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Original scientific paper

Research of the vascular endothelial growth factor expression in the placenta

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ABSTRACT: Introduction: The development of tissues in certain organs takes place simultaneously with the development of blood vessels. The placenta is an organ whose development provides a unique model for examining and understanding the process of organogenesis. The vascular endothelial growth factor (VEGF) is one of the first identified angiogenic factors and one of the most important regulators of normal and pathological angiogenesis. The aim of the study was to determine the localization and intensity of VEGF expression in the normal term placenta. Methods: The study analyzed ten normal term placental samples of healthy pregnant women (from the 38th to the 40th week of gestation). Placental tissue sections were stained with the standard hematoxylin-eosin method (HE) and with the immunohistochemical method for VEGF. Results: The small number of cells of the amniotic epithelium and trophoblast had positive VEGF expression of low intensity. Positive VEGF expression of moderate intensity was present in a small number of cells of the chorionic plate stroma, villi stroma, and basal plate. Positive VEGF expression of moderate intensity was present in more than 50% of endothelial cells (3+) of all placental blood vessels. Conclusions: Positive VEGF expression was present in the amniotic epithelium, mesenchymal cells of the chorionic plate, trophoblast, stroma cells of the villi, endothelial cells of the placental blood vessels, and the cells of the basal decidua. In the placenta, the most intensive immunoreactivity for VEGF was present in the endothelium of the placental blood vessels. Positive VEGF expression in the normal placenta indicates a significant role of VEGF in the development of the placenta.

Keywords: placenta, vascular endothelial growth factor A, immunohistochemistry.

INTRODUCTION

Placental angiogenesis is a key process for the development of the placenta, as well as for the development of all other tissues (Ortega et al, 2022). Angiogenesis is a process in which budding, lateral growth and branching of new blood vessels occur from preexisting blood vessels. The development of tissues in certain organs, such as the development of the retina of the eye, takes place simultaneously with the development of blood vessels. The organ whose development provides a unique model for examining and understanding the process of organogenesis is the placenta (Clark et al, 1988). The placenta is a temporary organ for the growth and development of the fetus that ensures the transfer of nutrients from the mother's organism. This discoid-shaped organ is the only source of oxygen and nutrients for the fetus. The specificity of the placenta as an organ lies in the complexity and extent of its functions, and its limited duration. The placenta lasts as long as the pregnancy lasts (Draganović, 2020).

Disorders such as pregnancy-induced hypertension (PIH) and intrauterine growth restriction (IUGR), which may not become obvious until the third trimester of pregnancy, are thought to be the result

of inadequate placentation in early pregnancy (Kaufmann, 2003). The pathogenesis of PIH is explained by hypoperfusion and ischemia of the placenta resulting from the failure in remodeling of spiral arterioles in the decidua. Analyzes of the placenta in IUGR indicate inadequate angiogenesis in the placenta, reduced vascularization of the terminal villi of the placenta, and reduced maternal and fetal blood flow (Warrander et al, 2012; Draganovic et al, 2021; Ljubojević et al, 2022, Jovičić et al 2020). VEGF is one of the first identified angiogenic factors and one of the most important regulators of normal and pathological angiogenesis (Helske et al, 2001; Wu et al, 2023). During pregnancy, VEGF participates in the proliferation, migration, and metabolic activity of trophoblasts and plays a key role in the formation of new blood vessels. During placental development, VEGF has a dual role in the placenta acting on both angiogenesis and trophoblast function. The aim of the study was to determine the localization and intensity of VEGF expression in the normal term placenta.

