Localization of matrix metalloproteinases in the placenta of cows with complicated pregnancy by placental insufficiency

V.S. Avdeenko1,*, D.I. Safronov1, and V.T. Akhmadov2

1St. Petersburg State University of Veterinary Medicine, St. Petersburg, Russia
2Chechen State University named after A.A. Kadyrov, Agrotechnological Institute, Grozny, Russia

Abstract. The placenta of 8 cows with physiological (PP) and placental insufficiency pregnancy (FPI) served as the material for the study. Analysis of the content of glycosaminoglycans (GAG) in the tissue of the uterine part of the placenta in cows with FPI indicated the degradation of the connective tissue matrix of cotyledons of chorionic villi and crypts of caruncles, due to the lack of interstitial substance structuring. Hyperplasia of crypts of caruncles is observed, which form glandular-like structures, with reduction in cotyledon chorionic villi in cotyledons. The level of MMP-3 metalloproteinase was detected in the structure of defragmented maternal crypts, and the presence of immunopositive cells was recorded only in the vascular region with active hyperplasia of crypt epithelial cells. The level of MMP-3 metalloproteinase was detected in the structure of defragmented maternal crypts, and the presence of immunopositive cells was recorded only in the vascular region with active hyperplasia of crypt epithelial cells. A decrease in the level of MMP-3 metalloproteinase in cotyledons of placental structures of cows was clearly noted in samples obtained from cows with placental insufficiency (FPI). Morphological changes in the placenta in cows with FPI, manifested before childbirth, are characterized by the free arrangement of cotyledons of chorionic villi in the crypts of caruncles formed by branches of uterine septa. Translocation of metalloproteinases of the MMR-3 type was found in the cotyledon space between the villi of the cotyledon chorion and the epithelium of the crypts of caruncles, especially in places where the villi are completely reduced. The conducted research may contribute to the development of enzyme immunoassay (ELISA) for the diagnosis of reproductive pathologies in mothers and newborns.

1 Introduction

Cows that give milk are often susceptible to postpartum uterine infections [1], which affects their fertility and economic performance. The main causes of late-stage pregnancy complications are factors of extragenital pathology. According to V. D. Kocharyan, et al. (2018), "... when pregnancy is complicated by eclampsia, it is usually accompanied by endogenous intoxication, changes in the protein and lipid composition of the blood, the
development of oxidative stress and the occurrence of a negative energy balance in the body" [2]. A number of authors [3, 4] with placental insufﬁciency, especially at the ﬁnal stage of gestation, highlight the rheological factor, in which there is an increase in erythrocyte aggregation, changes in blood coagulation properties with the development of symptoms of hepatopathy and nephropathy. K. M. Davenport et. al., [5] and S. F. S. Tavares Farias et. al. [6] in their publications argue that one of the decisive risk factors for complications at the end of pregnancy is a violation of microcirculation with damage to the endothelium of the capillaries of the uteroplacental carcas, mainly in the tissues of the maternal placenta, with the development of endocrine disorders leading to diffuse perfusion insufﬁciency of the placenta. According to the results of studies [7] and the prospects of using the determination of indicators of endogenous intoxication in blood serum and protein in urine in cows with symptoms of intrauterine developmental delay, which makes it possible to predict the development of reproductive pathologies in maternity patients. Matrix metalloproteinases (MMP) are zinc-dependent enzymes belonging to the family of metallopeptidases M10A and playing a key role in the destruction of the extracellular matrix (ECM). According to S. Heewon et. al., "...the contribution of MMP to matrix remodeling is extremely complex, since these endopeptidases have a wide range of functions" [8]. MMPs regulate the activity of various biologically active mediators, such as growth factors, cytokines and chemokines, both by direct cleavage and by disrupting their interaction with the extracellular matrix. Recently, a new classiﬁcation system has been proposed [9], which is based on the MMP structure, rather than on their specificity to the substrate. This system identiﬁes various categories of MMPs, such as archetypal MMPs, matrilysins, gelatinases, and furin-activated MMPs. Most MMPs are released from the cell into the environment as secreted enzymes. At the same time, there are membrane-type MMPs (MT-MMP), and some of them are found intracellularly, including the nucleus, where they function as transcription factors. The study focuses on the placenta of cows with normal and complicated pregnancies, where the main task is to determine the location of matrix metalloproteinases. This is extremely important because their activity is regulated by the TIMP family of proteins (tissue metalloproteinase inhibitors, α2-macroglobulin and the membrane-bound RECK protein, which induces the reversal of cysteine-enriched protein with Kazal motifs). This research may lead to the development of an ELISA rapid test.

The purpose of the study is to determine changes in the immunolocalization of matrix metalloproteinases in the placenta of cows when revealing demonstration markers.

2 Material and Methods

15 fragments measuring 1.0 x 1.0 cm² were analyzed in childbirth in Holstein cows, aged 3 to 5 years and weighing from 650 to 750 kg, with a milk yield of 11,000 to 15,000 liters per lactation. Newborn animals corresponded to the gestation period. The production of histological preparations was carried out according to a standard technique using light microscopy. Samples of caruncles and cotyledons are divided into fragments with a total area of up to 3 cm² and a thickness of at least 5 mm. Fixation was carried out in a solution of 10% neutral buffered formalin and poured in parafﬁn blocks. From the obtained blocks, a series of sections were prepared on a microtome in the entire thickness of the placentoma from the allantochorion to the endometrium. Next, the sections were stained with hematoxylin Carrazzi-eosin, according to Van Gieson, impregnated by Foot and stained with fuchselin according to Hart. Sections with a thickness of 5 microns were subjected to immunohistochemical analysis. These slices were attached to slides coated with poly-L-lysine. After dewaxing, endogenous peroxidase was inhibited by 20-minute incubation in 3% hydrogen peroxide solution. Immunohistochemical analyses were performed using the peroxidase-polymer imaging system in accordance with the manufacturer's recommendations.
(Lab Vision, Thermo, USA). The work used antibodies: recombinant bovine anti-MMP-2; new antibodies targeting specific proteins: some against MMP-3 and others against TIME-2. To visualize the activity of peroxidase, 3,3’-diaminobenzidine was used according to a standardized protocol. After that, the sections were stained with Mayer hematoxylin for further analysis.

