

Evaluation of the implantation site morphology in spontaneous abortion

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Abstract

The aim of this study was the characterization of the implantation site through histological and immunohistochemical exams and the evaluation of the changes that appear in the pregnancies ended by spontaneous abortion compared to normal pregnancies ended by requested abortion. One hundred eight patients were divided in two groups: the study group that included 58 patients with spontaneous abortion and the control group that included 50 patients with requested abortion. There has been made uterine curettage in all the cases after a complete preoperative evaluation and the obtained product was sent for histopathological evaluation and immunohistochemical study using a VEGF antibody. Studying the histological sections, we noticed the vasculogenesis stages chronology and then according to the histological aspects of normal pregnancy we noticed the histological changes that occurred at the site of implantation in the cases with pathological pregnancies ended by miscarriage. Our results from this study seem to indicate a correlation between decidual vascular changes and the appearance of miscarriage. In pregnancies ended by miscarriage, we found delays in the trophoblast development according to the gestational age at which the event abortifacient happened. The study emphasizes the temporal differentiation of utero-placental angiogenesis comparing to villous vasculogenesis and angiogenesis in the first trimester miscarriage and normal pregnancy. At the control group, VEGF expression was positive in 88% of cases, while in the study group, pregnancies ended by spontaneous abortion, positive expression of VEGF was present in only 31% of cases. Our data suggest vascular disorders and are in concordance with other histological and ultrasound studies postulating the idea of a link between miscarriage and placental vascular bed pattern changes.

Keywords: implantation site, spontaneous abortion, vasculogenesis.

Introduction

A critical step in the consolidation of pregnancy is the decidualization process when the endometrium suffers extensive changes regarding the morphology as well as the expression and secretion patterns for supporting the blastocyst implantation.

Clinical miscarriage is both a common and distressing complication of early pregnancy with many etiological factors like genetic factors, immune factors, infection factors but also psychological factors [1, 2]. In the last years, progress in the fields of cytogenetics and immunogenetics and a greater understanding of implantation and maternal–embryo interactions have offered new insights into the possible causes of this condition, and opened up new avenues for research into its prevention and treatment [3].

Angiogenesis is characterized by the increasing of the vascular permeability, the proliferation and migration of the endothelial cells and is controlled by various growth factors such as vascular endothelial growth factor (VEGF) – the first secreted factor with a high specificity for the endothelial line [4]. The VEGF family is known to regulate placental angiogenesis and maternal spiral artery remodeling [5].

The aim of this study was the characterization of the implantation site through histological and immunohisto-

chemical exams and the evaluation of the changes that appear in the pregnancies ended by spontaneous abortion compared to normal pregnancies ended by requested abortion.

Materials and Methods

Our study included a number of 108 patients with a first trimester pregnancy, which presented in "Filantropia" Clinical Municipal Hospital, Craiova, Romania, between 2010–2013. The patients were divided in two groups: the study group that included 58 patients with spontaneous abortion and the control group that included 50 patients with requested abortion. The patients for the study group were selected after an ultrasound examination that showed an empty gestational sac or stopping development pregnancy, accompanied or not by a first trimester bleeding. The patients for the control group were showing a normal evolution of first trimester pregnancy in clinical and ultrasound assessment.

We obtained the informed consent from all the patients and the study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

There has been made uterine curettage in all the cases after a complete preoperative evaluation and the obtained product was sent for histopathological evaluation.

The tissue fragments have been immediately fixed in solution of 10% neutral formalin and then processed by the classic method of wax embedding resulting paraffin blocks. These blocks were sectioned, at the paraffin microtome (Microm HM350) equipped with system of transfer of sections on water bath (STS, Microm), resulting sections with thickness of 4 μm . The sections were then stained by the usual method with Hematoxylin–Eosin (HE) and then analyzed at the optic microscope, evaluating comparative morphological aspects of uteroplacental vascular changes induced or not by endovascular trophoblastic invasion, and also vascular remodeling induced by the interstitial trophoblast. The vascular changes were evaluated both in the decidua, and in the placental villi.