METHODS

The study analyzed ten normal-term placental samples of healthy pregnant women. The gestation of the pregnant women was from the 38th to the 40th week of gestation. The study was performed at the university setting of the University of Banja Luka, Faculty of Medicine. The study was approved by The Ethics Committee of the University Clinical Center of Republic Srpska in Banja Luka and it was conducted in accordance with the Declaration of Helsinki. The collected placentas for histological analysis were without noticeable macroscopic damage and alterations. The placental tissue samples, size 1x1 cm were dissected at a medium distance from the center and margin of the placenta. The samples include whole-thickness of the placenta, from the basal to the chorionic plate. After fixation in 10% neutral buffered formalin, the tissues were processed and then embedded in paraffin wax. For histological analysis 5 μm thick tissue sections were obtained, and they were stained with the standard hematoxylin-eosin method and with the immunohistochemical staining method using anti-VEGF antibody (Dako). Antigen retrieval was performed for 20 min in citrate buffer at pH 6.0. For blocking endogenous peroxidase activity the tissue sections were incubated with a 3% hydrogen peroxide solution for 5 minutes at room temperature. Then, the sections were washed briefly with distilled water and after that with phosphate-buffer saline (PBS) for five minutes (Dako, EnVsion FLEX WASH BUFFER). Incubation with primary antibody (monoclonal ab-VEGF, clone VG1, 1:25 and 1:50) was performed for 30 minutes at room temperature. Afterward, the sections were washed three times for three minutes in PBS and reincubated with HRP polymer anti-mouse (Dako K4000) for 30 min. After washing in PBS the sections were treated with 3,3'-diaminobenzidine (DAB) chromogen was used and the reaction was monitored under a microscope. The counterstaining with Mayer hematoxylin was performed also.

All placental tissues in the placental sample were evaluated for VEGF immunoreactivity. The intensity of staining was classified into four categories: 0 - negative, 1 - weak, 2 - moderate, and 3 – strong. Depending on the percentage of immunopositive cells immunoreactivity was divided into five categories: 0 = all cells were negative; 1+ = from 0 to 25% of cells were positive; 2+ = if 25 – 49% of cells were positive; 3+ = if 50 – 75% of cells were positive; and 4+ = if >75% of cells were positive (Schiessl et al, 2009). VEGF expression in blood vessels of placental samples was estimated as positive if VEGF immunoreaction was present in endothelial cells in 10% or more blood vessels of the placenta (Holzer et al, 2013). The obtained data were statistically analyzed by the methods of descriptive statistics.

RESULTS

VEGF expression in the placenta was analyzed from the chorionic plate, villi to the basal plate of the placenta. In the amnion epithelium, the most of cells showed negative VEGF expression. Only a small part of

cells showed positive VEGF expression of very low intensity. VEGF expression in the amnion epithelium was evaluated as very weak, and the percentage of immunopositive cells was small 1+ (table 1, figures 1 and 2). In the stroma of the chorionic plate of the placenta, positive VEGF expression of medium intensity 2 was present in a small number of cells 1+ (table 1, figures 1 and 2). In the villous trophoblast, immunoreactivity for VEGF was very low and a small number of cells showed immunoreactivity for VEGF (table 1).

Table 1. The VEGF expression in normal term human placenta.

	VEGF expression in normal term placenta	
	Intensity of staining	**Percentage of immunopositive cells**
Amniotic epithelium	1	1+
Stroma of the chorionic plate	2	1+
Trophoblast	1	1+
Blood vessel endothelium	2	3+
Stroma of the villi	2	1+
Basal plate	2	1+

0 - negative, 1 - weak, 2 - moderate, and 3 – strong; 1+ = from 0 to 25% of cells were positive; 2+ = 25 – 49% of cells were positive; 3+ = 50 – 75% of cells were positive; and 4+ = >75% of cells were positive.

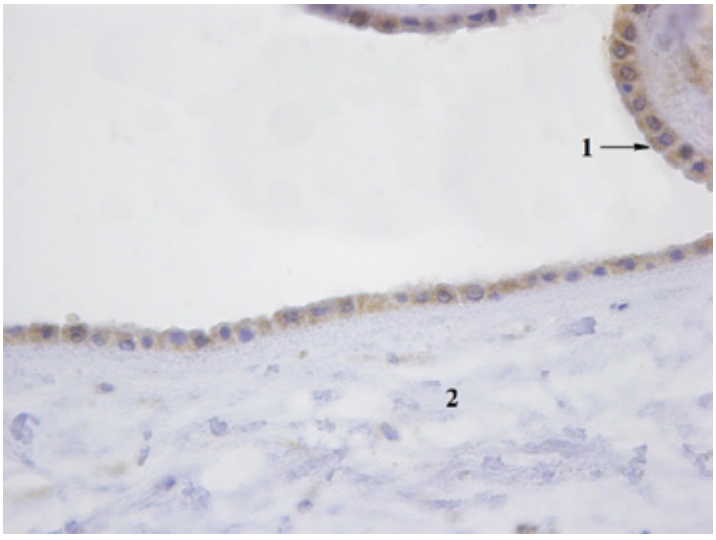

Figure 1. VEGF expression in the amniotic membrane: 1. positive VEGF expression in the amniotic epithelium, 2. amniotic membrane stroma (anti-VEGF 1:25, x 400).