Tissue samples in paraffin sections with a thickness of 5 microns, intended for immunofluorescence staining, were placed on slides pre-coated with Menzel poly-L-lysine (manufactured in Germany). After the dewaxing process, the sections were incubated in glycine solution to reduce autofluorescence, and then fixed and stained with antibodies conjugated with fluorochrome to identify specific cell populations. The antibodies were unmasked by boiling the slices at 100°C in a citrate buffer with pH = 6.0 for 10 minutes. After that, the slides with cuts were placed horizontally in a wet chamber, where two types of primary antibodies were applied: mice and rabbits, and left overnight at 4°C. The next day, the slides with cuts were washed in two shifts of 0.05 M Tris buffer for 20 minutes and first incubated for 1 hour with secondary antibodies conjugated with Alexa 594 (goat-anti-rabbit, Abcam), then for another 30 minutes with secondary antibodies conjugated with FITC (goat-anti-mouse, Abcam). After that, buffer washing was carried out for a total of 30 minutes, and several drops of the enclosing medium containing DAPI (Sigma) were applied to the cuts.

3 Results

Hyperplastic cotyledon epithelial cells inside the crypts were exposed to MMP-3 expression, as shown in Figure 1A. In case of FPI, translocation of matrix metalloproteinase type MMP-3 occurred in the cotyledons of the cavity of the crypts of caruncles, where the free space was significantly reduced, and the villi in these crypts were almost completely reduced, as shown in Figure 1B. Diffuse distribution of matrix metalloproteinases of the MMP-3 type (Figure 1A) was also noted in the cytoplasm of epithelial cells in the maternal septa of crypts of caruncles.

![Fig. 1](https://doi.org/10.1051/bioconf/202410803008)

**Fig. 1.** Immunolocalization of matrix metalloproteinases in the placenta of cattle: A – PP; B – FPI. (1 - Recombinant Bovine Anti-MMP-2 antibod; 2 - Antibodies: Recombinant Bovine anti-MMP-3, Papanicolaou smear. Staining with Mayer's hematoxylin). Total magnification is ×200.
When studying the ratio of MMP-3/TIMP-2 and MMP-2/TIMP-2 in the placentas of cows with PP, using double immunofluorescence staining, it was revealed that the content of the tissue inhibitor could visually be attributed to membrane-bound proteins, then with FPI they had cytoplasmic localization. Indirectly, this may indicate a violation of the integrity of cell membranes, Figure 2.

![Image](https://example.com/image.png)

**Fig. 2.** 1 - The ratio of MMP-2/TIMP-2 and 2 - MMP-3/TIMP-2 in placental structures of cattle (Immunofluorescence method. The cores are DAPI-stained). Total magnification is ×200.

In addition, MMP-9 remained present only in the decaying cotyledons of the maternal part of the placenta and surrounding vessels (Figure 2A). Weak expression of MMP-9 was observed in the cytoplasm of epithelial cells of the maternal part of the placenta. The ratio of the studied types of metalloproteinases in the placental structures of cows with FPI tended towards MMP-3. Analysis of the content of the tissue inhibitor TIMP-1 of the studied types of metalloproteinases in the placenta of cows did not show significant changes in the expression of this protein in placental structures. Nevertheless, it is worth noting a decrease in the level of the TIMP-1 inhibitor of matrix metalloproteinases in the cytoplasm of giant cells in placenta samples obtained from cows with FPI (Figure 2B).

## 4 Conclusion

In placentomas with fetal placental insufficiency (FPI), an increase in the number of blood capillaries penetrating between trophoblastic cells is observed. In the stroma of cotyledon villi, there is an increase in the number of collagen fibers and an increase in the number of fibroblasts with larger sizes than in physiological pregnancy (PP). Metalloproteinase of the MMP-9 type is reduced in the placental structures of cows with FPI, especially in the degrading cotyledons of villi entering the crypts of the maternal part of the placenta and around the villi vessels of the chorion of the fetal part of the placenta. Degradation of the connective tissue matrix of the crypts of caruncles is noted in the uterine part of the placenta of cows with FPI, which indicates the lack of structure of the interstitial substance. The translocation of metalloproteinases of the MMP-3 type is observed in the space between the cotyledon chorion villi cotyledon and the epithelium of the crypts of caruncles, especially in
places where the villi are completely reduced. In the cotyledons of the fetal part of the placenta of a healthy cow, MMP-9 is distributed throughout the cytoplasm of syncytiotrophoblasts and trophoblasts, covering the entire area of cells around the chorionic villi, with the exception of binucleated cells. Thus, increased activity and distribution of matrix metalloproteinases in cotyledons of cows with FPI before childbirth contributes to the degradation of the intracellular matrix of the maternal part of the placenta, which can cause reproductive disorders, and low or absent expression of matrix metalloproteinases may be a consequence of neonatal diseases of newborns. Consequently, a change in the balance of metalloproteinases in the placenta of cows may contribute to the development of reproductive pathologies.

Acknowledgements

The research was carried out within the framework of a grant from the Russian Science Foundation 23-26-00284, https://rscf.ru/project/23-26-00284/.

References