The immunohistochemical study was performed on fine sections of 3 μm , obtained by using the same equipment that were plated on slides pre-treated with poly-L-Lysine, and then immunohistochemically processed using the LSAB/HRP method. For this study has been used the monoclonal antibody anti-Hu Mo VEGF clone VG1, manufactured by Dako, dilution 1:300.

Results

Studying the histological sections, we noticed the vasculogenesis stages chronology, which is manifested through: the hemangioblast progenitor cells development and recruitment, hemangioblast cords formation, primitive endothelium differentiation, endothelial tube elongation, the presence of apoptotic bodies showing apoptosis, apoptosis that is required for vascular lumen formation.

Histological aspects in the control group – pregnancies ended by requested abortion

At 5–6 weeks of gestation, we noticed that villi appear dominated by a network of vascular elements. Vessels and cords are centrally and peripheral located, in contact with the cytotrophoblast.

We found the presence of bitrophoblastic villi with hemangioblast cords attached to the trophoblast of the mesenchymal villous, some learned from it and situated in the villous stroma (immature intermediate villi), the simultaneous presence of both types of villi, but the predominance of mesenchymal ones (Figure 1). In this phase, predominant are mesenchymal villi, which were previously called primary villi.

In cases of pregnancies at 7–8 weeks gestational age, the villi present a capillary network, which contains more vessels than cellular cords. We noticed the concomitant presence of mesenchymal villi and immature intermediate villi, the current name of primary and secondary villi (Figure 2). Predominance of immature intermediate villi, rare mesenchymal villi and extravillous trophoblast (former syncytial bud) are also noteworthy. The vessels from chorionic villi must be completed for vasculogenesis, to angiogenesis can occur; without channeling vessels the pregnancy may degrade.

Pregnancies at 9–10 weeks gestation are characterized by the presence of two large vessels centrally located and surrounded by capillary network, connected to the capillary network located on the peripheral side of the

villi. Predominant are immature intermediate villi. At this gestational age are seen villous projections containing capillaries, which ends in finger glove.

At 11–12 weeks gestational age, immature intermediate villi are characterized by the presence of two central vessels surrounded by a capillary network. The stem villi appear, which are monotrophoblastic villi wallpapered by syncytiotrophoblast, characterizing the stem villi showing a differentiated vascular network.

We found that in the late stage of pregnancy endometrium corresponds to morphological changes that accompany the implantation of the conception product.

Histological aspects in the study group – pregnancies ended by spontaneous abortion

According to the histological aspects of normal pregnancy, we aimed the histological changes that occurred at the site of implantation in the 58 cases with pathological pregnancies ended by miscarriage.

The products of the uterine curettage after miscarriage were collected in the interval of 4–12 weeks of amenorrhea, or 2–10 weeks of gestational age.

Typically, syncytiotrophoblastic and cytotrophoblastic cells have a dysmorphic growth pattern, developing the two-cell types, one close to the other.

In the miscarriages with small gestational age, we noticed that cytotrophoblastic and syncytiotrophoblastic cells are fairly well represented in comparison to the number of villi present.

Studying temporal differentiation of uteroplacental angiogenesis comparing to villous vasculo- and angiogenesis in the first trimester miscarriage, in cases with elder gestational age we found endovascular trophoblastic invasion with immature bitrophoblastic villi, nucleated erythrocytes and angioblasts; presence of hypocellular and hypovascular villi and extravillous fibrin deposits (Figure 3), compared to the lean endovascular trophoblast differentiation with important chronic and acute inflammatory process, diffuse and perivascular scattered, and also microthrombosis and intravillositary edema that we found in cases of spontaneous abortions at lower gestational age (Figure 4).

The cytotrophoblasts, specialized epithelial cells of the placenta, invades the decidual interstitium, internal third of myometrium and final uterine blood vessels. This invasion plays a critical role in establishing and maintaining an adequate amount of blood to the developing fetus.

