Environmental influence on the features of the formation of placental structures in cow

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Abstract. Normal organization of placental structures at different stages of pregnancy is critical for the growth and development of the fetus. The aim of this study is to characterize the localization and ratio of epithelial and mesenchymal cells in the placenta of cows with physiological and complicated preeclampsia pregnancy using the immunohistochemical method.

To date, it has been established that adverse environmental factors adversely affect the reproductive potential of animals regardless of their gender [1, 2, 3].

One of the most common complications of pregnancy in mammals is preeclampsia, a multisystem disease resulting from abnormal placentation due to insufficient invasion of trophoblasts into the spiral arteries of the hemochorial type of placenta [4, 5]. And if the pathogenesis of preeclampsia in mammals with hemochorial placentas is being studied quite actively, then the features of the initiation and development of this pathological state in mammals with other types of placenta remain outside the sphere of interest of researchers.

According to the modern classification based on a complex of morphological characters, the placenta of cows belongs to the synepitheliochorial type. An earlier assumption that the uterine epithelium
degrades during placentation, as a result of which the trophoblast is directly attached to the maternal connective tissue, turned out to be untenable. It has now been established that the placenta of a cow is formed by the migration and fusion of a certain type of trophoblastic cells with epithelial cells of the uterus, thereby forming a hybrid fetal-maternal syncytium, represented by trophoblastic binuclear / giant cells [6, 7].

The trophoblast cells are represented by the placental epithelium, which develops rapidly during pregnancy, creating an organ responsible for the transport of nutrients and gas exchange between the blood of the mother and the fetus. To maintain the integrity of the cell structure in the cytoplasm, there is an internal framework of intermediate filaments of types I and II - keratins, which are necessary for the normal development and functioning of the placenta. It has been established that defects in the keratin cytoskeleton of trophoblasts (degradation or, conversely, the formation of aggregates) can lead to embryonic lethality [8, 9, 10]. In turn, placental stromal cells are of extraembryonic mesenchymal origin and are important for the formation and maintenance of villi, while cytoskeletal mesenchymal cells consist of another type of intermediate filaments - vimentin.

The aim of this study is to characterize the localization and ratio of epithelial and mesenchymal cells in the placenta of the synepitheliochorial type in physiological and complicated preeclampsia pregnancy as a morphological criterion for changes in the placental structures of cows with the development of a pathological condition.

2. Materials and methods

For pathomorphological examination, samples of placentas from cows with normal pregnancy and diagnosed preeclampsia were fixed in 10% neutral formalin. Then the samples were dehydrated and clarified, and embedded in a paraffin medium according to a conventional technique. Sections, 5 μm thick, were mounted on glass slides and stained with hematoxylin-eosin.

Paraffin sections of placenta samples 5 μm thick, subject to immunohistochemical and immunofluorescence studies, were mounted on glasses treated with poly-L-lysine («Menzel»).

Immunohistochemical reactions were performed using a peroxidase-polymer imaging system according to the manufacturer’s instructions (LabVision). To block endogenous peroxidase, the sections after dewaxing were incubated for 20 minutes in 3% hydrogen peroxide. Antibodies were unmasked by boiling the sections at 4 °C in citrate buffer with pH = 6.0 for 10 minutes. The slides were incubated overnight at 4 °C with antibodies to keratin-18 (mouse, monoclonal, clone C-04) and vimentin (rabbit, monoclonal, clone EPR3776). Peroxidase was developed with 3-3-diaminobenzidine from the protocol kit. At the final stage of the reaction, the sections were counterstained with Mayer's hematoxylin. Preparations without incubation with primary antibodies in full compliance with the remaining stages of the protocol served as a negative control.

