The state of some protective factors of the vaginal mucosa in healthy cows and cows with mycoplasmosis

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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 specific treatment. This disease may lead to infertility. An obstacle to the introduction of pathogens into the epithelium of the mucous membrane is a variety of defense mechanisms. A study of the state of the protective properties of the vaginal mucosa in healthy and mycoplasma-infected cows was carried out by determining the concentration of hydrogen ions and immunoglobulins in vaginal secretions. The results of the study showed a shift in the reaction of vaginal secretions towards neutrality in mycoplasmosis. Thus, the persistence of mycoplasmas in the mucous membrane of the vagina of cows causes a change in the protective properties of the vaginal secretion, as a result of which conditions are created that promote the activation of pathogenic microflora.

1 Introduction

Recently, the attention of veterinary specialists has been drawn to diseases that are not accompanied by specific clinical manifestations and are characterized by a long course. This feature complicates their timely diagnosis and, as a consequence, the appointment 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. One of these diseases is mycoplasmosis, and in particular its genital form [1, 2]. It is known that long-term persistence of mycoplasmas in the organs of the genitourinary tract has a negative effect, including on the reproductive function of animals [3]. Genital mycoplasmosis can manifest itself not only by chronic urethritis and vaginitis, but also by infertility, fetal mortality, and early abortion. In livestock farms in the North-West region of the Russian Federation, urogenital mycoplasmosis is widespread, which negatively affects reproduction rates [4].

The possibility of introducing pathogens into the mucous membranes and their ability to initiate the inflammatory process is largely influenced by nonspecific and specific protection factors. Thus, a certain range of concentration of hydrogen ions in the vaginal

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secretion is comfortable for the natural microbiota and prevents the reproduction of pathogenic microorganisms. One of the most important specific protective factors is the immunoglobulin composition of vaginal secretions, especially the content of secretory immunoglobulin A (sIg A) in it. This class of immunoglobulins prevents the adhesion of infectious agents to the epithelial cells of the histohematogenous barrier due to the formation of immune complexes, and also neutralizes the biological activity of pathogens. Based on the foregoing, it was of interest to us to compare the state of protection factors of the vaginal mucosa in healthy cows and cows with genital mycoplasmosis.

2 Materials and methods

The studies were carried out in JSCOsminkoye, Slantsevsky District, Leningrad Region, on non-pregnant cows 3-4 years old. Were formed 2 groups of 8 animals each. The first group is healthy cows in which the PCR test for Mycoplasma spp. was negative. The second group consisted of cows with a positive PCR test for Mycoplasma spp., without pronounced clinical signs of vaginitis. [5] M. bovigenitalium was identified by serological method in these animals. In both groups of animals, vaginal secretions were collected from the walls of the vagina using a special spoon in the cervical region.

In the obtained secret, the concentration of hydrogen ions (pH) and the content of immunoglobulins and their classes were determined. Determination of the concentration of hydrogen ions was carried out using the test system "Colpo-test pH" [6]. For this, the test strip was immersed in the test secret for 2 seconds, removed, the excess secretion was removed, and the result was read after 15 seconds. The content of immunoglobulins of the classes Ig G, Ig M, Ig A and sIg A was determined by the method of radial immunodiffusion in a gel according to Mancini. The results obtained were statistically processed using the SPSS 22.0 computer program.

All conducted research is approved by the ethical commission of the university.

3 Results and discussion

The conducted studies have established significant differences in the concentration of hydrogen ions in the vaginal secretions of healthy cows and those with mycoplasmosis (Table 1).

| Animal groups                      | Concentration of hydrogen ions (pH) | Oscillation range | Average value |
|-----------------------------------|-------------------------------------|-------------------|---------------|
| Clinically healthy                |                                     | 4,2 – 5,5         | 4,65±0,02     |
| Cows with genital mycoplasmosis   |                                     | 6,0 – 7,0         | 6,56±0,02     |

The results of the study show that in animals with mycoplasmosis, an increase in the concentration of hydrogen ions by 1.91 units was observed, with a comparable range of fluctuations of this indicator.

The multidirectional nature of changes was observed when studying the content of classes of immunoglobulins in vaginal secretions in the studied groups of animals (Table 2).
Table 2. Content of classes of immunoglobulins in the vaginal secretions of healthy cows and mycoplasmosis cows.

| Classes of immunoglobulins | Clinically healthy | Cows with genital mycoplasmosis |
|----------------------------|--------------------|---------------------------------|
| Ig A, g/l                  | 0.024±0.003        | 0.018±0.001                     |
| Ig M, g/l                  | 0.039±0.002        | 0.054±0.003                     |
| Ig G, g/l                  | 0.19±0.008         | 0.183±0.008                     |
| sIg A, g/l                 | 0.067±0.005        | 0.096±0.007                     |

From the data in the table, it can be seen that in cows with mycoplasmosis in the vaginal secretions there is a decrease in the content of Ig A, an increase in Ig M and sIg A, against the background of a stable concentration of Ig G.

