Vascular Endothelial Growth Factor and its Receptors in the Placenta of Pregnant Women with Obesity

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Comparative morphological study of placentas from women with obesity and normal body weight was performed. Expression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1, VEGFR-2, VEGFR-3) was detected by immunohistochemical methods. Nonbranching angiogenesis predominated in the placentas from obese women. Immunohistochemical analysis showed reduced intensity of the reaction to VEGF in the syncytiotrophoblast and vascular endothelium of stem villi and enhanced VEGF expression in non-villous cytotrophoblast and endothelial cells of capillaries of mature intermediate and terminal villi; reduced expression of VEGFR-1 and increased levels of VEGFR-2 and VEGFR-3 in the studied structures were also noted.

Key Words: *obesity; placenta; vascular endothelial growth factor*

Among the types of extragenital pathology in obstetric practice, obesity in pregnant and parturient woman is of considerable importance, because the incidence of various complications in women with excessive body weight 2-3 times surpasses than in women with normal body weight [4,9].

It is well known that intrauterine development affects both the perinatal outcome and the health of the child, especially conditions associated with metabolic disorders [5,6]. Abnormal growth and development of the fetus are associated with the development of metabolic diseases after birth [8].

The placenta is a key regulator of fetal growth ensuring the delivery of nutrients and removal of metabolic products. It also produces a wide range of hormones and growth factors. Specific phenotype of the placenta, *e.g.* its size, structural features and blood supply, oxygen delivery, and transport of nutrients, was detected in various pathological conditions [14]. However, morphofunctional changes of the placenta in pregnant obese women have practically never been studied.

Transplacental nutrient and oxygen exchange directly depends on placental blood flow, which in turn depends on placenta vascularization. Vascular endothelial growth factor (VEGF) plays the key role in placental angiogenesis by regulating the development of new blood vessels through interaction with the corresponding tyrosine kinase receptors [2,12].

The purpose of this work was to perform immunohistochemical study of VEGF and its receptors (VEGFR-1, VEGFR-2, VEGFR-3) in the placenta of pregnant obese women.

MATERIALS AND METHODS

The work is based on morphological analysis of 20 placentas after timely delivery (39-40 weeks). The main group included 14 women (29-40 years) with alimentary-constitutional obesity; the control group included 6 patients (23-37 years) with normal body weight and physiological pregnancy.

After macroscopic study of the placentas, tissue fragments from the central zone were fixed in 10%

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neutral formalin. Histological examination 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 (Flt-4), mouse antibody to CD34 (clone QBEnd/10), and polymer detection system (Spring Bioscience). Primary retrieval of the antigens was performed by boiling in citrate buffer (pH 6.0); endogenous peroxidase was blocked by 15-min incubation with 0.3% hydrogen peroxide. The sections were counterstained with hematoxylin. Immunohistochemical reaction to VEGF and its receptors in the syncytiotrophoblast (SCT), non-villous cytotrophoblast (NVC), vascular endothelial cells of stem villi (VEC), and endothelial cells of the terminal and mature intermediate villi (ECV) was scored using a semiquantitative scale: no response (0), weak reaction (1), moderate reaction (2), and intensive reaction (3).

Morphometric analysis of the sections was carried out using an image analysis system on the basis of an Axio Imager M1 microscope and AxioVision software (Carl Zeiss). The area and perimeter of ECV, their capillaries, the number of capillaries and the relative area of the intervillous space were estimated. Vascularization index was calculated as the ratio of the total area of capillaries of the villi to the cross-sectional area of the villi (%). The quantitative data were processed statistically using Statistica 6.0 software.

RESULTS

During histological analysis of hematoxylin and eosin-stained placenta preparations from main group women, the correspondence of villous tree maturity to gestational age, moderate compensatory-adaptive and involutive-degenerative processes were registered.

The maturity of the villous tree corresponded to gestational age in 5 placentas and lagged behind it by 2-4 weeks in 9 placentas. In all observations, edema of villous stroma in varying degrees (Fig. 1, *a*) and wide intervillous space (Fig. 1, *b*) were detected in the placentas of the main group. Villous tree was mainly presented by villi of large diameter with low number of capillaries, and most villi were cut longitudinally. These changes in the placentas of obese women attested to the prevalence of nonbranching angiogenesis.