In an immunofluorescence study, sections after dewaxing were incubated in a glycine solution to reduce autofluorescence. Antibodies were unmasked by boiling the sections at 100 °C in citrate buffer with pH = 6.0 for 10 minutes. Then the slides were placed horizontally in a humid chamber, 2 types of primary antibodies were applied: mouse and rabbit, and left overnight at 4 °C. The next day, slides with sections were washed in two shifts of 0.05 M Tris buffer for 20 minutes and first incubated for 1 hour with secondary antibodies conjugated to Alexa 594 (goat anti-rabbit, Abcam), then for another 30 minutes with secondary antibodies conjugated to FITC (goat anti-mouse, Abcam). Washed with buffer for a total of 30 minutes, then a few drops of embedding medium containing DAPI (Sigma) were applied to the sections. Micro-preparations were studied and photographed using an AxioScope.A1 (ZEISS) microscope equipped with a high-resolution digital camera AxioCamMRc5. The resulting photos were processed using the ZET pro 2012 (ZEISS) program.
3. Results and discussion

3.1. Results of the study of the content of intermediate filaments of the epithelial and mesenchymal phenotype in the placenta of a cow during the physiological course of pregnancy

The results of the carried out immunohistochemical study demonstrated that the cytokeratin skeleton of the cells lining the lacunae of the uterine septa occupied the entire space of the cytoplasm. Chorionic villi giant trophoblast cells also tested positive for the keratin-18 intermediate filament, but the protein was recorded predominantly on the surface of the cytoplasmic membrane (Figure 1).

Figure 1. Immunolocalization of K-18 in the placenta of a cow during physiological pregnancy. The presence of keratin-18 in the cells of the lining of the uterine crypts, as well as in the trophoblasts of the chorionic villi. Antibodies to keratin-18. Method – «Applied Behavior Analysis». Staining with Mayer's hematoxylin. Total magnification 200.

The study of the immunolocalization of mesenchymal filaments revealed that vimentin + cells, mainly fibroblasts, constituted the connective tissue matrix of the uterine septa, and were also part of the stroma of the chorionic villi. In the central part of the villi, cells immunopositive to vimentin, forming the capillary walls - endotheliocytes, were also recorded (Figure 2).

A visual assessment of the number of capillaries showed that, according to morphological features, the bulk of the stem and terminal villi were mature and well vascularized, which ensured good intrauterine development of the fetus.

Figure 2. Immunolocalization of vimentin in the placenta of a cow during normal pregnancy. Vimentin-positive structures that are part of the uterine septa, as well as the stroma of the chorionic villi and capillary walls (indicated by arrows). Antibodies to vimentin. Method – «Applied Behavior Analysis». Staining with Mayer's hematoxylin. Total magnification 200.
3.2. Results of the study of the content of intermediate filaments of the epithelial and mesenchymal phenotype in the placenta of cows during pregnancy complicated by preeclampsia

Histological examination of the maternal part of the placenta of cows with pregnancy complicated by eclampsia revealed hyperplasia of the uterine crypts. At the same time, the free space of the crypts formed by the septa turned out to be critically reduced, and the villi in such lacunae were almost completely reduced.

The study of keratin-18 revealed a sharp decrease in the amount of protein in the epithelial cells of the lining of the uterine crypts. A pronounced decrease in the content of epithelial-type filaments on the surface of trophoblasts was also noted (Figure 3).

Figure 3. Immunolocalization of K-18 in the placenta of a cow in complicated pregnancy with eclampsia. Degradation of the keratin skeleton in cytotrophoblasts and lack of response to epithelial filaments in the cells of the lining of uterine crypts. Antibodies to keratin-18. Method – «Applied Behavior Analysis». Staining with Mayer's hematoxylin. Total magnification 200.

The results of the immunohistochemical reaction to vimentin confirmed the degradation of the connective tissue elements that made up the skeleton of the intermediate and terminal villi: only structureless masses and a small number of macrophage-like cells were recorded in the lumen of the lacunae. In the central part of the unchanged villi, a sharp decrease in the number of small capillaries was noted (Figure 4).

Figure 4. Immunolocalization of vimentin in the placenta of a cow in complicated pregnancy with eclampsia. Reduction of terminal chorionic villi (indicated by crosses) and decreased vascularization of the remaining villi (indicated by arrows) Antibodies to vimentin. Method – «Applied Behavior Analysis». Staining with Mayer's hematoxylin. Total magnification 200.

