

ИММУНОБИОЛОГИЧЕСКИЕ СВОЙСТВА ВАГИНАЛЬНОГО СЕКРЕТА У ЗДОРОВЫХ И БОЛЬНЫХ МИКОПЛАЗМОЗОМ КОРОВ

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Резюме. Генитальный микоплазмоз у коров — заболевание, сопровождающееся латентным течением, что затрудняет его своевременную диагностику и назначение адекватной терапии. Неспецифическая симптоматика микоплазмоза в совокупности с периодами бессимптомного течения приводит к развитию функциональных и морфологических изменений в органах половой системы коров, заканчивающихся в итоге бесплодием. Мониторинговые исследования животноводческих хозяйств Северо-Западного региона РФ показали, что инфицированность гениталий коров микоплазмами может составлять от 20% до 40% поголовья. Причем прослеживается четкая зависимость между высокой инфицированностью поголовья и низкими показателями воспроизводства. В связи с этим животноводческие предприятия несут существенный экономический ущерб. Одним из важных механизмов, предотвращающих внедрение различных патогенов в репродуктивный тракт, является резистентность слизистой оболочки влагалища. Среди этих факторов наиболее важная роль отводится вагинальной аутофлоре, десквамации эпителия, фагоцитозу, кислотности вагинального секрета, содержанию в нем иммуноглобулинов, лизоцима и ряду других неспецифических факторов защиты. Нами было проведено изучение изменения иммунобиологических характеристик вагинального секрета при субклиническом течении генитального микоплазмоза. Для исследования были отобраны здоровые коровы с отрицательным PCR-тестом на *Mycoplasma* spp. и инфицированные *Mycoplasma* spp., без клинических признаков вагинита. У обеих групп животных проводили определение в вагинальном секрете концентрации водородных ионов, активности лизоцима и иммуноглобулинов классов IgG, IgM, IgA и sIgA. Несмотря на то, что персистенция микоплазм во влагалище не сопровождается выраженными клиническими признаками вагинита, но состояние факторов защиты слизистой оболочки претерпевает значительные изменения. Проведенные исследования позволили установить, что длительное нахождение микоплазм во влагалище коров проявляется достоверным увеличением концентрации водородных ионов на 41% и снижением лизоцимной активности вагинального секрета в 2 раза, то есть наблюдается угнетение основных факторов предотвращающих колонизацию слизистой патогенной микрофлорой. Кроме того, происходит перераспределение классов иммуноглобулинов в вагинальном секрете. Это проявляется достоверным увеличением содержания IgM и sIgA на фоне тенденции к снижению IgA. Отмеченные изменения в совокупности создают благоприятные условия для внедрения секундарной микрофлоры и развитию бактериально-микоплазменных вагинитов, усугубляющих морфофункциональные изменения в репродуктивном тракте и повышающих риск возникновения бесплодия.

Ключевые слова: коровы, иммуноглобулины, вагинальный секрет, микоплазмоз, лизоцим

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Образец цитирования:

Р.М. Васильев, С.В. Васильева «Иммунобиологические свойства вагинального секрета у здоровых и больных микоплазмозом коров» // Медицинская иммунология, 2021. Т. 23, № 4. С. 987-990.
doi: 10.15789/1563-0625-IBP-2278
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For citation:

R.M. Vasiliev, S.V. Vasilieva "Immuno-biological properties of vaginal discharge in healthy and mycoplasmosis-infected cows", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2021, Vol. 23, no. 4, pp. 987-990.
doi: 10.15789/1563-0625-IBP-2278
DOI: 10.15789/1563-0625-IBP-2278

IMMUNO-BIOLOGICAL PROPERTIES OF VAGINAL DISCHARGE IN HEALTHY AND MYCOPLASMOSIS-INFECTED COWS

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Abstract. Genital mycoplasmosis in cows is a disease accompanied by a latent course, which complicates its timely diagnosis and the appointment of adequate therapy. The nonspecific symptomatology of mycoplasmosis, combined with periods of asymptomatic course, leads to the development of functional and morphological changes in the organs of the reproductive system of cows, resulting in infertility. Monitoring studies of livestock farms in the North-West region of the Russian Federation have shown that infection of the genitals of cows with mycoplasmas can be from 20 to 40% of the livestock. Moreover, there is a clear relationship between the high infection rate of the livestock and low reproduction rates. In this regard, livestock enterprises incur significant economic damage. One of the important mechanisms preventing the introduction of various pathogens into the reproductive tract is the resistance of the vaginal mucosa. Among these factors, the most important role is assigned to vaginal autoflora, epithelial desquamation, phagocytosis, acidity of vaginal secretions, the content of immunoglobulins, lysozyme and a number of other nonspecific protective factors in it. We studied the changes in the immuno-biological characteristics of vaginal secretions in the subclinical course of genital mycoplasmosis. Healthy cows with a negative PCR test for *Mycoplasma* spp. were selected for the study. and infected with *Mycoplasma* spp., without clinical signs of vaginitis. In both groups of animals, the concentration of hydrogen ions, the activity of lysozyme and immunoglobulins of the classes IgG, IgM, IgA and sIgA were determined in the vaginal secretions. Despite the fact that the persistence of mycoplasmas in the vagina is not accompanied by pronounced clinical signs of vaginitis, but the state of protective factors the mucous membrane undergoes significant changes. The conducted studies made it possible to establish that the long-term presence of mycoplasmas in the vagina of cows is manifested by a significant increase in the concentration of hydrogen ions by 41% and a decrease in the lysozyme activity of vaginal secretions by 2 times, that is, inhibition of the main factors preventing the colonization of mucous pathogenic microflora is observed. In addition, there is a redistribution of classes of immunoglobulins in the vaginal secretion. This is manifested by a significant increase in the content of IgM and sIgA against the background of a tendency towards a decrease in IgA. The noted changes in aggregate create favorable conditions for the introduction of secondary microflora and the development of bacterial-mycoplasma vaginitis, aggravating morpho-functional changes in the reproductive tract and increasing the risk of infertility.