Morphometric analysis of sections showed that the area of intervillous space in the placentas from main group women surpassed the normal values by 75.7% ($p<0.05$, Table 1). The mean area and perimeter of ECV did not differ from the control values ($p>0.05$). The mean number of blood capillaries in one villus also did not differ in the studied groups. However, the lumen area and perimeter of a single capillary of chorionic villi in the placenta from main group women were smaller than the corresponding normal values by 37.1 and 18.9%, respectively ($p<0.05$), while the total area and perimeter of capillaries in a villus were lower by 35.3 and 26.9%, respectively ($p<0.05$). Calculated vascularization indices of the villi were also lower (by 32%, $p<0.05$).

Immunohistochemical analysis of placentas in the control group revealed maximum expression of VEGF in SCT of the villi (Fig. 1, *c*; and Fig. 2) with characteristic cytoplasmic staining. In placentas from women of the main group, a decrease in the intensity of the reaction to VEGF in SCT (Fig. 1, *d*) and VEC (9 and 16.8%, respectively) and more intensive expression in NVC (Fig. 1, *e, f*) and ECV (15.2 and 38%, respectively, $p<0.05$) were observed. As a result, the maximum expression of VEGF in the placentas of the main group was noted in elements of NVC.

Immunohistochemical analysis of VEGF receptor distribution in normal placentas revealed lower levels of their expression in endothelial cells and trophoblast cells compared to that of the growth factor itself (Fig. 2). The most intensive reaction to VEGFR-1 (Fig. 3, *a*) and VEGFR-3 were found in NVC and to VEGFR-2 in VEC. Minimum expression of all three receptors was observed in ECV.

In placental tissue of women of the main group the decreased expression of VEGFR-1 and increased level of VEGFR-2 and VEGFR-3 were revealed. Thus, the intensity of the reaction to VEGFR-1 in vascular endothelial stem and terminal villi was by 43 and 28.3% below the normal values ($p<0.05$). In this case,

TABLE 1. Morphometric Parameters of Placental Villi (*M±m*)

Parameter	Control group	Main group
Villus area, μ²	3090.5±364.2	3075.6±323.5
Villus perimeter, μ	209.9±18.6	208.7±12.2
Number of capillaries in the villus	4.8±0.3	4.9±0.4
Mean capillary area, μ²	219.0±18.8	137.8±17.6
Mean capillary perimeter, μ	56.5±3.2	45.8±2.3
Total area of capillaries, μ²	1033.2±198.9	668.9±108.6
Total perimeter of capillaries, μ	304.9±24.2	223.0±17.6
Degree of vascularization, %	32.5±2.5	22.1±1.9
Relative area of intervillous space, %	21.8±2.3	38.3±2.9

