Research Article

Opportunistic Microorganisms in the Etiology of Nonspecific Andrological Diseases of Cattle and Comparative Effectiveness of Antibacterial Drugs

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Abstract | The article presents the study of the etiological factor of nonspecific andrological diseases of bulls in the Republic of Bashkortostan and changes in the morphological and biochemical composition of blood in these diseases. The influence of complex antibacterial therapy on the microbial landscape of the preputial cavity in the treatment of nonspecific andrological diseases is considered. The relationship between clinical signs, laboratory parameters of semen quality and biochemical and haematological blood parameters of animals before and after treatment is revealed. Treatment was performed using the following drugs: the first group was treated with Bicilin-3 (penicillins pharmacological group), Boflox (fluoroquinolones) was used to treat the second group of animals, and the third group was treated with Tetracycline hydrochloride (tetracyclines). The paper presents the results of the clinical examination of animals, the study of the reproductive system, the assessment of the degree of reflexes manifestation and the obtained sperm quality. The composition of the opportunistic pathogenic microflora of the preputial cavity of bulls with balanoposthitis and mixed andrological pathology (balanoposthitis, vesiculitis and prostatitis) was determined by the method of microbiological research. There is a significant decrease in the number of red blood cells, hematocrit and haemoglobin, while haemoglobin concentration in one red blood cell increases. The number of platelets increases significantly in comparison with clinically healthy animals. The animals were divided into groups according to the principle of pairs of analogues. Each drug was tested on animals with both balanoposthitis and mixed andrological pathology. Animals were isolated and kept in equal conditions. In the first group, the therapeutic efficacy was 25%, in the second – 100%, and in the third – 75%. Boflox showed high therapeutic effectiveness in treating all types of andrological pathology, including balanoposthitis and the adnexal sex glands inflammation. Tetracycline hydrochloride was also highly effective for treating balanoposthitis, but it was ineffective in mixed andrological pathology. Bicillin-3 showed low efficacy in both types of the pathological process. The results of the conducted therapeutic measures demonstrate the greatest effectiveness of Boflox, which belongs to the group of fluoroquinolones. Treated animals were studied, and opportunistic pathogenic microorganisms were not found.

Keywords | *Staphylococcus* sp., *Enterobacteriaceae* sp., *Streptococcus* sp., Balanoposthitis, Vesiculitis, Boflox

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INTRODUCTION

The main task for agricultural science and the agro-industrial complex is to provide the population with livestock products. Many factors hamper the increase in the number and productivity of animals. Nonspecific andrological diseases are among the most significant factors, especially those caused by opportunistic pathogenic

microflora, which in recent years has played a crucial role in animal disease (Chenoweth and Osborne, 1978; Cockcroft, 2015; Paray et al., 2018). According to some researchers, currently, there is a steady decrease in semen quality of producing bulls, its volume, concentration and motility of sperm cells, as well as a significant increase in the number of abnormal forms of germ cells (Comhaire et al., 1980; Skreekumaran, 2000; Davidson, 2003; Debasish, 2003; Ivanov et al., 2018). Early culling of producing bulls when they lose breeding value due to the reproductive system's diseases causes significant damage to animal husbandry (Saunders and Ladds, 1978; Cavalieri and Van Camp, 1997). In this connection, the problem of andrological diseases is most acute for livestock breeding (Schollum, 1997). For example, 128 out of 280 adult bulls were culled for different reasons. 73.4% of them had organic pathologies of the reproductive organs, and 49.2% were culled due to poor sperm quality (Perumal et al., 2013; Cockcroft, 2015; Ventsova and Safonov, 2021).

According to the literature data, vesiculitis is the most prevalent, and the currently existing therapy methods are not very effective (Cavalieri and Van Camp, 1997; Matiukhina, 2011; Norman et al., 2003).

Andrological diseases are also relevant for beef cattle breeding (Andreeva et al., 2020; Nikolaeva et al., 2020) as they lead to a decrease in weight gain, and in the case of complications and the absence of treatment, they can even cause the death of animals.

In andrological diseases, the main pathological changes occur in the reproductive organs, and any physiological and pathological changes in the body lead to changes in the blood (Amare, 2016; Ivanov et al., 2018; Vorobyov et al., 2019). The blood performs a protective function due to the presence of white blood cells in it. With antigen exposure to the body, their quantitative indicator changes. The criterion for the metabolism level indicator in the body is the biochemical composition of the blood. From the point of view of diagnosis, changes in blood parameters are, in most cases, nonspecific. So, the morphological characteristics of blood parameters are the most crucial component in the complex diagnosis of animal diseases.