Keywords: cows, immunoglobulins, vaginal secretions, mycoplasmosis, lysozyme

Introduction

In recent years, the attention of veterinary specialists has increasingly been drawn to diseases that are not accompanied by specific clinical manifestations and characterized by a long latent course. These features complicate early diagnostics and, as a result, the timely administration of adequate therapy. As a result, irreversible functional and morphological changes develop in the body, leading to the premature exclusion of animals from economic use, causing significant economic damage to livestock enterprises. One of such diseases is the genital form of mycoplasmosis [3, 8]. It is known that long-term mycoplasma persistence in the genitourinary system has a negative effect, including the reproductive function of animals [6, 7]. Genital mycoplasmosis can manifest not only by chronic urethritis and vaginitis, but also by infertility, fetal mortality, and early abortion. Quite often, farm veterinarians find no connection between these manifestations and mycoplasmosis. A study

on the major genital infections provided a negative result, and a biochemical blood test indicates a good metabolic status in animals. As a result, mycoplasmas continue to persist in the herd, increasing the number of infected animals. Monitoring of livestock farms in the North-West region of the Russian Federation showed that urogenital mycoplasmosis is widespread and shows a clear correlation with low reproduction rates [10].

The vaginal mucous membranes display a wide range of protective factors to resist invasion and reproduction of pathogenic microorganisms. Among them, a significant role is played by the vaginal microflora, desquamation of the epithelium, phagocytosis, acidity of the vaginal secretion and level of immunoglobulins, lysozyme and other nonspecific protective factors therein. Thus, the natural vaginal microbiota in combination with a certain range of hydrogen ions concentration prevents the penetration and reproduction of most pathogenic microorganisms. Immunoglobulins contained in vaginal secretions,

especially secretory immunoglobulin A (sIgA), prevent the adhesion of infectious agents to epithelial cells of the mucous membrane via formation of immune complexes, and also activate the complement system neutralizing the pathogen biological activity. Lysozyme hydrolyzes murein contained in the cell wall of many microorganisms.

Taking into account the features of the mycoplasma biology, which allows to escape from some arms of the immune response, it would be worth studying what changes are observed in the cow local immunity of the vaginal mucosa during their long-term persistence.

Materials and methods

The studies were carried out in JSC "Osminskoye" of the Slantsevsky district of the Leningrad region on non-pregnant cows aged 3–4 years old. There were formed 2 groups per 8 animals in each. The group 1 contained healthy cows with negative PCR test for *Mycoplasma* spp.; group 2 consisted of cows with a positive PCR test for *Mycoplasma* spp., without overt clinical signs of vaginitis. For the diagnosis of mycoplasmosis, the test system "MIC-COM", LLC "InterLabService", Russia was used. *M. bovis genitalium* was identified by the serological method in such animals. In both groups, vaginal discharge was collected from the walls of the vagina using a special spoon in the area of the cervix.

The concentration of hydrogen ions (pH), the activity of lysozyme, level of total immunoglobulins and their classes were determined in the samples. Concentration of hydrogen ions was measured by using the test system "Colpo-test pH" LLC "Biosensor AN", Russia. For this, the test strip was immersed in the vaginal sample for 2 seconds, removed, excess material was removed, and the result was assessed 15 seconds later. The level of immunoglobulins classes IgG, IgM, IgA and sIgA was determined by the method of radial gel immunodiffusion according to Mancini, using antisera against cattle immunoglobulins manufactured by Hangzhou Sunlong Biotech Co., Ltd. China. Lysozyme activity was determined by the nephelometric method according to V.G. Dorofeychuk method using a test culture of *Micrococcus lysodeikticus* strain No. 2665. The results obtained were statistically processed by using the SPSS 22.0 computer software.

Results and discussion

The studies carried out have established significant changes in the immuno-biological characteristics of vaginal discharge in cows with mycoplasmosis.