In the cases with miscarriage, the characteristics aspects were minimum interstitial trophoblast invasion, with leukocyte fibrin thrombus, perivascular trophoblastic invasion with rich acute and chronic inflammatory process.

We noticed also a number of elements characteristic for abortion and compromised pregnancy, represented by: secretory endometrium with decidualized stroma, rich lymphocytic infiltrate, glands with advanced secretory changes, decidualized stroma with necrobiosis and vascular thrombosis, lymphocytes in the decidua and perivascular, periglandular, vascular microthrombosis.

We noticed in the study group spongy and compact decidua (secretory glands) with abundant vascularization

with large and small vessels, normal and dilated, both in decidua and in villous axis. Vascular changes were evident and different types: decidua with necrobiosis injuries and hemorrhagic areas in 14 cases (Figure 5); redness, inflammation and vascular thrombosis in 39 cases, both in large vessels and in small collapsed vessels (Figure 6). In six cases, we found the presence of intra-luminal hemorrhage associated with acute inflammatory infiltrate.

Delays of histological development stage were encountered in histological assessment of trophoblast obtained from the pregnancies included in the study group.

We note that we did not have cases with only one type of histological delay. In all cases, the more pregnancy was advanced in gestational age, more elements combined we had, that have shown us that lack of normal histological development at the appropriate time, may compromise pregnancy development.

In this study, at the control group, VEGF expression was positive in 88% of cases, while in the study group, pregnancies ended by spontaneous abortion, positive expression of VEGF was present in only 31% of cases.

VEGF expression at the control group was positive and highly positive in the decidua (Figure 7), the trophoblast (Figure 8), in villous axis, at the glandular epithelium, and stromal cells. This indicates that VEGF is an important vasculogenetic factor involved in early placentation strictly necessary to maintain pregnancy.

A feature of VEGF expression in pregnancies ended by spontaneous abortion was the variable expression of VEGF. We met a variable positivity both in distribution and intensity at the level of villi (Figures 9–11), syncytiotrophoblast cells, cytotrophoblast cells, and villous axis, regardless of the degree of maturation of chorionic villi (Figure 12).

In generally, the VEGF expression was positive at the level of villous axis and weakly positive at the level of syncytio- and cyto-trophoblast these issues meeting at the level of the same villi. In the study conducted in pregnancies ended by spontaneous abortion, we met another characteristic of the VEGF expression, which consists in reduced expression of the VEGF antibody in decidual cells (Figure 13) and epithelium of endometrial glands (Figure 14).

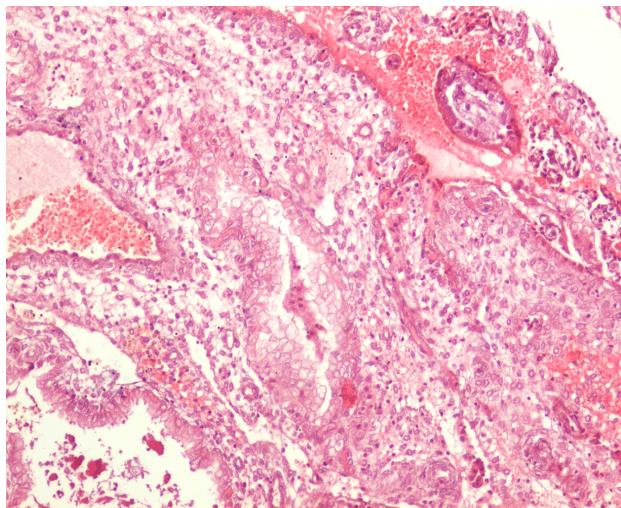


Figure 1 – Presence of villi nearby the decidua (HE staining, $\times 100$).