The results of modern studies show that, in most cases, the causes of nonspecific balanoposthitis are the following opportunistic pathogenic microorganisms: Escherichia coli, Staphylococcus sp, Streptococcus sp, Pseudomonas aeruginosa (Comhaire et al., 1980; Andreeva et al., 2020). The presented microorganisms, in most cases, are isolated by associations. Assessing the etiological factor of the adnexal glands' nonspecific inflammation, the authors agree that the leading role belongs to Staphylococcus, Streptococcus, Pseudomonas aeruginosa (Debasish, 2003; Galin et al., 2020), and Escherichia coli, Staphylococcus aureus, Enterobacter spp, Streptococcus spp. (Amare, 2016; Paray et al., 2018; Kalaeva et al., 2019). Davidson notes that bacterial inoculations mostly have *E. coli*, *Staphylococcus* spp., *Streptococcus* spp. (Davidson, 2003; Robert, 2016; Henkel, 2020). In this regard, it is crucial to identify and treat andrological diseases at early stages. So, when treating balanoposthitis, it is possible to avoid the development of ascending infection and damage to the adnexal sex glands, while the quality of sperm does not deteriorate. With inflammation of the adnexal sex glands, the timely treatment allows complete restoration of animals' reproductive qualities due to the absence of damage to the testes and their appendages.

The degree of manifestation of the pathological process depends on the isolated microorganisms. So, in animals with signs of inflammation of the adnexal sex glands, *Staphylococcus* sp., which has plasma-coagulating properties, was isolated. According to the literature data, it is known that this microorganism has excellent pathogenic properties (Barry, 2014).

For preserving animals reproductive ability, it is essential to identify and treat sick animals since it's associated with the peculiarities of these diseases the aetiology and pathogenesis. So, in case of balanoposthitis, main pathological changes occur in the tissues of the mucous membrane of the penis and preputial cavity. Therefore, the quality of sperm doesn't change, but if untreated, inflammation of the accessory genital glands may develop by a mechanism of ascending infection. With vesiculitis and prostatitis, the pathological process occurs in the adnexal sex glands. The chemical composition of their secretions changes and becomes unsuitable for the life of sperm cells. Simultaneously, with effective complex therapy, it is possible to restore the animal's reproductive ability completely. Otherwise, the transition of the inflammatory process to the testes and their appendages is possible, which, in most cases, means an unfavourable prognosis for the reproductive ability. In some cases, animals can even die. For solving this problem, it is necessary to find the most effective antibacterial therapy.

In this connection, the purpose of the research was to study the microbial landscape of cattle's preputial cavity with nonspecific andrological diseases and to search for the most effective antibacterial drug. The following tasks were set to achieve the goal: (1) To determine the generic composition of opportunistic microflora in animals with nonspecific andrological pathology; (2) To investigate the clinical manifestation of diseases and correlate the data with the results of microbiological and biochemical blood tests and sperm quality assessment; (3) to determine the comparative therapeutic effectiveness of antibacterial drugs

in nonspecific andrological diseases.

Research hypothesis: The antibacterial drug Boflox is highly effective in the treatment of andrological diseases. The etiological factor is the microorganisms sensitive to this drug (*Staphylococcus* sp., *Streptococcus* sp., *Enterobacter* sp. and *Pseudomonas* sp., including plasma-coagulating *Staphylococcus* sp. are all resistant to many drugs and have great pathogenic properties). The concentration of Boflox in the tissues of the reproductive organs is high. Since it is mostly excreted unchanged, its therapeutic effect on the reproductive organs continues even when dissolved in the urine. The conducted research confirms the proposed hypothesis.

Scientific novelty

(1) The generic composition of opportunistic microorganisms that cause nonspecific andrological diseases, characteristic of the Republic of Bashkortostan, was determined; (2) The Boflox antibacterial drug, belonging to the fluoroquinolones group, showed high therapeutic effectiveness in treating nonspecific andrological diseases of cattle.

MATERIALS AND METHODS

Materials

Experimental studies were conducted from January to May 2020 in a livestock breeding complex of the Republic of Bashkortostan, Russia. Bulls with non-specific andrological diseases are the object of the study. This research work was performed at the Department of Infectious Diseases, Zoohygiene and Veterinary and Sanitary Examination of the Bashkir State Agrarian University, State Farm "Alekseevskiy" of the Ufimskii district of the Republic of Bashkortostan and State Budgetary Institution "Bashkir Scientific-Production Veterinary Laboratory". The study's object was 50 bulls. Their medical examination revealed twelve animals with clinical signs of the reproductive system's diseases, including eight animals with balanoposthitis symptoms. Four animals had both balanoposthitis and inflammation of the adnexal sex glands (vesiculitis and prostatitis).

