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FEATURES OF VASCULAR DECIDUA IN THE WOMEN WITH INTRAUTERINE SEPTUM

ОСОБЕННОСТИ СОСУДИСТОЙ СИСТЕМЫ ДЕЦИДУАЛЬНОЙ ОБОЛОЧКИ У ЖЕНЩИН С ВНУТРИМАТОЧНОЙ ПЕРЕГОРОДКОЙ

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Summary

The density of microcirculatory vessels in parietal and basal parts of deciduas (PD and BD) was less when compared with the control by 28,8% (P<0,05) and 29,7% (P<0,05) correspondently in women with complete intrauteral septum who had infertility period, which lasted not less than 5 years (from 5,0 up to 13,5 years) with the consequent first trimester miscarriage have been investigated. The general square of microcirculatory vessels in secretory part of decidual endometrium exceeded control value by 24,0% (P<0,05), while lumen square of vessels in PD and BD exceeded control data by 57,3% and 50,5% (P<0,05) correspondently. Established differencies of microcirculatory vessels are considered as a pathogenetic of the infertility and miscarriage in women with intrauteral septum.

Key words: vessels of decidua, intrauteral septum, miscarriage, infertility.

Реферат

У женщин с полной внутриматочной перегородкой, у которых наблюдался период бесплодия период бесплодия составил не менее 5 лет (от 5,0 до 13,5 лет) с последующим спонтанным прерыванием беременности в первом триместре, плотность сосудистой сети в париетальной и базальной частей децидуальной оболочки (ПД и БД) была меньше чем в группе контроля на 28,8% ($P<0,05$) и на 29,7% ($P<0,05$) соответственно. Общая площадь сосудистой сети в секреторном эндометрии децидуальной оболочки была на 24,0% больше, чем в контроле ($P<0,05$), а площадь поперечного сечения сосудов в ПД и БД превышала соответствующие показатели в группе контроля – соответственно на 57,3% и на 50,5% ($P<0,05$). Установленные различия сосудистой сети рассматриваются в качестве патогенетических механизмов развития бесплодия и невынашивания при наличии внутриматочной перегородки.

Ключевые слова: васкуляризация децидуальной оболочки, внутриматочная перегородка, невынашивание, бесплодие.

Infertility and miscarriage among women with uterine septum is a problem that can be associated with the presence of the septum itself, and systemic metabolic, hormonal features of the woman's body [3, 10]. Vascularization of decidua is a factor of successful implantation of fertilized ovum. Decidualization, changes in the vascular net and invasion of immunocompetent cells are the most important processes that occur in the first weeks after implantation [7; 14].

Remodeling of the vasculature (vascular adaptation) includes angiogenesis, i. g. formation of new blood vessels on the background of the existing system of microcirculation activity preservation [5]. The newly formed vessels are characterized by increased permeability, endothelial cell proliferation and migration, which is regulated by various growth factors including vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and angiopoietins (Ang), and proteases such as membrane matrix metalloproteinases (MT -MMP) [12; 15]. Violations of the process of remodeling the vasculature are crucial for

pathogenesis of miscarriage. It has been established that the higher density of decidual vessels [9], and increase of VEGF expression and its receptors correlate with recurrent pregnancy loss [19].

The objective of this study is to investigate vascularizations of decidua in women with intrauterine septum with a history of infertility and miscarriage with spontaneous abortion in the first trimester of pregnancy.

Materials and methods. 17 women with intrauterine septum, suffering from infertility from 1.0 to 3.0 years, followed by a pregnancy, resulted in spontaneous abortions in the first trimester have been under examination. Thus, the criteria for inclusion of the women in the observation were: 1) confirmed diagnosis of complete uterine septum; 2) the presence in the history of infertility period of no more than 3 years; 3) presence of spontaneous abortion in the first trimester, observed after a period of infertility and marked at least twice. The fulfillment of the requirements in accordance with the criteria mentioned allowed to set the relationship of the parameters under study not only with the factors that contribute to miscarriage, but also to consider their relationship with infertility. Tissues of decidua of practically health women (n=12) were used as control. They underwent induced abortion in the terms of the first trimester of pregnancy comparable with the treatment group. All the examinations have been conducted in compliance with the requirements of Ukrainian Ministry of Health Care order № 281, dated 01.11.2000 and approved by the Bioethics Commission of Odessa National Medical University.

Decidua samples were obtained by vacuum aspiration during the abortion. Termination of pregnancy in spontaneous abortion was verified by ultrasound examinations. The aspirated tissue have been analyzed for the absence of pathologic lesions and if the latter were present, they were excluded from the study. Pathological changes in women violated implantation and gestation (hemostasis disorders, autoimmune diseases); mismatching of pregnancy, defined by the date of the last menstrual period with gestational age of the embryo were other exclusion criteria.