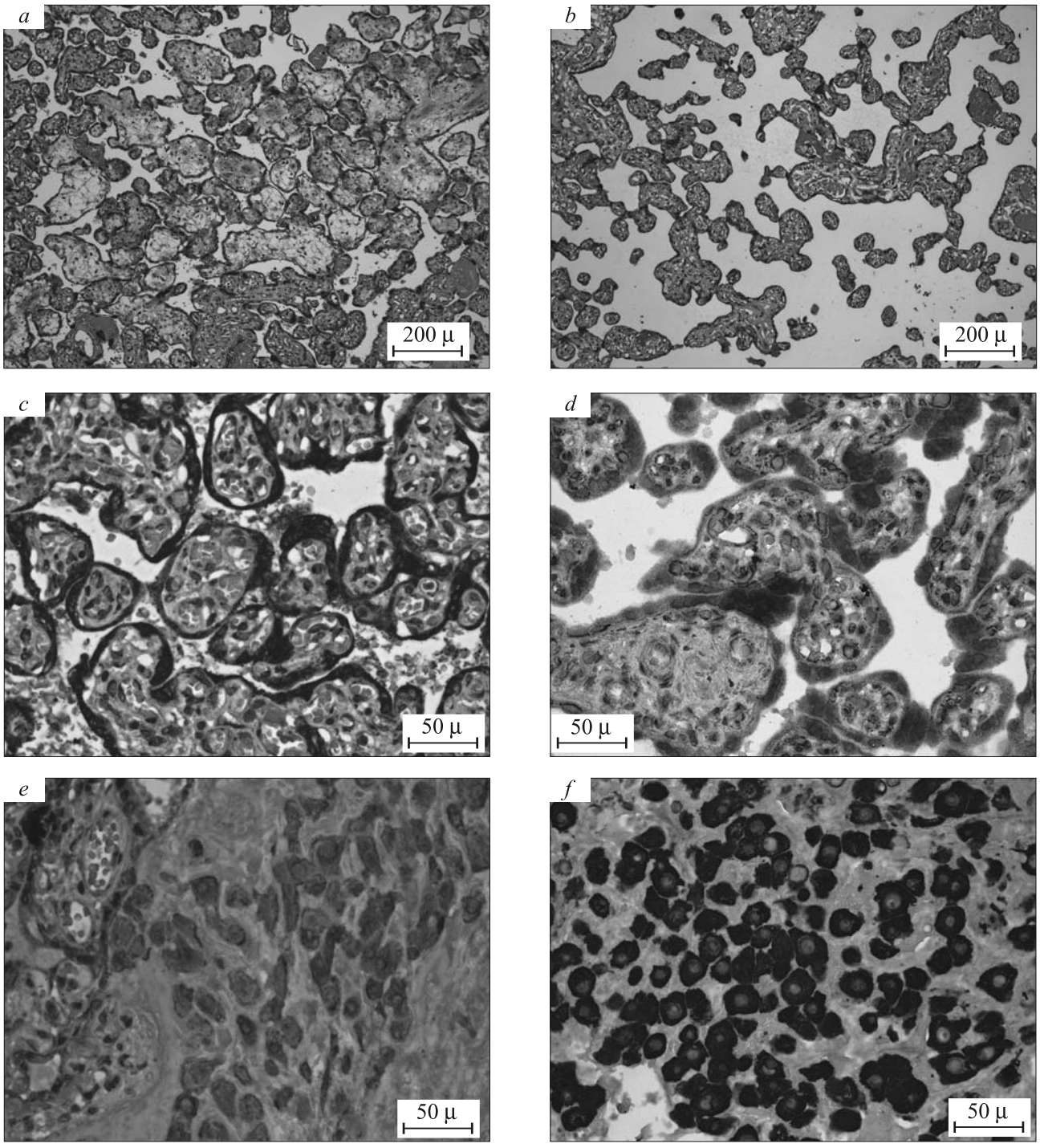

Fig. 1. Histological and immunohistochemical features of placentas from obese women (*a*, *b*, *d*, *f*) and women woth normal body weight (*c*, *e*). *a*: edema of villi; *b*: widening of the intervillous space, *c*, *d*: VEGF expression in SCT and ECV; *e, f*: VEGF expression in the cytoplasm of NVC. *a, b*: hematoxylin and eosin staining , ×100; *c-f*: immunoperoxidase method, ×400.

in elements of SCT and NVC it was lower by only 2.7 and 2.8%, respectively ($p>0.05$). In contrast, expression of VEGFR-2 in ECV exceeded control values by more than 4 times ($p<0.01$; Fig. 3, *b, c*), in VEC by 28.7%, and in NVC cells by 55.3% ($p<0.05$; Fig. 3, *d, e*). Changes in VEGFR-3 were less pronounced. The intensity of its reaction in SCT was above the normal level at 33.2% ($p<0.05$, Fig. 3, *f*), in VEC at 30% ($p<0.05$), and in NVC it was lower at 2.8% ($p>0.05$).

Analysis of receptor/growth factor expression intensity ratios yielded very interesting results. VEGFR-1/VEGF ratio in almost all studied structures (ex-