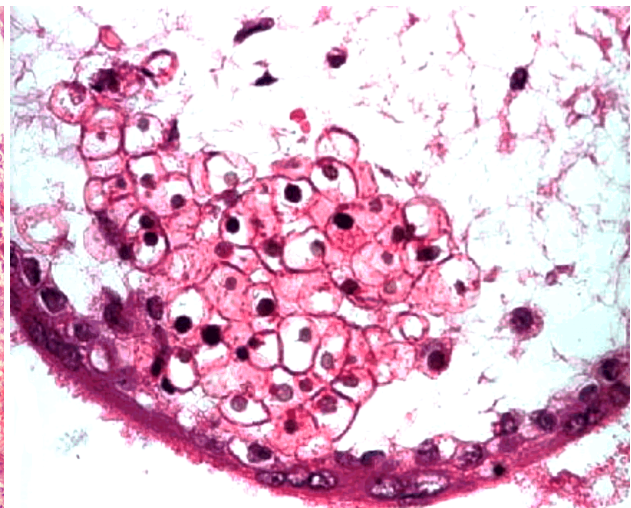


Figure 2 – Immature intermediate villi in evolution (HE staining, $\times 200$).

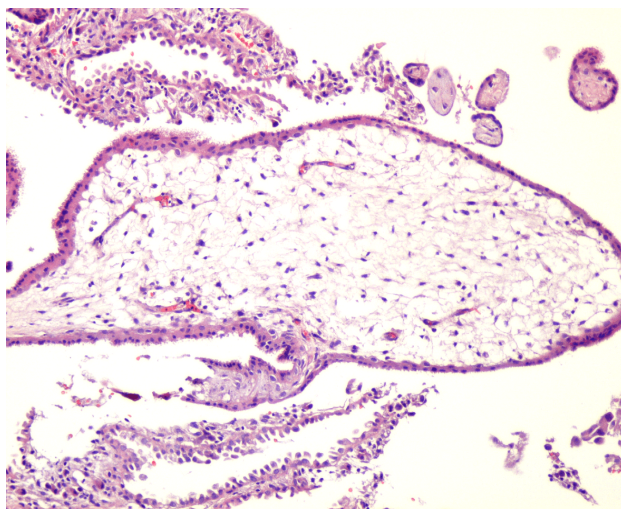


Figure 3 – Secondary villous with edema in the villous axis and small vessels with poorly visible lumen in the periphery (HE staining, $\times 100$).

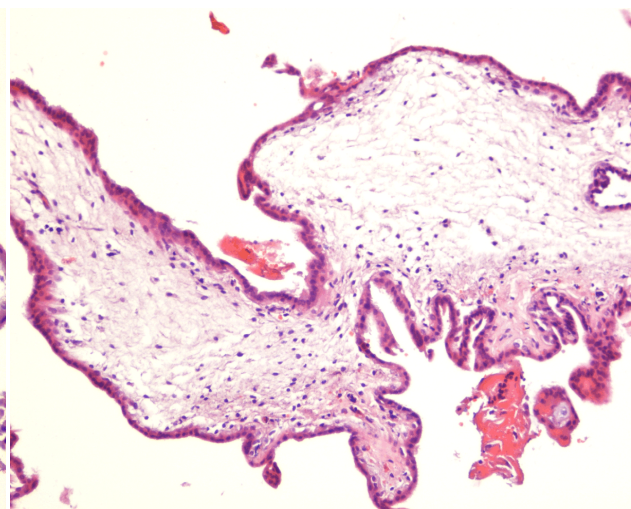


Figure 4 – Primary and secondary villi dilated by edema (HE staining, $\times 100$).

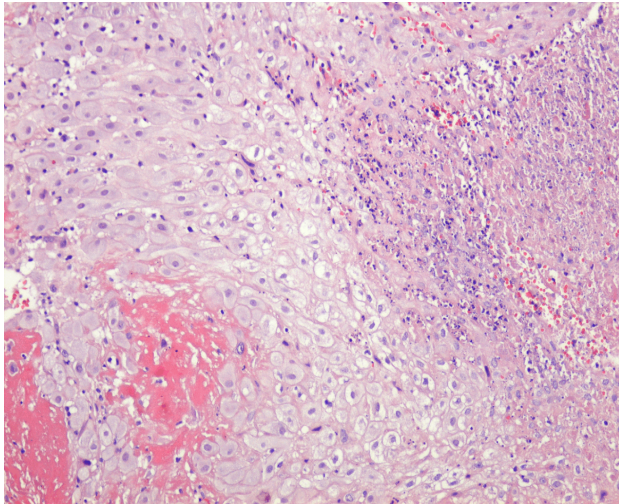


Figure 5 – Compact decidua with necrobiosis (HE staining, ×100).