Samples decidua derived from vacuum aspirate have been fixed in formalin and then paraffin treated. Then hematoxylin-floxin-safran staining of the sections was done. Hematoxylin (50 g of potassium aluminum sulfate, 1 g of hematoxylin, 500 mg of citric acid, 25 g of chloral hydrate, 200 mg $NaJO_3$ in 1000 ml of aqua distillate) stained the cell nuclei stained in purple-magenta; floxin (0.25g of floxin in 100 ml of distillate water) stained cell cytoplasm in pink; safranin (3 g of safranin in 1000 ml of 100% alcohol) was used for staining of collagen fibers orange-yellow. This staining allowed to differentiate decidua and its

secretory part - secreting decidual endometrium (SDE). Basal and parietal part of decidua (BD and PD, respectively) differ based because of presence or absence of non - villous trophoblast using anti - cytokeratin staining [8]. The results obtained in women with an accurate verification of decidua all components (SDE, BD and PD) have been analyzed.

Verification and examination of vasculature staining was performed on the presence of antigen CD_{34} presenting in the endothelium of arteries, veins and capillaries and is an endothelial marker [8]. CD_{34} is also defined in the endothelial cells of lymphatic vessels of various tumors, but it does not exist in those cells under normal conditions [18]. The study [13] concluded about the absence of CD_{34} in the endothelium of uterus's lymphatic vessels. Therefore, in our study we dealt with the endothelium of blood vessels as a compartment of the antigen mentioned.

Stained on the CD_{34} antigen slices were investigated in several fields of view [9]. At that, in each tested sample of decidua plots we studied 2-4 sections per power field x 100. The indexes of the vessels stained in such a way total area and a number of vessels per unit of field of vision were taken into account. The latter was done automatically using the software Qwin, Leica Microsystems. The results obtained were used to calculate the number of blood vessels per mm^2, the total area occupied by the vessels per mm^2 (mm^2 / mm^2) and the cross sectional area of the vessels (mm^2 / vessel).

The results were statistically processed using conventional in biomedical research criteria for assessing the significance of differences between groups (method ANOVA + Newman- Keuls test).

Results of the study. The density of blood vessels was significantly higher in the SDE (118.5 ± 9.2 vessels / mm^2) compared with PD (38.6 ± 2.9) and BD (26.3 ± 2.7) ($P < 0.01$), Fig. 1). The density of blood vessels in PD was higher than in BD (at 31.9%) ($P < 0,05$). At the same time in intrauterine septum (IUS) patients a trend to the increase of the index under study as compared to the control group by 10.7% ($P > 0,05$) (Fig. 1) was marked. In PD and BD samples the parameters under study were less than those in the control group by 28.8% ($P < 0.05$) and 29.7% ($P < 0.05$). The investigational index in PD was higher than that in BD to 32.7% ($P < 0.05$) (Fig. 1).

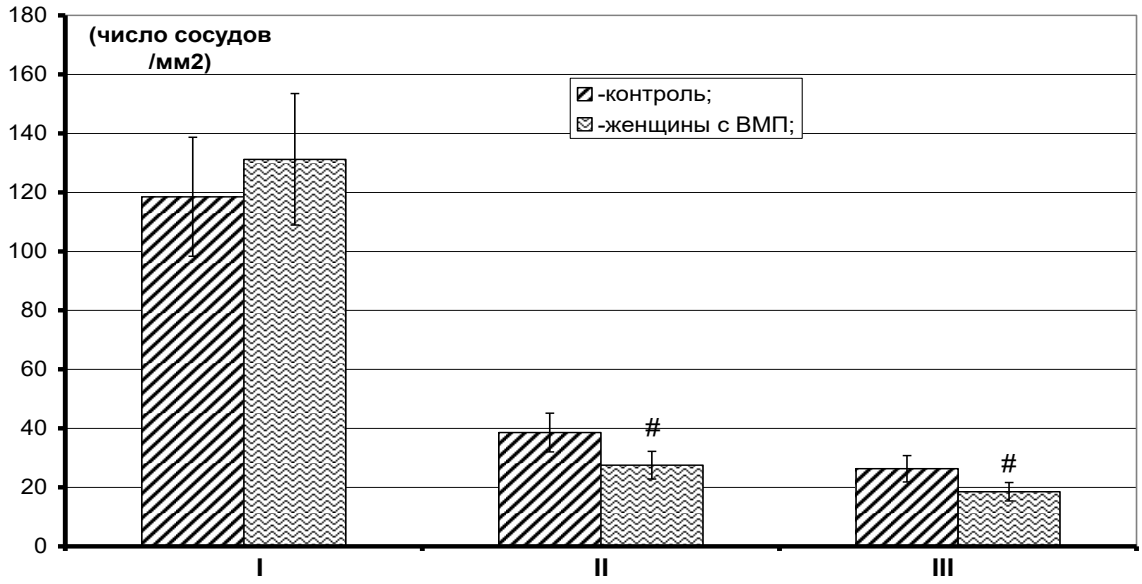

Fig. 1. Density of vascular net in the parts of desidua in the women with intrauterine septum

Letters of references: abscise axis: I- SDE; II- PD; III- BD; axis of coordinates – index under study.

