytiotrophoblast and the minimum in mesenchymal cells (Fig. 1, *a*). In women with DM1, more intensive reaction of VEGF in structures of terminal villi was noted (Fig. 2). The most intensive expression surpassing the control level by 29.9% ($p<0.05$) was found in syncytiotrophoblast cells (Fig. 1, *b*). VEGF expression in endotheliocytes surpassed the normal values by 21.1% ($p<0.05$), while in mesenchymal cells by only 6.1% ($p>0.05$).

Immunohistochemical analysis of VEGFR distribution in control placentas revealed lower levels of their expression in cells of terminal villi in comparison with the expression of the corresponding growth factor. VEGFR-3 was more intensively expressed, especially in the syncytiotrophoblast. The level of VEGFR-2 expression was minimum, especially in mesenchymal cells (Fig. 1, *c*).

In placentas from women with DM1, expression of the three studied receptors EGFR was enhanced and this increase was more marked in comparison with the expression of VEGF. The maximum intensity of the reaction was noted for VEGFR-2 (Fig. 1, *d*): it surpassed the control level in mesenchymal cells of terminal villi by 2.4 times and in capillary endothelial cells and syncytiotrophoblast cells by 1.8 times ($p<0.05$). Maximum differences in VEGFR-1 expression in DM1 (Fig. 1, *e*) and normal physiological pregnancy (Fig. 1, *f*) were also observed in mesenchymal cells. Minimum values, but surpassing the control level by 43.1% ($p<0.05$) were observed in endothelial cells of terminal villi. In turn, the most intensive expression of VEGFR-3 (by 57.8% surpassing the normal) was detected in syncytiotrophoblast cells and minimum expression (but still surpassing the control by 27.4%) was noted in mesenchymal cells.

Thus, placentas of women with DM1 were characterized by uneven increase in the expression of the studied VEGF receptors in cells of terminal villi. This conclusion was confirmed by the analysis of relative intensity of the expression of VEGF and its receptors (Table 1).

Indeed, the calculated coefficients in DM1 were higher than in physiological pregnancy. Different degree of elevation of these values in different cell types is worthy of note, which probably attests to placental dysfunction of DM. The most pronounced deviation from the normal were revealed for VEGFR-2/VEGF (by 130.5%, $p<0.01$) and VEGFR-1/VEGF (by 78.9%, $p<0.05$) in mesenchymal cells of terminal villi.