50 bulls were subject to the medical examination. Twelve of them (24%) had andrological diseases, including four animals with mixed andrological pathology, i.e., balanoposthitis, vesiculitis and prostatitis (33%). Eight animals had only clinical signs of balanoposthitis (67%).

Design of research

Sick animals (n=12) were isolated in a separate cowshed and divided into three groups (n=4) by the pairs-analogues principle to study the therapeutic effectiveness of antibacterial drugs, which were used according to the scheme and the instructions. Groups of animals were kept in isolation from each other under identical conditions.

The antibacterial drug Bicillin-3 was used in the first group (n=4). It was administered intramuscularly, at a dose of 10 000ED / 1 kg of live weight, for ten days. A hydrogen peroxide solution was used three times a day for ten days to wash the preputial cavity.

Treatment of the second (n=4) group was carried out using antibacterial drug Boflox, belonging to the fluoroquinolones group. It was administered intramuscularly, at a dose of 1 ml/ 50 kg of live weight, once a day, for five days. The preputial cavity was washed with a solution of potassium permanganate, three times a day, for five days. Katozal was also used to stimulate natural resistance.

Animals of the third group (n=4) were intramuscularly injected with tetracycline hydrochloride at a dose of 6 mg / 1 kg of live weight. The powder was dissolved immediately before administration. The preputial cavity was washed with a solution of chlorhexidine, three times a day, for seven days.

Katozal was used in all three groups to stimulate nonspecific resistance. It was administered intramuscularly, at a dose of 20 ml per animal.

Research methods

Sperm quality (its volume, colour, smell and consistency) was evaluated macroscopically using a graduated test tube. The concentration, density and number of living and pathological forms of sperm cells were also determined microscopically. Using a methylene blue solution, the respiration rate of sperm cells was determined, taking into account the time spent on the solution discolouration. The nature of crystallization was evaluated using the method of Sviatovets (1981) by mixing a drop of sperm plasma with a drop of 0.9% sodium chloride solution. After that, the resulting drug was evaluated microscopically.

To isolate the opportunistic microflora, bacteriological studies were conducted using generally accepted methods. Preputial cavity swabs were taken according to the method of Mikhailov and Zudilin (1975) with the help of probe-tampons.

To study the blood morphological parameters, blood samples were taken from the caudal vein using vacuum tubes with the anticoagulant K3 EDTA. To determine the blood biochemical parameters, its samples were taken using vacuum tubes with a coagulation activator. Samples were taken before treatment and after complex therapy. Blood was examined in the conditions of the State Budgetary Institution Bashkir Research and Production Laboratory.

Blood morphological parameters were determined using an automatic hematological analyzer. To determine blood biochemical parameters the Biolab-100 biochemical analyzer was used. The Biolab-100 device is manufactured by Lumex, Russian Federation, and is listed in the state register of measuring instruments under the number 49809-12. The device is subject to annual verification.

Statistical analysis

The data obtained were statistically processed using the Microsoft Office Exel program. The reliability of the indicators was determined using the Student's criterion. The average values of haematological and biochemical parameters and the deviation "± m" were determined for each group, including clinically healthy animals. Then the differences between each group and clinically healthy animals were compared. After therapeutic measures, the results of each group before and after treatment were compared. To assess the statistical significance of the differences, the table of critical values of the Student's t-test was used, considering the difference to be statistically significant at $t < 0.5$. The qualitative assessment of bacteriological parameters was made according to the colonies in the nutrient medium. The generic affiliation of microorganisms was determined. Plasma-coagulating properties of representatives of Staphylococcus sp. were determined through reaction.

RESULTS AND DISCUSSION

Sexual reflexes of healthy animals were clearly manifested. The time of manifestation of all reflexes was within two minutes. The mucous membrane of the preputial cavity and penis is pale pink, painless, and excretion is free. The scrotum skin is smooth, straightened, the testes are large, oval in shape, facing the bulge cranial, mobile and easily displaced upwards. The volume of sperm obtained from apparently healthy animals was 12-15 ml. It was milky white, without smell, its consistency is creamy, homogeneous, without foreign inclusions. When examined using a microscope, the entire field of view is filled with sperm cells. There is almost no gap between them, which allows estimating the sperm as thick. 90% of sperms have a rectilinear translational movement and standard physiological shape.

During the study of sick bulls, their testes were pulled up to the abdominal wall. The scrotum skin was wrinkled and tightened. But the testes, the appendages of the testes and the scrotum had no pathological changes. Palpation causes pain. The volume of sperm obtained from them was 1-4 ml. The sperm is grey, with a greenish tinge, indicating the presence of pus impurities.