#-P<0.05 in comparison with the control group index.

The total area of the vessels in the control group was significantly lower in SDE (0.019 ± 0.002 mm^2 / mm^2) compared with PD (0.041 ± 0.003) and BD (0.048 ± 0.005). At the same time BD's vascular area significantly exceeded that in SDE (P <0.01) (Fig. 2). In IUS patients the total area of vessels in SDE amounted 0.025 ± 0.002, which was at 24.0% higher if compared to the control group rate (P <0.05). A similar excess of indexes compared with the control in PD and BD averaged 8.9% and 22.6% (P>0.05), correspondingly. At that the parameters under study in IUS women was significantly lower in the SDE compared to PD (at 44.5%) (P <0.05), and less in PD compared to BD by 27.4% (P < 0.05) (Fig. 2).

The cross sectional area of the vessels was significantly less in SDE as compared to PD (153 and 1320 mm^2/vessel, respectively), and BD (2532 mm^2/vessel); at that statistically significant difference between PD and BD indexes was observed (P <0.01) (Fig. 3). In IUS patients in PD and BD a significant increase of the index under investigation in comparison with those in the control group by 57.3% and 50.5% (P <0,05) took place. Besides, there was a significant difference of the vessels diameter between portions SDE, PD and BD (Fig. 3).

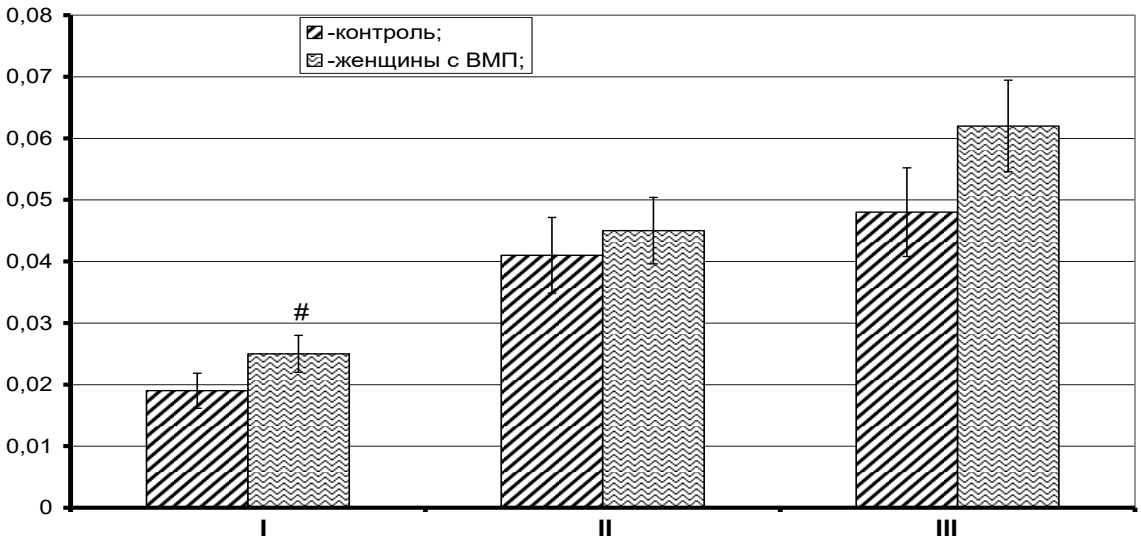

Fig. 2. Total area of vessels (mm^2/mm^2) in the parts of desidua in the women with intrauterine septum

Letters of references: the same as in Fig.1.