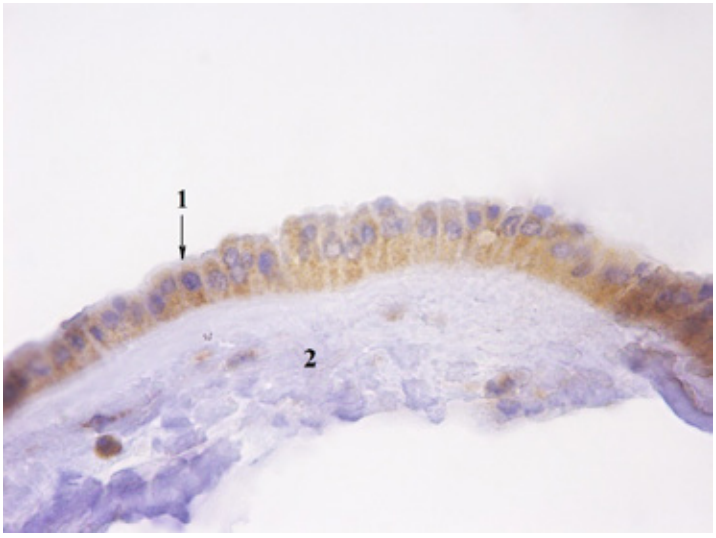

Figure 2. VEGF positive amniotic epithelium: 1. positive VEGF expression in the amniotic epithelium, 2. stroma of the amniotic membrane (anti-VEGF 1:25, x630).

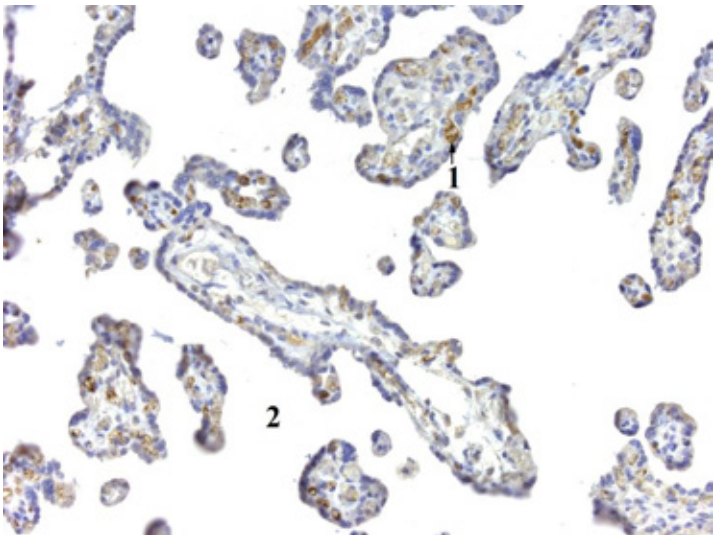

Figure 3. VEGF expression in the normal placenta: 1. positive VEGF expression in the endothelium of capillaries in the terminal villi. 2. intervillous space (anti-VEGF 1:50, x200).

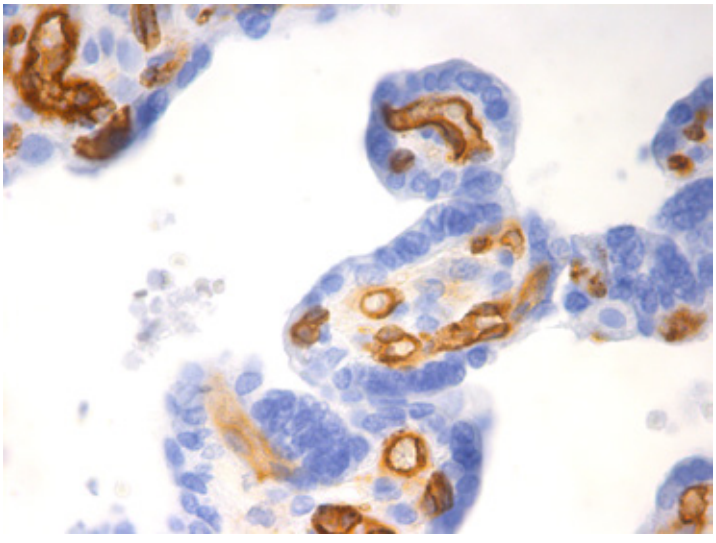

Figure 4. Positive VEGF expression in cells of the stroma of the villi and the endothelium of the villous capillaries (anti-VEGF 1:50, x630).