Microscopy of sperm revealed many immobile, dead sperm cells (more than 50%) and the presence of a significant number of white blood cells and inclusions. Leucospermia is one of the signs of vesiculitis and prostatitis. The methylene blue solution was not discoloured within the study time. The estimation of the crystallization nature revealed many inclusions in the form of polygonal crystals and lumps of oval and irregular shape, which means the presence of inflammatory exudate.

Three experimental groups were formed to determine the effectiveness of various antibacterial drugs.

In the first group (n=4), treatment lasted 10 days—one animal out of four recovered. The remaining three animals had clinical signs until the tenth day. The therapeutic efficacy of Bicillin-3 was 25%.

The pathological process proceeded differently. In one of the animals with only clinical signs of balanoposthitis, there was a decrease in the manifestation of mucosa hyperemia of the preputial cavity and penis on the eighth day. The nodules become invisible, the rounded thickening at the end of the preputial cavity disappears. Ten days later, this animal had a pale pink mucous membrane, the removal of the penis was free and painless, and the frequency of urination was within the physiological range. The sexual reflexes have recovered to normal and are estimated at 4 points. The sperm quality is good. In an animal that had a mixed andrological pathology (balanoposthitis + vesiculitis + prostatitis) the symptoms persist on the tenth day after the treatment: frequent and painful urination, urethra discharge and inclusions in the urine and sperm in the form of white flakes at the end of urination.

Sexual reflexes are significantly repressed and estimated at 1 point. Ejaculation is painful. Sperm volume is reduced to 1 ml. Methylene blue solution does not discolour within the study time. Microscopy reveals a large number of white blood cells, dead sperms prevail. Rectal examination revealed an increase in local temperature; the prostate gland is painful; the vesicular glands lobulation disappears, and the vesicular syndrome is pronounced.

At the microbiological examination of the first group (n=4), on the tenth day after the treatment, representatives of the genus Staphylococcus sp. were isolated in all four animals. Animals with only balanoposthitis (n=3) also had Enterobacteriaceae sp. And in one animal with a mixed andrological pathology, an association of Staphylococcus sp. and Streptococcus sp. was found. Twenty days after treatment, the animals were examined again. The examination revealed that in two animals, the association of Staphylococcus sp. and Enterobacteriaceae sp. is still present. In other animals, only representatives of the genus Staphylococcus (monoculture) were found.

Haematological and biochemical parameters in the first group of animals before treatment were as follows: the number of white blood cells increased ($p < 0.01$) 39.02 ± 7.37 $\times 10^3$ / µl (Table 1). Total protein increased ($p < 0.001$) to 78.04 ± 4.08 g / l, mineral metabolism indicators reduced: phosphorus concentration 1.81 ± 0.13 mmol/l, calcium 2.41 ± 0.05 mmol/L, potassium ($p < 0.05$) 4.34 ± 0.49 mmol/l. Ten days after the recovery, the indicators were within the physiological range.

On the tenth day after treatment, an animal with a mixed andrological pathology (balanoposthitis + vesiculitis + prostatitis) still had the symptoms of inflammation of the adnexal sex glands. Table 1 shows that there is an increase in the number of white blood cells ($p < 0.01$), granulocytes ($p < 0.001$), while the relative content of lymphocytes and a mixture of monocytes, basophils, eosinophils decreases ($p < 0.001$).

In the second group (n=4), the treatment of animals with balanoposthitis lasted 5 days and animals with mixed andrological pathology were treated for 6 days in.

After the treatment, all four animals got recovery. The therapeutic effectiveness of Boflox was 100%. At the bacteriological examination, ten days after the start of treatment, staphylococcus and Enterobacteriaceae associations were found in one of the animals having balanoposthitis and staphylococcus and streptococcus in another animal. In the other two animals with mixed andrological pathology (balanoposthitis, vesiculitis, and prostatitis) the analysis for plasma-coagulating staphylococcus was negative. Twenty days after the course of treatment, opportunistic microorganisms were not detected. The other two animals of this group had mixed andrological pathology (balanoposthitis, vesiculitis and prostatitis). On the fourth day of their treatment, there was a significant decrease in clinical signs manifestation.