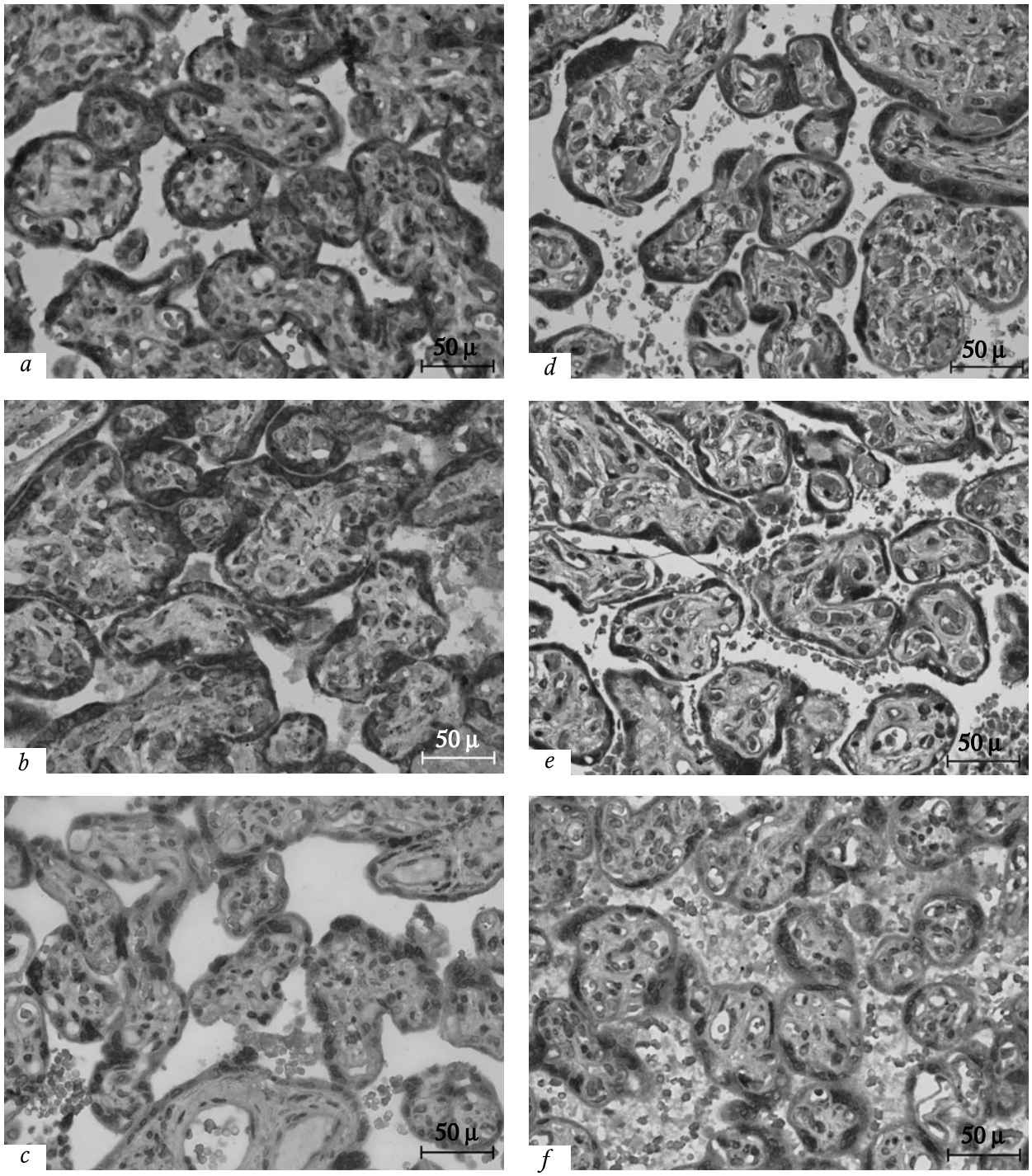


**Fig. 1.** Immunohistochemical changes in placental villi in the control group (*a*, *c*, *e*) and women with DM1 (*b*, *d*, *f*). *a, b)* VEGF expression; *c, d*) VEGFR-2; *e*, *f*) VEGFR-1. Immunoperoxidase staining, ×400.

In capillary endothelial cells and syncytiotrophoblast cells, the maximum increase was noted for VEGFR-2/VEGF, by 47.8 and 368%, respectively ($p<0.05$).

In general, changes in the placentas from women with DM1 were characterized by predominance of angiogenesis processes with the formation of branched vessels and more pronounced expression of VEGF and its receptors in all studied structures of terminal villi in comparison with normal placentas.

The observed changes agree with published results of morphological and molecular genetic studies of the placenta in DM [1,4]. It was found that VEGFR-2 is the main transmitter potentiating the mitogenic and angiogenic effects of VEGF [3,12]. Binding of VEGF

**TABLE 1.** Ratio of Receptor to Growth Factor Expression in Structures of Placental Villi in DM1 (*M*±*m*)

| Cells | VEGFR-1/VEGF | | VEGFR-2/VEGF | | VEGFR-3/VEGF | |
|---|---|---|---|---|---|---|
| | control | DM1 | control | DM1 | control | DM1 |
| SCT | 0.62±0.02 | 0.73±0.02 | 0.50±0.02 | 0.69±0.02 | 0.74±0.03 | 0.89±0.03 |
| EC | 0.56±0.02 | 0.67±0.02 | 0.46±0.02 | 0.69±0.02 | 0.65±0.02 | 0.73±0.03 |
| Mes | 0.53±0.02 | 0.95±0.03 | 0.39±0.02 | 0.89±0.03 | 1.39±0.05 | 1.67±0.06 |