Thus, the concentration of hydrogen ions in the vaginal discharge of healthy cows ranged from 4.2 to 5.5 and averaged 4.65 ± 0.02 . In cows with genital mycoplasmosis, it ranged from 6.0 to 7.0 and averaged 6.56 ± 0.02 .

The study of the immunoglobulin class level in the vaginal discharge showed the multidirectional pattern of the changes. The concentration of IgG in healthy cows and cows with mycoplasmosis practically did not differ and reached to 0.19 ± 0.008 g/l and 0.183 ± 0.008 g/l, respectively. The IgM level in healthy cows was 0.039 ± 0.002 g/l, while in those infected with mycoplasma it was increased – 0.054 ± 0.003 g/l. The level of IgA in the vaginal secretion of healthy animals was 0.024 ± 0.003 g/l, while in animals with mycoplasmosis it was slightly decreased – 0.018 ± 0.001 g/l. The data on the level of secretory immunoglobulin A were as follows: 0.067 ± 0.005 g/l – in healthy cows and 0.096 ± 0.007 g/l – in infected cows. It should be noted that the total level of immunoglobulins in the vaginal discharge of healthy and mycoplasma-infected cows did not differ significantly – 0.32 g/l and 0.351 g/l, respectively.

Assessing the lysozyme activity in vaginal discharge showed that in healthy animals it was $11.71 \pm 0.41\%$, whereas in animals with mycoplasmosis it was significantly lowered – $5.86 \pm 0.33\%$.

Although the persistence of mycoplasmas in the vagina was not accompanied by pronounced clinical signs of vaginitis, the state of the mucous protection underwent significant changes [2, 5].

The concentration of hydrogen ions in vaginal secretions in cows infected with mycoplasmas increased by 41% ($p < 0.001$) and became close to neutral pH value. Such a shift in pH value exerted a suppressive effect on the vaginal microflora and, at the same time, was more favorable for pathogenic microorganisms. As a result, an environment is created in the vagina promoting to development of secondary bacterial vaginitis.

The study of the immunoglobulin level showed that its total amount in the vaginal discharge in healthy and mycoplasma-infected cows was virtually similar suggesting that they do not actively migrate through the histohematogenous barrier. However, the immunoglobulin class composition was markedly altered. The level of IgG in the discharge of sick animals was by 0.007 g/l lower than in healthy animals, which was insignificant ($p > 0.05$). Such changes indicate that the persistence of mycoplasmas in the vaginal mucosa does not trigger the opsonization mechanism nor activate the complement system. The decrease in IgA level by 25% observed in cows with mycoplasmosis was also insignificant. Along with this, the sIgA level in infected animals was higher by 43% than in healthy cows, and such changes had a high degree of significance ($p < 0.001$). Since secretory immunoglobulin A prevents the adhesion of pathogens on the mucous membranes and stimulates the phagocytic activity of macrophages and neutrophils, an increase in its concentration indicates potentiated protection of the mucous membranes

from their further colonization by mycoplasmas [9]. A slight decrease in the amount of IgA is probably associated with its transport into epithelial cells to form sIgA. Changes in the concentration of class A immunoglobulins of similar nature on some mucous membranes of cows with mycoplasmosis were also noted elsewhere [1, 4]. While studying the content of IgM, its significant ($p < 0.01$) increase by 38.5% in the vaginal discharge of mycoplasma-infected cows was established. Since IgM is produced first in response to contacting any antigen, long-term maintenance of its high concentration suggest about systematic mycoplasma antigenic stimulation of the vaginal mucosa. The antigenic polymorphism of the pathogen can contribute to long-term stimulation, and the high homology to the structural proteins of the animal host allows it to evade the pre-synthesized antibodies.

While studying the lysozyme activity of vaginal discharge, it was found that in cows infected with mycoplasmas, it was lowered by 2-fold compared to healthy animals. It might be due to the fact that the

structure of the mycoplasma cytoplasmic membrane differs significantly from the structure of the bacterial cell wall, destruction of which is directed by lysozyme, as well as the possible production of metabolites that suppress the activity of lysozyme-producing cells.

Thus, the persistence of mycoplasmas in the vaginal mucous membrane of cows causes multidirectional changes in protective properties. This is manifested by a significant increase in the concentration of hydrogen ions and decreased lysozyme activity of vaginal discharge, i.e., inhibition of the main factors preventing the colonization of the mucous layers by pathogenic microflora is observed. In addition, immunoglobulin classes were redistributed in the vaginal discharge. Such changes along with favorable conditions were generated for invasion of secondary microflora and the development of bacterial-mycoplasma vaginitis. The established changes will allow to outline a rational protocol for treatment of cows with genital mycoplasmosis.

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Поступила 15.03.2021

Отправлена на доработку 01.06.2021

Принята к печати 09.06.2021

Received 15.03.2021

Revision received 01.06.2021

Accepted 09.06.2021