Animals of the second group with mixed andrological pathology (n=2) recovered after the treatment measures. Table 1 shows that after the treatment measures in the second group of animals, there were changes in the following haematological parameters: the number of white blood cells decreased ($p < 0.01$), the relative content of lymphocytes significantly ($p < 0.001$) increased, the mixture of monocytes, eosinophils and basophils increased ($p < 0.001$) too; but the quantitative indicators of granulocytes decreased ($p < 0.001$). The absolute content of lymphocytes decreases ($p < 0.001$); the amount of monocytes, eosinophils, basophils; and granulocytes decreases significantly ($p < 0.001$). There was the increase in the content of red blood cells ($p < 0.01$), the concentration of hemoglobin ($p < 0.01$) and the haematocrit ($p < 0.01$) by 32.52 ± 0.77%. The blood platelet concentration is significantly reduced ($p < 0.01$). The total protein index significantly decreased ($p < 0.001$). The mineral metabolism indicators increase slightly.

Thus, after the treatment with the antibacterial drug Boflox, the haematological parameters came to the physiological norm, correlating with the clinical and andrological examination and bacteriological studies.

In the third group (n=4), the manifestation of clinical signs decreases in three of the four animals with balanoposthitis on the sixth day of the treatment. On the eighth day, clinical recovery occurs. One animal with a mixed andrological pathology still had clinical signs on the tenth day. Thus, the therapeutic efficacy of tetracycline hydrochloride was 75%.

During the bacteriological examination, plasma-coagulating staphylococcus is not detected. In all four animals of this group, ten days after the treatment, an association of representatives of staphylococci and Enterobacteriaceae's genus was found. Table 1 shows that before treatment, the number of white blood cells ($p < 0.01$), the relative content of lymphocytes ($p < 0.01$) significantly increased in the animals of the third group, and there was a decrease in the amount of mixture of monocytes, eosinophils and basophils ($p < 0.001$). However, granulocytes relative content ($p < 0.001$) exceeds the same indicator in clinically healthy animals.

After treatment, the following haematological indicators were revealed in animals with clinical signs of only balanoposthitis: the total number of white blood cells decreased by 63.8%. Red blood cell indicators increased. Thus, the number of red blood cells increased by 48.7%, amounting to 5.8 $\times 10^6$ / µl, by 62.2% (6.15 $\times 10^6$/µl) and by 46.3% (6 $\times 10^6$/µl). The blood platelet count decreased by 44.1%, amounting to 177 × 10^3 / µl, by 42.1% (237 $\times 10^3$/µl) and by 49.5%, amounting to 159 $\times 10^3$/µl in the remaining animal.

After treatment, on the tenth day of observation, the third group's remaining animal with mixed andrological pathology (balanoposthitis + vesiculitis + prostatitis) still has clinical signs. The following indicators were determined during the haematological study: the total number of white blood cells decreased by 59.8%, amounting to 20.1 $\times 10^3$ / µl. The relative content of lymphocytes increased by 5% (39.7%); the relative amount of a mixture of basophils, eosinophils, monocytes remains unchanged (3.1%); the relative content of granulocytes increased by 3.8% (57.2%). The red blood cells indicators have also changed. The number of red blood cells increased by 54.4% (4.2 $\times 10^6$ / µl). The number of blood platelets decreased by 17.5% (381 $\times 10^3$ / µl) (Table 1).

Table 1: Haematological and biochemical parameters of blood before and after treatment.