**Note.** SCT: syncytiotrophoblast; EC: capillary endothelial cells; Mes: mesenchymal stromal cells.

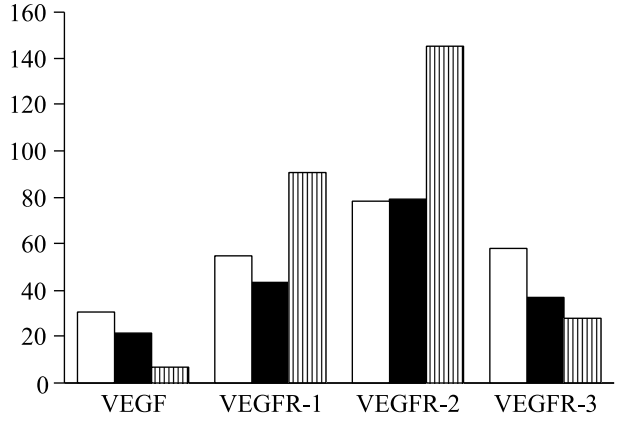


**Fig. 2.** Changes in the expression of VEGF and its receptors in placental villi in DM1. Ordinate: % above the normal values. Open bars: syncytiotrophoblast; dark bars: capillary endothelial cells; shaded bars: mesenchymal stromal cells of villi.

with VEGFR-2 activated angiogenesis via stimulation of proliferation and growth of endothelial cells. VEGFR-2 induces the growth of vessels via activation of the Raf-Mek-Erk pathway. The key role of this receptor in vasculo- and angiogenesis is also confirmed by the fact that vascular islets and blood vessels do not form in mice lacking these receptors, which leads to intrauterine death of these embryos on gestation days 8.5-9.5 [11].

Hyperglycemia and moderate hypoxia are associated with enhanced expression and binding of VEGF with VEGFR-2 in endothelial cells of villous vessels, which leads to hypercapillarization of intermediate and terminal villi [8]. It should be noted that pregnancy began against the background of decompensation of carbohydrate metabolism and compensation was achieved later (gestation weeks 6-8) under conditions of pump insulin therapy.

Insulin, in turn, enhances VEGF expression through direct interaction with its promoter [10]. During pregnancy complicated by DM, hyperglycemia in the maternal body induces hyperinsulinemia of the fetus, which stimulates production of VEGF by endothelial cells of the fetoplacental vessels. In turn, insulin therapy can enhance VEGF expression in villous trophoblast.

Thus, considerable changes in immunohistochemical profile of VEGF and its receptors in cells of placental villi were revealed despite effective correction of glucose level in all women. These changes in immunophenotype of terminal villi attest to placental abnormalities and can disturb the development of the fetus and newborn. Hence, the development of new methods of correction of metabolic disturbances in pregnant women with DM is an urgent problem.

## REFERENCES

1. M. O. Babawale, S. Lovat, T. M. Mayhew, *et al., Diabetologia*, **43**, No. 9, 1185-1196 (2000).
2. J. Djelmis, G. Desoye, and M. Ivanisevic, *Diabetology of Pregnancy*, Karger; Basel (2005).
3. N. Ferrara, H. P. Gerber, and J. LeCouter, *Nat. Med.*, **9**, No. 6, 669-676 (2003).
4. E. Geva, D. G. Ginzinger, C. J. Zaloudek, *et al., J. Clin. Endocrinol. Metab.,* **87**, No. 9, 4213-4224 (2002).
5. U. Hiden, I. Lang, N. Ghaffari-Tabrizi, *et al., Curr. Vasc. Pharmacol.,* **7**, No. 4, 460-466 (2009).
6. E. Jauniaux and G. J. Burton, *Placenta,* **27**, Nos. 4-5, 468-474 (2006).
7. K. Kumazaki, M. Nakayama, N. Suehara, and Y. Wada, *Hum. Pathol.,* **33**, No. 11, 1069-1077 (2002).
8. L. Leach, A. Taylor, and F. Sciota, *J. Anat*., **215**, No. 1, 69-76 (2009).
9. L. Pietro, S. Daher, M. V. Rudge, *et al., Placenta*, **31**, No. 9, 770-780 (2010).
10. V. Poulaki, W. Y. Qin, A. M. Joussen, *et al., J. Clin. Invest*., **109**, No. 6, 805-815 (2002).
11. F. Shalaby, J. Rossant, T. P. Yamaguchi, *et al., Nature*, **376**, 62-66 (1995).
12. C. P. Sibley, M. A. Turner, I. Cetin, *et al., Pediatr. Res*., **58**, No. 5, 827-832 (2005).