The most intense immunoreactivity for VEGF is present in the endothelium of the villi capillaries and larger blood vessels, arterioles, and arteries of the placenta (figure 3). Positive VEGF expression of moderate intensity was present in more than 50% of endothelial cells. VEGF expression in the endothelium was 3+ (table 1). Also, according to the number of blood vessels of the placenta that had positive VEGF expression, and with the limit of positivity which is more than 10% of all blood vessels, the VEGF expression in the blood vessels of the placenta was determined as positive. Immunoreactivity for VEGF was present in the stroma of the villi. Immunoreactivity of medium intensity – with category 2, was present in a smaller number of stromal cells of the villi – with category 1+ (table 1, figure 4).

In the basal plate, between the decidual cells, cells with a size smaller than the decidua cells and with positive VEGF expression in the cytoplasm were present. VEGF positive expression of basal decidua cells was of medium intensity - with category 2, and their number was category 1+ (table 1 and figure 5).

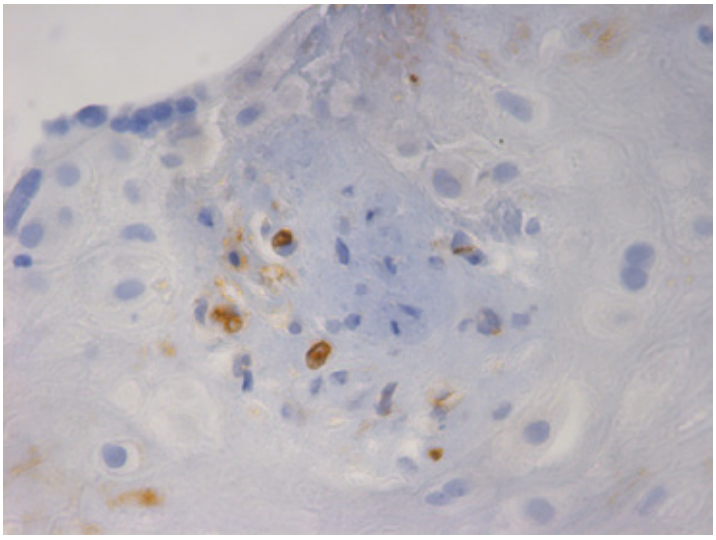

Figure 5. Positive VEGF expression in cells located between the decidual cells (anti-VEGF 1:50, x630).

DISCUSSION

Vascular endothelial growth factor, basic fibroblast growth factor, b-FGF, and endothelial nitric oxide are positive regulators of angiogenesis (Hendrix et al, 2019). They are strongly expressed during embryonic and fetal development, especially in the first trimester of pregnancy.

VEGF, also known as VEGF-A, is a protein that increases vascular permeability. It was originally obtained by purifying the liquid secreted by the tumor. Independently of the isolated protein from the fluid secreted by the tumor, a few years later, another protein with angiogenic activity was purified and isolated and named VEGF. Molecular cloning revealed that these two proteins are identical and they are encoded by a single gene.

The VEGF family of proteins includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, placental growth factor (PlGF), and VEGF-E (Orf-VEGF). Except for the last member of the family, for the rest of these proteins of the VEGF family, there are five genes in the genomes of mammals, including humans.

In this study, positive VEGF expression was found in the amniotic epithelium, mesenchymal cells of the chorionic plate, trophoblast, stroma cells of the villi, endothelial cells of the placental blood vessels, and cells of the basal decidua. In the placenta, the most intensive immunoreactivity for VEGF was present in the endothelium of blood vessels. Immunoreactivity was medium to strong intensity, present in more than 50% of endothelial cells, with category 3+.

In the study by Schiessl and co-authors, VEGF expression was analyzed in the normal placenta through all three trimesters of pregnancy. Placental bed biopsies were obtained at 8–10, 12–14, 16–20, and 37–42 weeks of gestation (Schiessl et al, 2009). VEGF-A was localized in endovascular, intramural, and extravillous trophoblast at all examined gestational ages. There was no difference in the VEGF-A expression in the trophoblast across the gestational age groups.

Özgökçe and coworkers found in the normal placenta, between thirty and thirty-eight gestational weeks, moderate positive VEGF expression in the villi, blood vessel endothelial cells, syncytial cells, and Hofbauer cells (Özgökçe et al, 2022).