Haematological and biochemical parameters	Clinically healthy animals	Before treatment				After treatment	
		Group 1 (n = 4)	Group 2 (n = 4)			Group 1 (n = 4)	Group 2 (n = 4)
white blood cells, X×10³/µl	8.05±0.45	39.02±7.37**	43.35±4.74***	34.5±7.6**	26.88±6.85	10.68±0.53**	14.55±2.34*
lymphocytes, (rel.), %	70.25±2.23	42.65±4.56**	42.15±0.77***	48.07±3.96**	48.2±3.45	67.85±1.55***	56.33±6.58
a mixture of monocytes, eosinophils, and basophils (rel.), %	6.67±0.26	3.8±0.13***	3.58±0.25***	3.73±0.24***	4.47±0.66	6.17±0.16***	5.3±0.85
granulocytes (rel.), %	23.07±2.45	53.55±1.82***	54.28±0.96***	48.2±4.12***	47.33±4.11	25.98±1.63***	38.38±7.42
lymphocytes (abs.), X×10³/µl	5.57±0.4	16.38±2.43**	18.2±1.76***	16±2.66*	12.45±2.6	7.22±0.19***	7.58±0.58*
mixture of monocytes, eosinophils and basophils (abs.), X×10³/µl	0.53±0.03	1.42±0.16***	1.5±0.05***	1.23±0.21*	1.12±0.18	0.65±0.03***	0.72±0.07
granulocytes (abs.), X×10³/µl	1.85±0.19	21.23±4.8***	22.65±2.96***	17.25±4.9*	13.3±4.18	2.79±0.33***	5.97±2.17
red blood cells, X×10⁶/µl	8.7±0.25	3.41±0.24***	3.29±0.42***	3.63±0.36***	4.15±0.65	5.79±0.18**	5.54±0.52*
hemoglobin, g / l	141±2.42	84±4.14***	83±6.65***	87.5±4.96***	93±9.6	117.75±4.58**	113.75±7.32*
haematocrit, %	42±0.84	20.73±1.18***	19.8±2.45***	21,63±1,84***	24.2±3.07	32.52±0.77**	30.77±2.28*
blood platelets, X×10³/µl	403.25±33.46	403.25±33.46	476.75±58.17***	376±41.84*	331.75±50.21	154.5±12.44**	214.25±33.04*
total protein, g / l	58.97±1.47	78,04±4,08***	78.47±2.58***	73.28±3.77*	59.48±4.89	41.67±1.75***	48.22±20.1
phosphorus, mol/l	1.44±0.18	1.81±0.13	1.53±0.12	1.42±0.15	2.05±0.25	1.58±0.09	1.59±0.08
calcium, mol/l	3.56±0.83	2.41±0.05	2.44±0.06	2.33±0.07	4.03±0.82	3.62±0.57	3.01±0.27
potassium, mol/l	6.54±0.42	4.34±0.49*	4.89±0.62	6.25±2.37	6.41±0.44	5.86±0.28	4.24±0.08

Note: The difference is statistically significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In the third group of animals after the treatment measures, the number of white blood cells significantly decreased ($p < 0.05$). The relative content of lymphocytes and the content of a mixture of monocytes, eosinophils and basophils increased slightly. The content of granulocytes decreased amounting to 38.38±7.42%. The absolute content of lymphocytes is significantly lower ($p < 0.05$), while the mixture of monocytes, eosinophils, basophils, and granulocytes decreases slightly. The content of red blood cells and haemoglobin and hematocrit concentration increased ($p < 0.05$). The blood platelet concentration decreased ($p < 0.05$). Changes in the biochemical composition of the blood are insignificant.

The conducted research results prove the connection between bacterial culture tests, clinical signs, sperm quality examination results, haematological and biochemical blood parameters of animals.

The research revealed that the etiological factor of the development of nonspecific andrological diseases characteristic of our Republic, in most cases, is the association of opportunistic microorganisms *Staphylococcus* sp. and *Streptococcus* sp., and *Enterobacteriaceae* sp., which causes balanoposthitis. The association of plasma-coagulating *Staphylococcus* sp. and *Enterobacteriaceae* sp. causes mixed andrological pathology (balanoposthitis, vesiculitis and prostatitis), which is explained by the large pathogenic properties of plasma-coagulating staphylococcus. In more weakened animals, the association of staphylococcus and Enterobacteriaceae balanoposthitis can cause balanoposthitis. The peculiarity of the generic composition of the opportunistic microflora, characteristic of the Republic of Bashkortostan conditions, is the absence of Pseudomonas representatives in all animals, regardless of the pathological process's severity.

In andrological diseases, the main changes occur in the reproductive organs. Still, there are also the following changes in haematological and biochemical parameters: a significant ($p < 0.001$) increase in the number of white blood cells, total protein ($p < 0.001$) and blood platelets ($p < 0.001$). The content of haemoglobin in one red blood cell increases too, which is associated with the presence of a pronounced inflammatory process. An increase in the number of white blood cells occurs due to granulocytes ($p < 0.001$) and the relative content of lymphocytes decreases, which is a sign of bacterial infection. There are prominent features of anaemia, a decrease in the content of red blood cells, and, accordingly, haemoglobin, and hematocrit, which is associated with a general weakening of the body. There is a metabolic disorder, and the concentration of potassium, calcium and phosphorus decreases.

The influence of nonspecific andrological diseases on the quality of sperm and reproductive abilities directly