Despite the fact that in a large number of cases, genital mycoplasmosis proceeds without pronounced signs of vaginitis, changes are observed in the vaginal secretion from the side of local protection factors [7].

When studying the pH of vaginal secretions, a shift of this indicator to the neutral side is observed. In cows with mycoplasmosis, compared with healthy cows, the concentration of hydrogen ions was 41% higher and the change was significant (P <0.001).

An immunological study shows that the total content of immunoglobulins in the vaginal secretion in healthy and mycoplasmosis animals was almost the same 0.32 g / l and 0.351 g / l, respectively. However, the distribution of immunoglobulins by class had significant differences. The Ig A concentration in cows with mycoplasmosis was 0.018 ± 0.001 g / l, which was 25% less than in healthy cows - 0.024 ± 0.003 g / l, but the difference was not significant (P> 0.05). The level of Ig G in vaginal secretions in healthy people and patients with mycoplasmosis was almost the same - 0.19 ± 0.008 g / l and 0.183 ± 0.008 g / l. The content of immunoglobulin M in the vaginal secretion of healthy cows was 0.039 ± 0.002 g / l, while in patients with mycoplasmosis it was significantly higher - 0.054 ± 0.003 g / l, this change was significant (P <0.01). A similar trend was observed for secretory immunoglobulin A (sIgA). In healthy cows, the sIgA level was 0.067 ± 0.005 g / l, while in patients with mycoplasmosis it was 43% higher - 0.096 ± 0.007 g / l, and the differences had a high degree of reliability (P <0.001).

The results obtained allow us to assert that the persistence of mycoplasmas in the mucous membrane of the vagina of cows causes a chronic inflammatory process, leading to an imbalance in a number of its protective factors.

A decrease in the acidity of the vaginal secretion in mycoplasmosis has a depressing effect on the vaginal autoflora and creates an opportunity for the introduction of pathogenic microorganisms into the mucous membrane. As a result, sick animals often develop mixed infections with severe clinical signs of vaginitis.

Analyzing the data obtained in the study of the content of immunoglobulins in vaginal secretions, it can be said that mycoplasmas do not cause active migration of immunoglobulins across the histo-hematic barrier, but a redistribution of their class composition is observed. The identical content of Ig G in vaginal secretions in healthy and mycoplasmonic animals indicates that the pathogen, in terms of its biological properties, is capable of escaping opsonization and does not activate the complement system. A significant increase in sIgA in animals with mycoplasmosis indicates the activation of the protection of the vaginal mucosa from further colonization by mycoplasmas [8]. An increase in Ig M levels indicates that mycoplasmas provide systematic antigenic stimulation of the immune system. On the one hand, this occurs due to the antigenic polymorphism of
the pathogen, and on the other hand, the high homology to the structural proteins of the animal body allows it to evade the immune response.

4 Conclusion

The results of the studies conducted allow us to assert that the persistence of mycoplasmas in the vagina of cows causes a change in the protective properties of the mucous membrane. This is manifested by an increase in the concentration of hydrogen ions, as well as a redistribution of classes of immunoglobulins in vaginal secretions. These changes create favorable conditions for the introduction of secondary microflora and the development of bacterial-mycoplasma vaginitis.

References

1. M. Hazelton, J. Morton, J. House, Journal of Dairy Science 103 (12), 11844-11856 (2020)
2. L. Sabirzianova, P. Anipchenko, A. Yashin [et al.], Journal of Animal Science 97 (S3), 214-215 (2019) DOI 10.1093/jas/skz258.439
3. C. Kirkbride, Veterinary Clinics of North America: Food Animal Practice 3(3), 575-591 (1987)
4. R. Vasiliev, International Bulletin of Veterinary Medicine 3, 15-16 (2008)
5. S. Makavchik, A. Sukhinin, S. Abgaryan, I. Belkina, Research Journal of Pharmaceutical, Biological and Chemical Sciences 10 (1), 2004-2012 (2019)
6. S. Makavchik, A. Sukhinin, Y. Danko [et al.], Reproduction in Domestic Animals, 54(S3), 98 (2019)
7. S. Jimbo, M. Suleman, T. Mainga, T. Prysiak, M. Mulongo, J. Perez-Casal, Veterinary Immunology and Immunopathology, 188, 27-33 (2017)
8. L. Corbeil, P. Bier, J. Duncan, Theriogenology, 6 (1), 39-44 (1976)