The study of Alahakoon and coworkers analyzed the immunolocalization of the angiogenic factor in different placental tissue types. VEGF was detected in syncytiotrophoblast, cytotrophoblast, extravillous trophoblast, endothelium, and Hofbauer cells (Alahakoon et al, 2018). Macrophages, decidua cells, and trophoblasts produce VEGF which is critical for the process of implantation (Wheeler et al, 2018).

Clark and coauthors showed with I-VEGF (VEGF labeled with sodium iodide) the major sites of action for VEGF, which were the endothelial cells within the fetal villi (Clark, 1998).

Invasive cytotrophoblast in early gestation has positive expression of VEGF-A, VEGF-C, placental growth factor, VEGFR-1, and VEGFR-3. At the time of delivery, the placenta has a positive expression for VEGF-A, PlGF, and VEGFR-1.

The trophoblast migrates in a retrograde direction along the wall of spiral arterioles (endovascular trophoblast), which it transforms into tubes of large diameter and low resistance. Endovascular trophoblast invasion occurs in a two waves. The first wave in the decidual segments of the spiral arterioles takes place from the 8th to the 10th week of gestation. The second wave of endovascular trophoblast invasion into segments of spiral arterioles in the myometrium takes place from the 16th to the 18th week of gestation. This physiological transformation is characterized by the gradual loss of the normal musculoelastic structure of the arterial wall and its replacement by an amorphous fibrinoid material in which trophoblast cells are embedded. These physiological changes are necessary for a successful pregnancy.

The roles of VEGF, b-FGF, and eNOS in placental angiogenesis may be altered in conditions such as IUGR. Placental angiogenesis, i.e. the regulation of vascular development, depends on complex relationships between these factors, which play an important role in the development of IUGR (Helske et al, 2001; Wu et al, 2023).

Positive expression of VEGF is present in villous and extravillous trophoblast. Evidence suggests that VEGF regulates trophoblast function by stimulating the release of nitric oxide.

In this study, the presence of cells with positive VEGF expression was found in the stroma of the villi and in the basal decidua. These VEGF positive cells may be macrophages. Research has shown the presence of macrophages called Hofbauer cells in the stroma of the villi, and the presence of macrophages of maternal origin in the decidua basalis. Recent studies have shown that macrophages synthesize VEGF.

At the point of contact between maternal tissue and fetal tissue, where fetal trophoblast cells enter the maternal decidua and remodel spiral arteries, decidual macrophages, and natural killer cells or NK cells are the largest and most significant population of immune cells (Thomas et al, 2021; Lash et al, 2022).

They promote angiogenesis and tissue remodeling. HBCs are also pro-angiogenic because they produce large amounts of VEGF and Sprouty proteins - Spry 1, 2, and 3 (Reyes et al, 2018). With the secretion of VEGF-A, osteopontin (OPN), MMP-9, and TIMP-1 they have a key role in the remodeling of blood vessels in the placenta (Lash et al, 2022). Also, they participate in the vasoregulation of placental blood vessels because they have the ability to produce prostaglandin E2 and thromboxane (Reyes et al, 2018). With the secretion of interleukins and chemokines, they participate in inflammation, and these factors also

have proangiogenic properties. In addition, HBC signal to placental fibroblasts via IL-6 and to villous cytotrophoblasts via osteopontin and GM-CSF (Thomas et al, 2021). HBCs secrete IL-1β, IL-6, IL-8, IL-10, chemokines CCL2, CCL3 and CCL4, TGF-β, low levels of FGF-2. HBCs through interaction with endothelial cells, fibroblasts, and trophoblasts support the growth of the placenta and its homeostasis (Thomas et al, 2021; Lash et al, 2022; Reyes et al, 2018).

CONCLUSIONS

Positive VEGF expression is present in all parts of the normal term placenta. Positive VEGF expression of low intensity was present in a small number of cells of amniotic epithelium and trophoblast. Positive VEGF expression of moderate intensity was present in a small number of cells of the chorionic plate stroma, the stroma of the villi, and the basal plate. Positive VEGF expression of moderate intensity was present in more than 50% of endothelial cells of all placental blood vessels. Positive expression of VEGF in the cells of all parts of the placenta indicates a significant role of VEGF in the development of the placenta.

CONFLICT OF INTEREST

There are no conflicts of interest.

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