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## VASCULARIZATION OF CHORION VILLI IN THE FIRST TRIMESTER OF GESTATION WITH PHYSIOLOGICAL COURSE AND IN RECURRENT MISCARRIAGE WITH UNDERLYING CHRONIC ENDOMETRITIS

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***Aim*. To study stages of vasculo- and angiogenesis in uncomplicated course of the first trimester of gestation and to identify disorders in vascularization of the villous chorion in recurrent miscarriages and chronic endometritis. *Materials and Methods*. The main group – villous chorion of 5-12 weeks of gestation obtained from female patients with recurrent miscarriage and chronic endometritis (n=35); comparison group – villous chorion obtained from clinically healthy females in official abortions (n=30). On the basis of the results of morphological examination the analysis of stages of vascularization of villous chorion was carried out. *Results*. In recurrent miscarriage associated with chronic inflammation of the**

**endometrium, structural changes of the villous chorion were detected manifested by retardation of differentiation of villi starting from the 5th week of gestation, by delay of vasculogenesis at the stage of formation of hemangioblastic cords typical of the 6-7th week with its inhibition in the subsequent periods. In villi of the 8-12th weeks of gestation no further differentiation of vessels was seen, hence, branched angiogenesis did not form. *Conclusion*. Disorders in differentiation of villi, in vasculo- and angiogenesis in the stroma of chorion villi with the underlying chronic inflammation of the endometrium that produced negative effect on further development and successful completion of gestation, were proved.**

***Keywords:*** *recurrent miscarriage, chronic inflammation of the endometrium, vasculogenesis, angiogenesis, villous chorion.*

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The problem of recurrent miscarriage (RM) continues to remain important, it occurs in 15-50% of cases and affects the reproductive function of 1-5% of married couples [1, 2]. Understanding of RM differs: some authors understand RM as 3 and more interruptions of gestation, others think it possible to refer to this pathology 2 and more successive spontaneous abortions [2,3]. Etiology of RM includes such factors as genetic, infectious, autoimmune diseases, malformations of uterus, endocrine disorders [3]. A special role in the genesis of RM is attributed to chronic endometritis (CE) which often takes asymptomatic clinical course [3,4]. Inflammation of the endometrium is accompanied by inhibition of synthesis of growth factors and by enhancement of cytopathic and cytolytic effects of inflammatory cytokines [5] that may influence vasculo- and angiogenesis, the key factors for formation of embryo-placental blood flow and hemochorial type of trophism in the developing embryo [6].

It is known that 80% of reproductive losses occur in early gestation [1], therefore study of the sequence of vascularization of chorion villi in the first trimester of uncomplicated course of gestation permits to identify disorders in formation of blood vessels in villi and their participation in mechanisms of RM in female patients with CE on the 5-12th week.

At present some works are known dedicated to study of vasculo- and angiogenesis in the villous chorion of the first trimester of gestation interrupted by medical abortion [6-11]. In morphological examination it was found that formation of vessels in chorion villi starts with the process of vasculogenesis (formation of blood vessels de novo) on the 20-22nd day of gestation after ovulation (a.o.). In the prevasculogenic stage corresponding to the mentioned periods, mesenchymal cells of stroma differentiate in 3 directions: into primitive placental macrophages, cells-precursors of hemangioblasts, and pericytes. Further on hemangioblasts form cords of angioblasts with gradually increasing lumen. Sequential structural changes end in formation of capillaries. Formation of capillary network occurs by angiogenesis – development of new vessels from the existing ones. There are distinguished 2 types of angiogenesis: “branching” angiogenesis – branching of vessels, and “non-branching” one – increase in length of vessels [6-8]. Angioblasts and endotheliocytes may be visualized in the stroma of chorion villi by immunomorphological method with CD31 and CD34 markers. Foreign authors used 3D method of visualization of chorion villi in the first trimester of physiological course of gestation [8-10]. There exist a number of research works dedicated to structural changes in chorion villi in spontaneous abortions and in missed miscarriage with the distinctive morphological sign being avascularized villi evidencing disorders in formation of vascular component [11-13].

The aim of the work was to study stages of vasculo- and angiogenesis in the first trimester of uncomplicated course of gestation and to identify disorders in vascularization of villous chorion in RM with the underlying chronic endometritis.

## Materials and Methods

The villous chorion (30 cases in comparison group) obtained in official abortions of clinically healthy females on the 5-12th week of gestation, and scrapes from the uter-

ine cavity of females with CE and RM in the mentioned periods (35 cases of the main group) were studied.

The biological material was subject to sequential morphological processing according to methodology described in the "Guidelines" by D.S. Sarkisov, Yu.L. Perov [14]. For evaluation of the structural changes in chorion villi on the tissue and cellular levels cuts were prepared 4-5 μm thickness from paraffin blocks and stained with hematoxylin and eosin. Vasculo- and angiogenesis in chorion villi were studied in the material with one week gestation interval, with gestation identified by an obstetrician-gynecologist by the first day of the last menstruation.