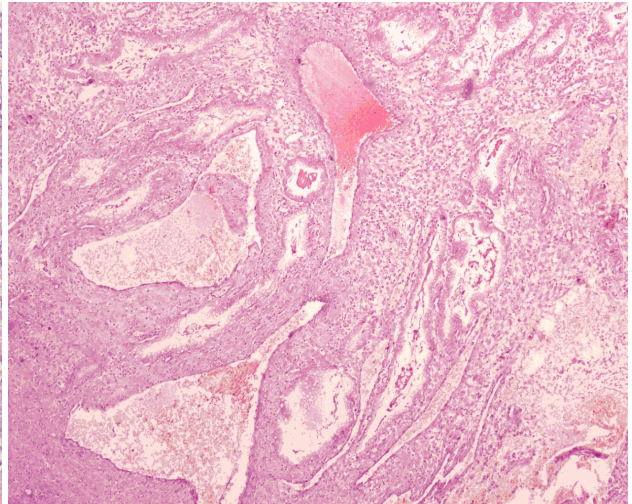


Figure 6 – Spongy decidua with dilated vessels with vascular hyperemia (HE staining, ×40).

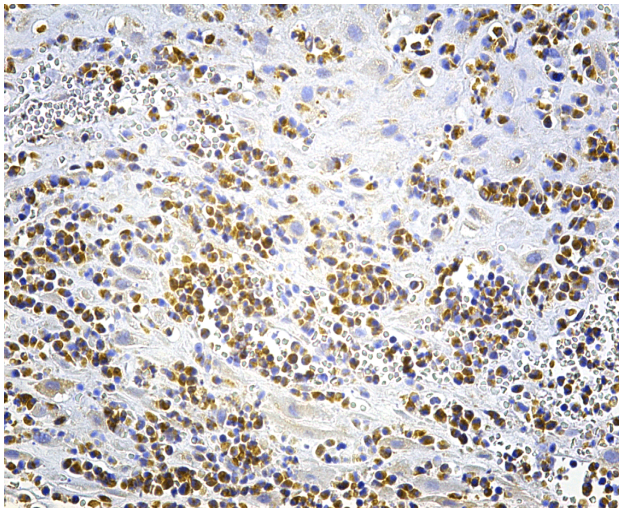


Figure 7 – Decidua with necrobiosis and frequent VEGF-C positive cells, ×200.

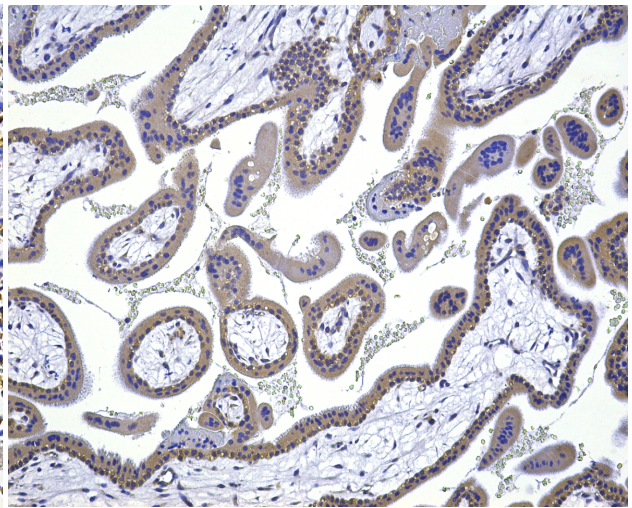


Figure 8 – Positive immunostaining for VEGF-C in the trophoblast, ×100.