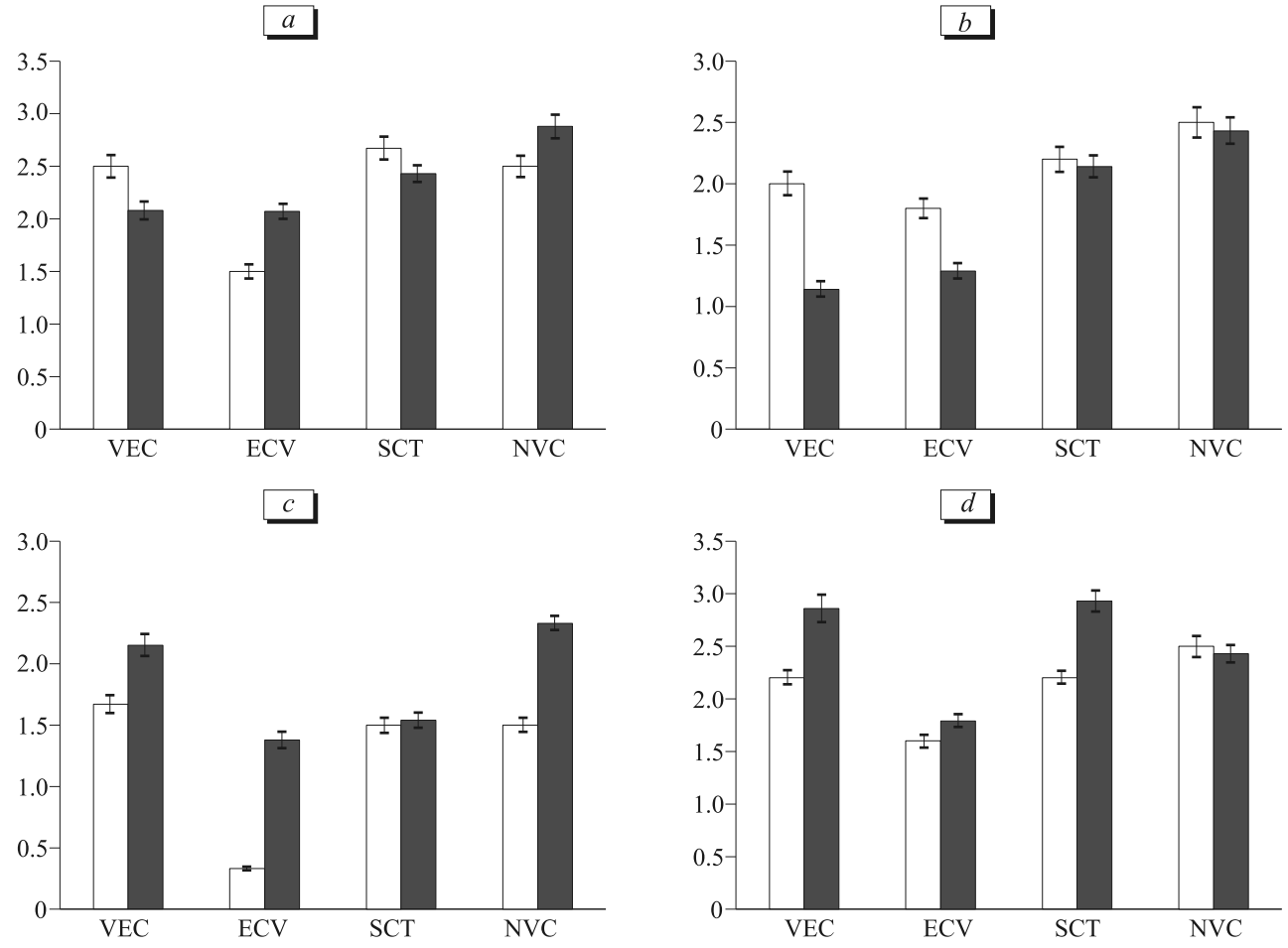

Fig. 2. Expression of VEGF (*a*), VEGFR-1 (*b*), VEGFR-2 (*c*), VEGFR-3 (*d*) in the placental tissue. Ordinate: intensity of the reaction (score).

cept SCT) was below the normal values, while VEGFR-2/VEGF ratios were above the normal. VEGFR-3/VEGF ratio exceeded the control values in VEC and SCT, but was below the normal in ECV and NVC.

Thus, the results of immunohistochemical study of normal placenta were generally consistent with published data on the distribution of VEGF and its receptors [1,3,12]. We found predominance of nonbranching angiogenesis in the placentas from obese women. In all studied structures, the more pronounced expression of VEGF in ECV and the NVC and its receptor VEGFR-2 in comparison with normal placentas was noted. Under normal conditions, binding of VEGF to VEGFR-2 activates the process of angiogenesis by stimulating proliferation and growth of endothelial cells [13].

Under conditions of moderate hypoxia, binding of VEGF to its receptor VEGFR-2 causes hypercapillarization of intermediate villi by the classical feedback mechanism that is accompanied by a pronounced expression of VEGF and VEGFR-2 in endothelial cells of villous vessels [10,15]. Despite the fact that obesity creates conditions for the development of preplacental hypoxia and that we found a combined increase in the expression of VEGF and VEGFR-2 in ECV, histological study revealed predominance of nonbranching angiogenesis. These changes were probably determined by the influence of factors and mechanisms preventing binding of VEGF to its receptor and thus inhibiting angiogenesis with branching characteristic of hypoxia. According to some authors [11], high concentrations of lipids and blood glucose levels (observed in obesity and diabetes), contributing to the closure or masking of receptors can play a role of these factors.

In addition, we found decreased expression of VEGF in SCT of villi releasing the synthesis products primarily into the maternal blood. These results agree with previous reports on reduced serum concentration of VEGF in obese women after 20 weeks of gestation [7].