**Results and Discussion**

The analysis of material of comparison group (obtained from clinically healthy women in official abortions) permitted to obtain the following data. On the 5$^{th}$ week of gestation vasculogenesis in the tertiary mesenchymal villi was in 2 cases represented by subepithelial and central accumulation of angioblasts in the stroma of villi, and in another 2 cases by endothelial tubules with narrow optically empty lumens visualized in stroma. In the villous chorion of 6 weeks' age vessels formed from the endotheliocytes were arranged subepithelially, and in the lumens erythroblasts were visualized. Results obtained on the 5-6$^{th}$ week of gestation, correspond to the morphological and immunohistochemical data earlier obtained on the 4$^{th}$ week of gestation (a.o.) [6,8]. Other authors, on the result of 3D examination, referred such changes to the 5-6$^{th}$ week of gestation (by ultrasound examination) [9,10].

On the 7$^{th}$ week of gestation first capillaries (1-2) were formed under epithelial layer on the periphery of large chorion villi, and endothelial tubules increased in number with nucleated erythrocytes in their lumen.

In villi of 8 weeks of gestation the amount of capillaries increased to 7-8 which evidences formation of the capillary network by angiogenesis. Capillaries were located not only on the periphery, but also in the paracentral and central zones of stroma. In the lumen of vessels erythroblasts were present. The obtained results comply with the hypothesis suggesting that formation of capillaries and increase in their quantity is associated with formation of capillary network by angiogenesis accompanying growth of chorion villi [6-9].

The 9$^{th}$ week of gestation was characterized by appearance of first denucleated erythrocytes. In capillaries located subepithelially, the lumen was twice increased. In larger centrally located vessels with optically empty lumens the structure of vessel walls complicated due to differentiation of the first myoblasts and their circular arrangement. The formed vessels differentiated into arterioles and venules [8].

On the 10$^{th}$ week, according to the authors' data, no significant and distinctive transformations of vessels were seen. With this, in the lumens only mature erythrocytes were visualized without erythroblasts. By the 11-12$^{th}$ week differentiation of arterioles and venules was completed in single stem villi.

Thus, in the first trimester of gestation in the villous chorion formation of capillary network and of major vessels of arteriole and venule type vital for formation of hemochorial type of nutrition providing adequate metabolism of substances and gases for the growing embryo, was completed [6,8].

In parallel, histological examination of material obtained in early period (5-12 weeks) from women with CE and RM, was conducted that revealed hypo- and avascularization of villous chorion in all studied periods of gestation. Thus, on the 5$^{th}$ week not only initial signs of formation of capillaries but also angioblasts were not visualized in the secondary mesenchymal villi of chorions. On the 6$^{th}$ week of gestation accumulations of angioblasts appeared in single villi forming angioblastic cords. Within the 7$^{th}$ week of gestation 70-80% of villi remained avascularized as before, and only 15-20% of them contained 1-2 subepithelially located vessels in stroma similar in structure to endothelial tubules with a narrow lumen. On the 8-9$^{th}$ week of gestation the number of capillaries in few vascularized villi increased 3-4-fold, and in their lumens first erythroblasts were visualized.

From the 10$^{th}$ to 12$^{th}$ weeks avascularized villi continued to predominate despite an in-

crease in gestational age. Here, on the 10-11th weeks in vascularized villi 1-2 capillaries were present as before located on the periphery of villi and containing erythroblasts in their lumen, and only on the 12th week of gestation first erythrocytes appeared in the vessel lumen.

Thus, in RM associated with chronic inflammation of the endometrium structural changes of the villous chorion were detected manifested by retardation of differentiation of villi after the 5th week of gestation, delay in vasculogenesis at the stage of formation of hemangioblastic cords typical of 6-7 weeks, with its inhibition in the subsequent period. In villi of 8-12 weeks of gestation further differentiation of vessels did not occur, so, branched angiogenesis was not formed. The main cause of disorders in differentiation of villi, of vasculo- and angiogenesis in stroma of chorion villi was suggested to be chronic inflammation of the endometrium that produces a negative effect on further development and successful completion of pregnancy. In general, the results obtained in the main group (RM with the underlying CE) were similar to those earlier described in such complication of pregnancy as spontaneous abortion and missed miscarriage [11-13].

## Conclusions

Development and formation of vessels in the villous chorion in the first trimester of gestation corresponding to the gestation age are essential for establishment of full-scale metabolic processes between mother and fetus that provide further development of embryo.

Histological examination of the material obtained from women with recurrent miscarriage in early periods (5-12 weeks) with the underlying chronic inflammation of the endometrium (n=35) in comparison with the results of histological examination of the material obtained from clinically healthy women in official abortions (n=30) confirmed existence of retardation of differentiation of villi in combination with delay of erythropoiesis, vasculo- and angiogenesis in chorion villi.

Authors believe that further examination of villous chorion in the given obstetrical pathology with use of immunohistochemical methods and electronic microscopy is required for identification of additional structural factors playing an important role in the pathogenesis of recurrent miscarriage with the underlying chronic endometritis.

*Authors have no conflict of interest to declare.*

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