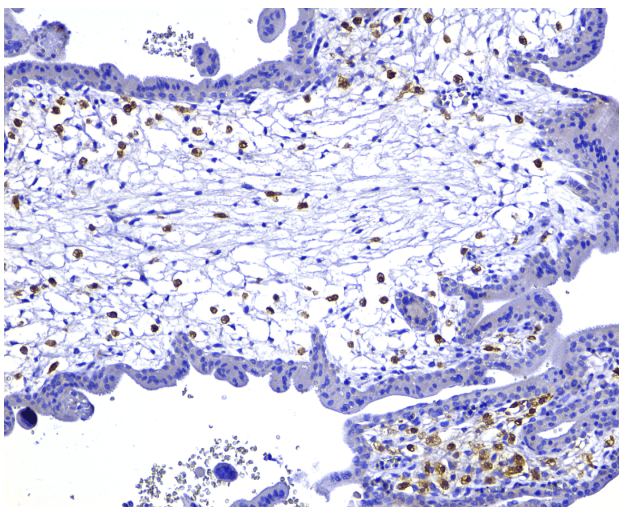


Figure 9 – Bitrophoblastic chorionic villi, some with frequent VEGF-C positive cells in the villi axis and others with rare VEGF-C positive cells, ×100.

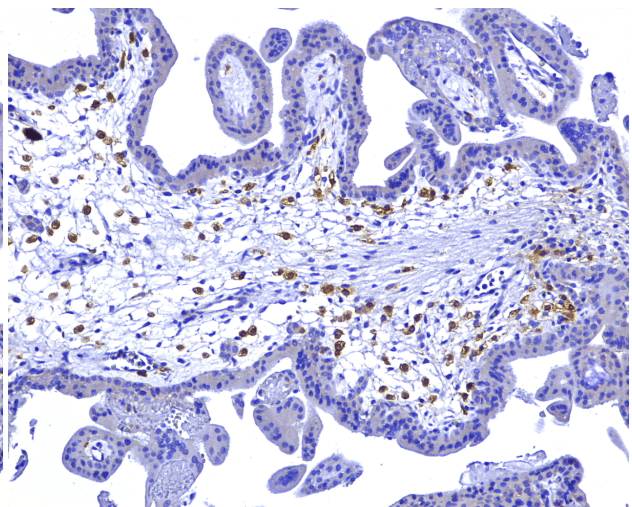


Figure 10 – Frequent VEGF-C positive cells in villous axis (layout compatible with partial mole), ×100.

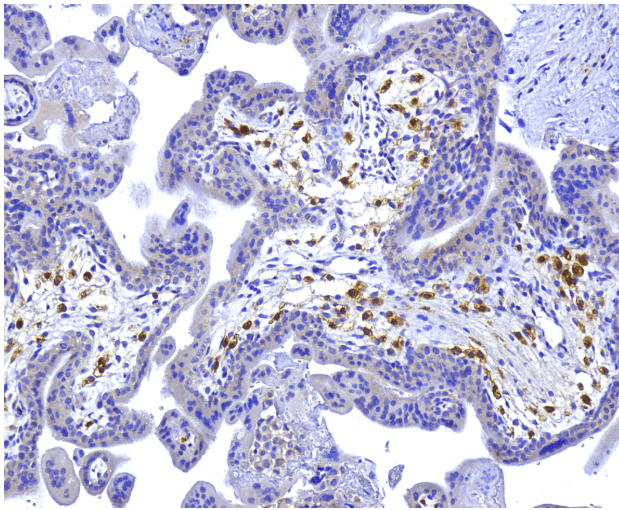


Figure 11 – Large villi with trophoblast proliferation), ×100.