depends on the severity of the pathological process. In balanoposthitis, there is a repression of sexual reflexes and difficulty in removing the penis, which is explained by the mucous membrane's pronounced soreness. Painful mucous membrane causes inhibition of sexual reflexes, leading to a decrease in sperm release volume, mainly through an indirect negative effect on the formation of prostate secretions during sperm production. Simultaneously, sperm cells without pathological changes, mainly have a rectilinear translational movement, since adnexal sex glands and testes are not affected. Thus, the sperm quality remains good, but the reproductive abilities of the animal are suppressed. However, it is crucial to consider that without treatment, complications caused by an ascending infection of the adnexal sex glands may develop. In mixed andrological pathology (balanoposthitis, vesiculitis and prostatitis), sperm becomes unusable its volume significantly decreases. There are many inclusions of white blood cells and inflammatory exudate, and sperm cells are mostly dead. This is due to a change in the chemical composition of the secret of the adnexal sex glands, which becomes unsuitable for the life of sperm cells. Besides, the prostate gland increases, leading to a narrowing of the lumen of the urogenital canal, which disrupts urination and has an irritating effect on the bladder, causing constant attempts to urinate in the animal. There is significant pain radiating to the scrotum, preputial cavity and anus. There is an increase in general and local body temperature. With early and effective treatment, the sperm quality and reproductive abilities are restored, because the testes and their appendages are not affected by the pathological process.

CONCLUSIONS AND RECOMMENDATIONS

The following composition of opportunistic microflora was revealed in the washings from the preputial cavity of bulls with andrological diseases: *Staphylococcus* sp., *Enterobacteriaceae* sp. and *Streptococcus* sp. association in animals showing signs of balanoposthitis, and the association of plasma-coagulating staphylococcus with Enterobacteriaceae in animals with signs of inflammation of the adnexal sex glands.

The following changes are noted in the biochemical composition of the blood: the content of total protein increases significantly; the content of calcium, potassium and phosphorus in the blood of animals decreases.

The use of Bicillin-3 in the first group showed low efficacy for treating balanoposthitis and mixed andrological pathology. After the treatment, clinical recovery was noted in one animal, an improvement was noted in another animal, and in two animals pathological signs persisted. The therapeutic effectiveness was 25%.

In the second group treated with Boflox, clinical signs of andrological diseases were not observed in all animals. Sexual reflexes and sperm quality were restored. The haematological and biochemical parameters came to the physiological norm; the therapeutic effectiveness was 100%.

In the third group, where tetracycline was used, one animal retained clinical signs of balanoposthitis; three animals had no clinical signs of andrological diseases. The quality of sperm and sexual reflexes were restored, even though one animal still had Staphylococcus sp. Animals with clinical signs of balanoposthitis recovered after the use of this drug. But the animal with mixed pathology (balanoposthitis, vesiculitis and prostatitis) still had clinical signs after the treatment. The therapeutic efficacy was 75%.

Thus, Boflox has 100% therapeutic effectiveness for treating balanoposthitis and mixed andrological pathology when used according to the following scheme: 1 ml per 50 kg of live weight 1 time per day, for five days in balanoposthitis, vesiculitis and prostatitis.

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NOVELTY STATEMENT

The Boflox antibacterial drug, belonging to the fluoroquinolones group, showed high therapeutic effectiveness in treating nonspecific andrological diseases of cattle.

AUTHOR'S CONTRIBUTION

Alexander Ivanov - Methodology, Validation, Investigation, Writing – Original Draft Preparation; Vacheslav Ignat`ev - Conceptualization, Validation, Formal Analysis, Writing – Original Draft Preparation; Alfia Andreeva - Methodology, Validation, Resources, Writing – Review & Editing; Oksana Nikolaeva - Validation, Investigation, Resources, Writing – Review & Editing; Zuleykha Ilyasova - Software, Validation, Formal Analysis, Data Curation.

CONFLICT OF INTERESTS

The authors have declared no conflicts of interest.

REFERENCES

• Amare E (2016). Incidence of gross pathological conditions of the reproductive organs in bulls. Ind. J. Vet. Pathol., 4(40): 305–