3.3. Results of the study of the ratio of intermediate filaments of the epithelial and mesenchymal phenotype in the placenta of cows with physiological and complicated preeclampsia pregnancy

The results of studying the ratio of epithelial and mesenchymal elements using the immunofluorescent method of double staining of antibodies on one preparation confirmed that a significant decrease in keratin filaments both in trophoblasts of the villous tree of the fetus and in epithelial cells of the maternal...
part occurred in the placenta of cows during the development of preeclampsia in the last stages of pregnancy, compared to physiological values (Figure 5). In this case, vimentin filaments also underwent partial reduction, which indicated a premature degradation of the extracellular matrix.

|       | Control | Preeclampsia |
|-------|---------|--------------|
| **Merge** | ![Control Merge](image1) | ![Preeclampsia Merge](image2) |
| **Vimentin** | ![Control Vimentin](image3) | ![Preeclampsia Vimentin](image4) |
| **Keratin-18** | ![Control Keratin-18](image5) | ![Preeclampsia Keratin-18](image6) |

**Figure 5.** The ratio of epithelial and mesenchymal elements in placental structures during physiological pregnancy and complicated preeclampsia. Immunofluorescence method. Kernels are finished with DAPI. Total magnification × 200.
4. Conclusion
Understanding the patterns of development and involution of placental structures at different stages of gestation is of great importance for the early diagnosis of pathological conditions in farm animals, especially cattle. However, in the scientific literature there is a lack of experimental data on what molecular mechanisms underlie the pathogenesis of preeclampsia in cows, while the state of pathological retention of membranes has been studied in sufficient detail.

The analysis of the content of intermediate filaments of the epithelial and mesenchymal phenotype in the placental structures of a cow during the physiological course of pregnancy made it possible to identify the main morphological criteria in the structure of the organ:
1. The presence of a large number of small capillaries both in the chorionic villi and in the uterine septa.
2. Equilibrium ratio of mesenchymal and epithelial structures in the placenta during normal pregnancy.
3. Content of a large amount of keratin-18 intermediate filament in crypt lining cells, which play an important role in the formation of hybrid fetal-maternal syncytium.

On the other hand, the analysis of epithelial and mesenchymal filaments in the placenta of a cow during pregnancy complicated by preeclampsia demonstrated the following morphological signs of the pathological condition:
1. Reduction of vimentin filaments, accompanied by a decrease in vascularization of chorionic villi.
2. Degradation of keratin filaments involved in the formation of cell contacts between cytotrophoblasts of the villous tree of the fetus and epithelial cells of the maternal part of the placenta.

It should be noted that the processes of degradation of the extracellular matrix of the placenta also proceed normally, however, the time frame of these changes is shifted towards the end of pregnancy and, together with vascular disorders of the local type, they cause prenatal transformation of placental structures.

As our study has shown, for the physiological course of pregnancy, an appropriate balance of keratin and vimentin filaments in the structures of the placenta is required. Thus, it has been established that the keratin cytoskeleton in the trophoblasts of the mouse and human placenta is necessary to maintain the structural integrity of these cells and the development of the placenta, and keratin deficiency causes both defects in trophoblast differentiation and the integrity of the villi. In turn, vimentin filaments play a significant role in villous contractility and modulation of the intervillos space, thereby affecting the efficiency of blood circulation between the mother and fetus. Therefore, changes in the balance or simultaneous degradation of keratin and vimentin filaments in the structures of the placenta of cattle can be a criterion for the disruption of the placentation process with the subsequent development of preeclampsia.

Thus, our study showed that in the placenta of cows with preeclampsia, degradation of the cytokeratin skeleton, which is involved in the formation of cell contacts in the hybrid fetal-maternal syncytia, occurs, which ultimately disrupts the intrauterine development of the fetus. Therefore, the ratio of keratin and vimentin intermediate filaments can be used as a histological criterion for changes in the placental structures of cows during the development of preeclampsia.

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