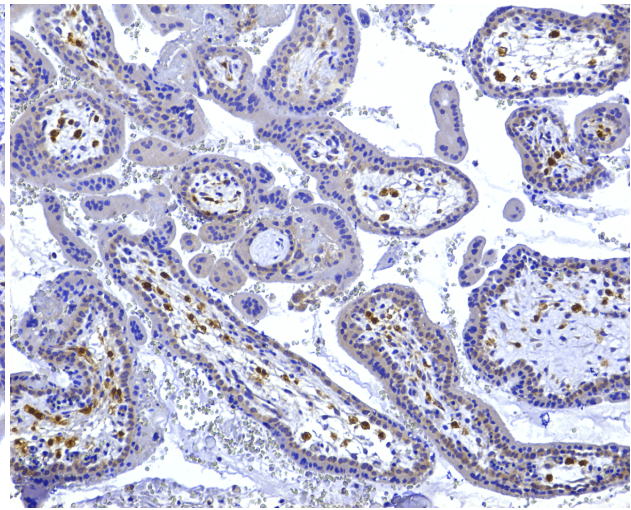


Figure 12 – Unequal villi with variable number of VEGF-C positive cells in villi axis and weak positive reaction in cytotrophoblast, ×100.

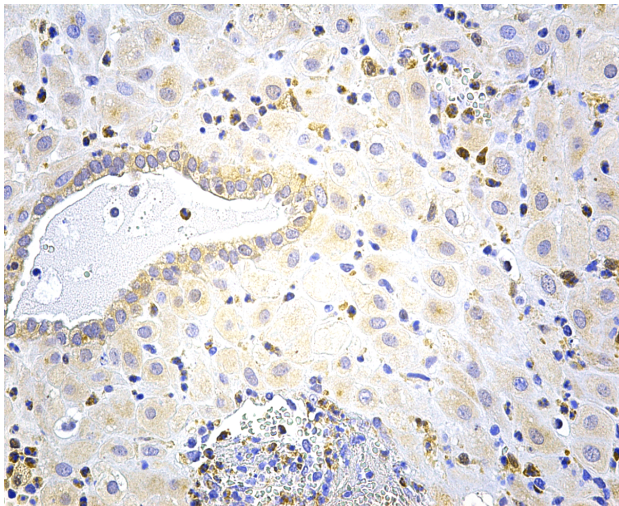


Figure 13 – Spongy decidua with weak positive immunostaining for VEGF-C both in decidual cells and endometrial glands and in rare stromal cells, ×200.

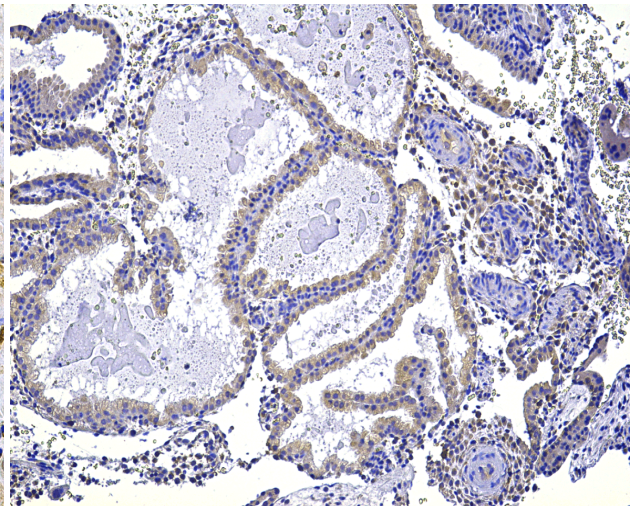


Figure 14 – Rare endometrial glands low positive for VEGF-C adjacent to the villi, ×100.

Discussion

The main function of the intermediate trophoblastic cell from the implantation site is to establish maternal-fetal circulation by invading the basal plate spiral arteries during early pregnancy [6].

The mechanisms involved in trophoblast survival are very little known [7].

These invasive fetal cells replace the endothelial layer of the uterine vessels, making them large caliber vessels. This vascular transformation allows the increase of uterine blood flow needed to support fetal development during pregnancy [8].

Studies of normal vascular chorionic villi showed that embryonic capillaries first appear between 6 and 20 days after conception [9].

The development of the vascular system of chorionic villi seems to be characterized in particular by maturation of luminal vessels from primitive hemangioblast cell cords and marginalization due to decrease of villous stroma area and increase of total vascular area [10].