Therefore, the observed peculiarities of immunohistochemical distribution of VEGF and its receptors in the placental tissue of obese women, to some extent, reflect the processes of compensation and disturbances in the functioning of the mother–placenta–fetus system under conditions of hyperlipidemia and hypoxia.

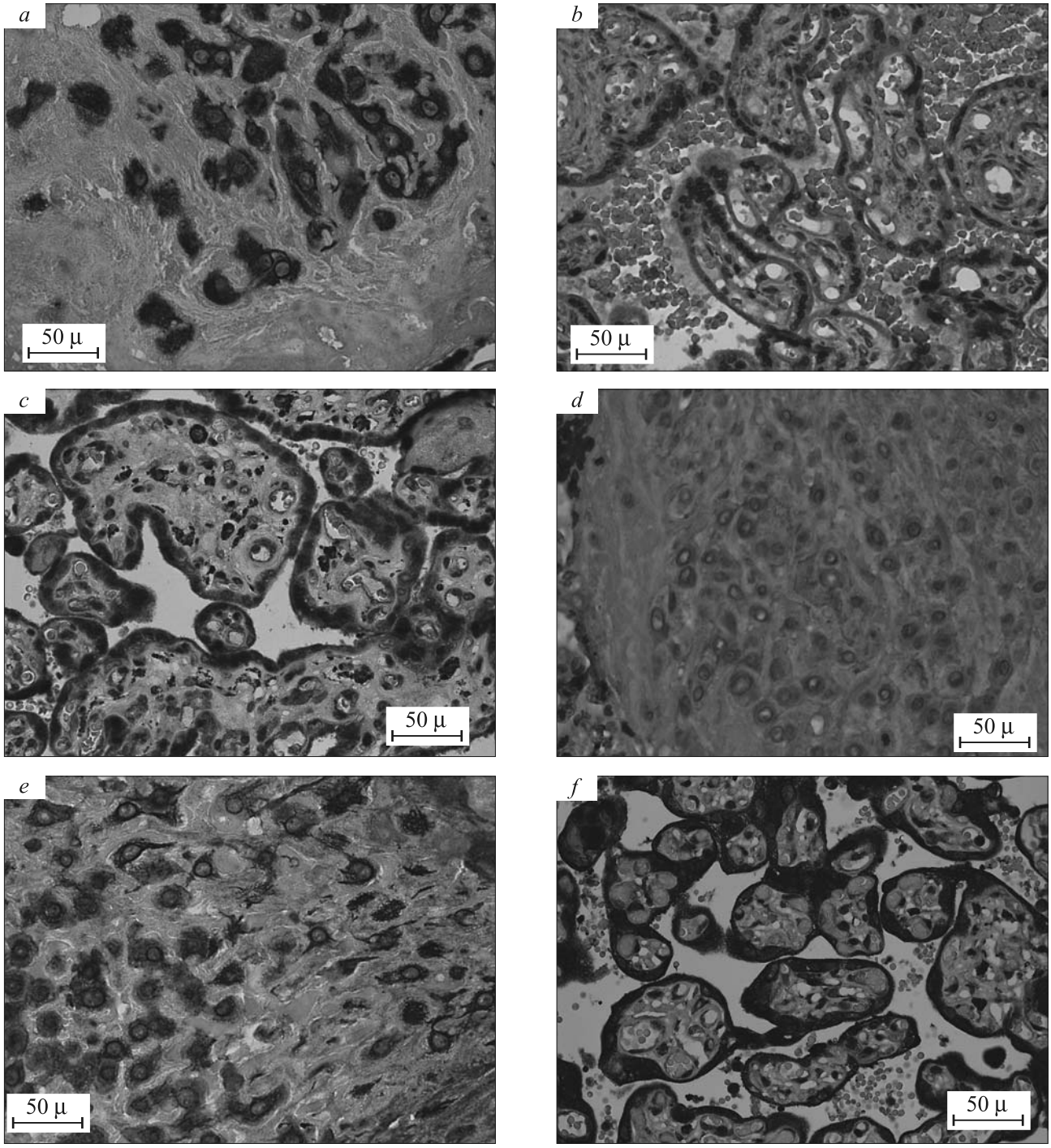

Fig. 3. Immunohistochemical features of placentas from obese women (*a*, *c, e*, *f*) and women with normal body weight (*b*, *d*). *a*: VEGFR-1 expression in NVC; *b*, *c*: VEGFR-2 expression in SCT and ECV; *d*, *e*: VEGFR-2 expression in cells of NVC; *f:* VEGFR-3 expression in SCT and ECV. Immunoperoxidase method, ×400.

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