#-P<0.05 in comparison with the control group index

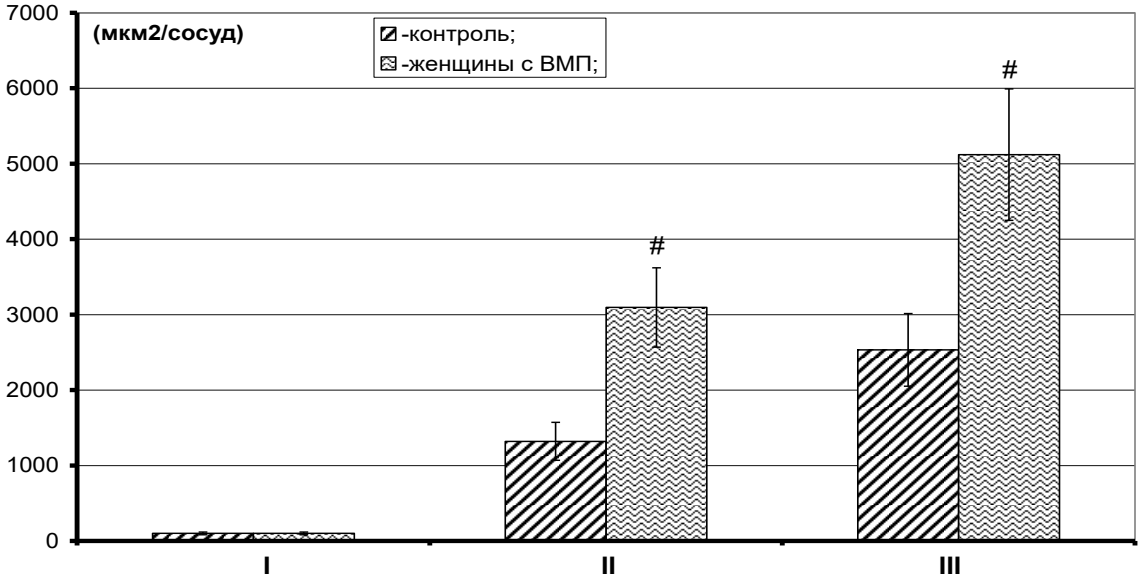

Fig. 3. Area of the vessels cross section in the parts of desidua in the women with intrauterine septum.

Letters of references the same as in Fig. 1

#-P<0.05 in comparison with control group index.

Thus, the results obtained may indicate a correlation between decidua's vascularization and miscarriage in IUS women. However, taking into account that among the criteria for the patinets' inclusion in the study was infertility period, the results mentioned are also essential for the understanding of the pathogenesis of infertility in this group of patients.

In decidua of control groups the density of blood vessels in its certain parts is different with the highest value of this indicator in the area of SDE. At the same time, the relative total area, and the cross sectional area of the vessels in SDE had the lowest indexes, while in BD they were the highest. Similar differences were noticed in IUS women. The density of the vasculature in IUS women was less in PD and BD tissues compared to the control at 28.8% ($P < 0.05$) and 29.7% ($P < 0.05$), correspondingly. The total area of the vasculature of SDE was 24.0% more than in the control ($P < 0.05$), and the vessels' cross sectional area in PD and BD was higher than in the control group at 57.3% and 50.5% ($P < 0.05$).

Thus, during the transition from SDE to BD there is a decrease of vascular density at the background of the increase of their relative area, and enlarging the vessels' diameter. This pattern is typical for both healthy women and IUS women. A similar result for women with recurrent abortion has been established in the studies [8].

Considering the possible mechanisms of decidua vascularization changes in IUS women, it should be noted that recently regulatory role of trophblast's villi, immune cells and the hormones securing pregnancy course as for vascularization of decidua [8]. Decrease of blood vessels density and increase the vascular space in BD and PD may be due a result of changes in the balance of angiogenic regulatory factors: VEGF content changes, angiopoietins and their receptors was associated with the formation of dilated or enlarged in size vessels of microcirculatory bed [4, 11].

In addition, changes in vascular pattern in a consequence SDE - PD - BD can be explained by the action of mechanical factors [8]. Thus, the lower density of blood vessels and the increase in the cross-section of the vessel in BD may be associated with increased blood perfusion of this area of decidua which occurs especially near the implantation zone [1; 8; 17]. The increase in the lumen of vessels may also be a result of the relative decrease in oxygen tension in the blood [6].

These differences of vascularization compared with the control group resemble those noted in the early and late phases of the first trimester of pregnancy, which may indicate abnormally quick "maturation" of the vasculature in IUS women [2; 8]. It can be assumed that this early maturation may facilitate access of maternal blood components to the intervillous space at an early stage of pregnancy in IUS women. This was confirmed in the women with

recurrent pregnancy loss using ultrasound studies [10]. This result may testify in favor of increasing oxygen and activation of peroxide processes as a consequence of changes in the vasculature, increasing the pericellular proteolytic activity and expression of angiogenic factors [16, 20].

Conclusions:

1. In the women with intrauterine septum with a history of infertility of at least 5 years (5.0 - 13.5 years), the density of vasculature in parietal and basal parts of decidua was less than in control at 28. 8% ($P < 0.05$) and 29.7% ($P < 0.05$).

2. The total area of microcirculatory network in secretory endometrium of decidua was 24.0% more than in the control ($P < 0.05$), and the cross-sectional area of parietal and basal decidua was higher than in the control at 57.3% and 50.5% ($P < 0.05$).

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