We found that morphological aspects of mesenchymal

villi characterize normal pregnancies, in development, at the gestational age of 5–6 weeks. With the evolution of pregnancy, at 7–8 weeks of gestational age, the evolution of pregnancy was correlated with the presence of mesenchymal villi and immature intermediate villi appearance. At 9–10 weeks of gestation, immature intermediate villi prevailed, and were still present cytotrophoblasts and fetal angioblasts [11]. Characteristic for pregnancies at 11–12 weeks of gestation was the presence in addition to the aforementioned villi of monotrophoblastic stem villi with completed vasculogenesis, capable of vascular remodeling done by angiogenesis.

Disorders occurring in vascular development may play a role in the pathogenesis of miscarriage [12, 13].

Angiogenesis is a key element in the endometrial development. An abnormal vascular development of the endometrium has been associated with spontaneous abortion increasing the amount of perivascular smooth muscle cells. A recent immunohistochemical study performed a genuine map of the spatial and temporal expression of several angiogenic growth factors and their receptors. The study revealed a number of patterns

of the expression of these angiogenic growth factors both spatially and temporally during a normal endometrial cycle. Furthermore, the immunolabeling intensity for angiogenic growth factors was modified for patients with previous spontaneous abortion, especially at the level of the vascular smooth muscle cells [14].

VEGF along with its receptors VEGFR1 and VEGFR2 are early involved in vasculogenesis. The results of a recent study suggest that decreased expression of VEGFR1 in decidua and weaker VEGF and VEGFR2 expression in placental villi and decidua may be associated with early pregnancy loss [15]. The earliest expression of the VEGF in human placenta has been in the 22 embryonic days, expressed in villous cytotrophoblast and the Hofbauer cells from villi center [16].

Endometrial VEGF is likely to be a critical molecule regulating implantation [17]. Uterine expression of VEGF appears to be cycle dependant, with increased levels of VEGF reported during the mid-secretory period [18]. In contrast, increased mid-secretory VEGF-A expression is not evident in women experiencing repeated IVF failures [19] suggesting that VEGF may have a role in successful implantation. Although the VEGF has important roles in normal and complicated pregnancy, the current predictive value of the VEGF family as biomarkers appears to be limited to early-onset pre-eclampsia [20].

One of the latest researchers concern in this area is represented by the links between the gene polymorphism of vascular endothelial growth factor (VEGF) and risk of spontaneous abortion. A meta-analysis of the literature conducted in 2012 suggests that the polymorphisms of the VEGF gene -2578C/A, -1154G/A did not show a statistically significant association with the risk of spontaneous abortion, while polymorphisms -634G/C and +936C/T were associated with an increased risk of spontaneous abortion within specific genetic models [21].

In our study, in the control group, VEGF expression was positive in 88% of cases, while in the study group, pregnancies ended by spontaneous abortion, positive expression of VEGF was present in only 31% of cases. A feature of VEGF expression in pregnancies ended by spontaneous abortion was the variable expression of VEGF. Perhaps this variability in the VEGF expression may lead to an imperfect vasculogenesis that threaten the pregnancy outcomes.

These data suggest that the VEGF expression and function are closely controlled in the maternal decidua and the early placenta to ensure an adequate vasculogenesis and angiogenesis during implantation and early placentalation.

Disruption of this balance could theoretically contribute to implantation failure and pregnancy loss. Further studies are needed to a better understanding of the way in which the control of the VEGF expression is made at the level of maternal-fetal interface, of its functions in the first period of pregnancy and of the consequences on reproduction of its aberrant expression in humans.

✉ Conclusions

Our results from this study seem to indicate a correlation between decidual vascular changes and the appearance of abortion. In pregnancies ended by mis-

carriage, we found delays in the trophoblast development depending on the gestational age at which the abortifacient event happened, the immature differentiation of intermediate trophoblast – multinucleated trophoblastic giant cell type, being supposed to be one of the causes of this pathology. These issues were encountered in our cases with miscarriage, confirming the literature data and are in concordance with other histological and ultrasound studies postulating the idea of a link between miscarriage and placental vascular bed pattern changes.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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