311. https://doi.org/10.5958/0973-970X.2016.00072.9
- Andreeva A, Nikolaeva O, Altynbekov O, Galieva C, Ilina K (2020). Influence of interferon-based drugs on immunological indices in specific prevention. Vet. World, 13: 238–244. https://doi.org/10.14202/vetworld.2020.238-244
- Barry AB (2014). Applied andrology in horses. In: Animal andrology: theories and applications. University of Kentucky, Lexington, USA, pp. 254–275.
- Cavalieri J, Van Camp SD (1997). Bovine seminal vesiculitis. A review and update. Vet. Clin. North Am. Food Anim. Pract., 13(2): 233–241. https://doi.org/10.1016/S0749-0720(15)30337-6
- Chenoweth PJ, Osborne HG (1978). Breed differences in abnormalities of the reproductive organs of young beef bulls. Aust. Vet. J., 54(10): 463–468. https://doi.org/10.1111/j.1751-0813.1978.tb00285.x
- Cockcroft PD (2015). Bovine medicine. Wiley-Blackwell, Hoboken. https://doi.org/10.1002/9781118948538
- Comhaire F, Verschraegen G, Vermeulen L (1980). Diagnosis of accessory gland infection and its possible role in male infertility. Int. J. Androl., 3(1-6): 32–45. https://doi.org/10.1111/j.1365-2605.1980.tb00093.x
- Davidson JR (2003). Prostatic diseases of the dog. Waltham Focus, 2: 4–10.
- Debasish S (2003). Studies on ante and post mortem pathological conditions of male genital organs of cow bull and buffalo bull. MA thesis. West Bengal University of Animal and Fishery Science, Kolkata.
- Galin P, Galyaveeva A, Bataev H, Safonov V (2020). The role of micronutrients and vitamins in the prevention and remote treatment of heart failure. Rev. Latinoam. Hipertens, 15(1): 26–32.
- Henkel R (2020). Infection in infertility. In: Parekatti SJ, Esteves SC, Agarwal A, eds. Male infertility. Springer Nature, Cham., 409–424. https://doi.org/10.1007/978-3-030-32300-4_32
- Ivanov AI, Andreeva AV, Skovorodin EN, Shaimukhametov MA, Altynbekov OM, Sultangazin GM, Nikolaeva ON (2018). Anaerobic microflora impact on pathomorphogenesis of swine dysentery. J. Eng. Appl. Sci., 13(S11): 8796–8802.
- Kalaeva E, Kalaev V, Efimova K, Chernitskiy A, Safonov V (2019). Protein metabolic changes and nucleolus organizer regions activity in the lymphocytes of neonatal calves during the development of respiratory diseases. Vet. World, 12(10): 1657. https://doi.org/10.14202/vetworld.2019.1657-1667
- Matiukhina EV (2011). Etiological role of opportunistic microflora in the occurrence of acropostites, balanopostites and vesiculites of bulls producers. PhD dissertation. Altai State Agrarian University, Barnaul.
- Mikhailov NN, Zudilin VA (1975). Method of treatment of bulls in pseudomoniasis. Veterinariia. 12: 85–86. https://doi.org/10.1109/MSPEC.1975.6368720
- Nikolaeva O, Andreeva A, Altynbekov O, Mishukovskaya G, Ismagilova E (2020). Probiotic drugs impact on the innate immunity factors. J. Glob. Pharma Technol., 12(1): 38–45.
- Norman ST, Parry S, Ladds P, Brown A (2003). The identification and management of reproductive disease of bulls. J. Sain Vet., 21(1): 6–18.
- Paray AR, Bhakat M, Lone SA, Mohanty TK, Sinha R, Rahman JU, Khanday ZB, Danish Z (2018). Role of preputial washing in reducing microbial load and improving bovine semen quality. Asian Pac. J. Reprod., 7: 97–102. https://doi.org/10.4103/2305-0500.233570
- Perumal P, Chamuah JK, Srivastava N, Vupru K, Srivastava SK (2013). Infectious causes of infertility in Buffalo bulls (Bubalus bubalis). Int. J. Bio-Res. Stress Manage., 4(1): 84–90.
- Robert AF (2016). Male genital system. Jubb Kennedy Palmer's Pathol. Domest. Anim., 3: 465–510. https://doi.org/10.1016/B978-0-7020-5319-1.00016-5
- Saunders PJ, Ladds PW (1978). Congenital and developmental anomalies of the genitalia of slaughtered bulls. Aust. Vet. J., 54(1): 10–13. https://doi.org/10.1111/j.1751-0813.1978.tb00261.x
- Schollum LM (1997). The microbiology of bovine semen and the antimicrobial activity of bovine seminal plasma. PhD dissertation. Massey University, Auckland.
- Skreekumaran T (2000). Structural and functional changes in the testis and epididymis of cross bred bulls with impaired fertility. PhD dissertation. Faculty of Veterinarian and Animal Science Kerala Agricultural University, Mannuthy.
- Sviatovets GD (1981). Evaluation of bulls by sperm production. The genetic basis of selection of cattle. Nauka Publishing, Kyiv.
- Ventsova I, Safonov V (2021). The role of oxidative stress during pregnancy on obstetric pathology development in high-yielding dairy cows. Am. J. Anim. Vet. Sci., 16(1): 7–14. https://doi.org/10.3844/ajavsp.2021.7.14
- Vorobyov V, Vorobyov D, Polkovnichenko P, Safonov V (2019). Evaluation of hematological and metabolic parameters in small ruminants with trace elements deficiency under different biogeochemical conditions. Worlds Vet. J., 9(4): 311–316. https://doi.org/10.36380/scil.2019.wvj39