ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 16(3), 9-23
Received 27.04.2025 Revised 16.08.2025 Accepted 23.09.2025

UDC 636.2.09:618.14-002:637.146.3
DOI: 10.31548/animal.3.2025.09

Efficiency of use of lactic acid bacteria for the treatment of cow endometritis

Anatolii Ugnivenko
Doctor of Agricultural Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0001-6278-8399

Stepan Demchuk
PhD in Veterinary Sciences, Leading Researcher
Institute of Animals Breeding and Genetics named after M.V. Zubets of National Academy of Agrarian Sciences of Ukraine
08321, 1 Pohrebniaka Str., Chubynske, Ukraine
https://orcid.org/0000-0002-4708-0955

Dmytro Nosevych*
PhD in Agricultural Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-2495-2084

Andriy Getya
Doctor of Agricultural Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-4747-9261

Oleksandr Kolisnyk
Doctor of Agricultural Sciences, Leading Researcher
Livestock Farming Institute of National Academy of Agrarian Sciences of Ukraine
61026, 1-A Tvarynnykyv Str., Kharkiv, Ukraine
https://orcid.org/0000-0002-0602-3575

Suggested Citation:
Ugnivenko, A., Demchuk, S., Nosevych, D., Getya, A., & Kolisnyk, O. (2025). Efficiency of use of lactic acid bacteria for the treatment of cow endometritis. *Animal Science and Food Technology*, 16(3), 9-23. doi: 10.31548/animal.3.2025.9.

*Corresponding author

Abstract. There is a necessity to find alternative methods to treat endometritis in cows that would not have disadvantages of traditional anti-microbial drugs in particular the occurring of resistance forms of bacteria and limitation of milk usage. The aim of the study was possibility of treating endometritis with a suspension of the probiotic, which includes strain B-2691 of *Lactobacillus acidophilus*. Cows of Ukrainian Black-and-White dairy breed with confirmed endometritis were injected into the uterine cavity with a suspension of the drug (500 billion microbial cells of lactic acid bacteria in 100 cm^3) from one to 3 times with an interval of 6 days between application. The overall effectiveness of treatment of cows of all groups after 3 times of the drug administration was 90%. 100% recovery of cows with subclinical, mild and severe clinical endometritis, as well as 73% recovery of cows with morphological changes in the uterus was observed. Intrauterine injection of the drug (300 billion cells per 100 cm^3 of saline) led to stabilisation of the number of bacteria in the mucus. On the sixth day after administration, the number of bacteria in the uterine mucus of cows with endometritis and of some clinically healthy cows decreased, so in all animals it did not exceed 100 thousand colony-forming units per 1 cm^3. The presence of lactic acid bacteria was detected in all cows. Therapeutic concentrations of *Lactobacillus acidophilus* from 0.5 to 3 billion/cm^3 negatively affected the activity of bull sperm during incubation in a thermostat. At the maximum concentration of lactobacilli, sperm death occurred during the first hour of incubation, and at the minimum concentration – within 2 hours. In the control sample, live sperm were detected after 4 hours of incubation. The probiotic should be used as an alternative method for the treatment and prevention of cow endometritis, but due to the negative effect of therapeutic concentrations of *Lactobacillus acidophilus* on sperm survival, insemination should be performed no earlier than 6 days after administration of the drug, when a decrease in the number of bacteria in the uterine mucus is confirmed

Keywords: cattle; *Lactobacillus acidophilus*; uterine mucus; mobility of sperms; colony-forming units

Introduction

Endometritis is a common disease of dairy cows occurring after calving and causes a deterioration in reproductive capacity. Previous studies have shown that endometritis is related to housing conditions. Appearance of impending calving and duration of preparation for calving depended on the cows keeping, and in fetal membranes of 93% of cows kept tethered, lacked mucins and postpartum catarrhal endometritis were diagnosed (Ugnivenko *et al.*, 2020). In pasture-based housing, catarrhal endometritis was observed in only 4.8% of the population. According to I. Sheldon *et al*. (2019), up to 40% of cows have postpartum uterine diseases. In Holstein cows, cytologically diagnosed endometritis was associated with impaired reproductive function, which was manifested in particular in an increase in the number of open days and a decrease in the percentage of pregnancy after the first insemination. In cows that tested positive for endometritis, in addition to the above-mentioned problems, an increase in the number of inseminations to obtain pregnancy and an increased duration of anestrus after calving were also observed. Endometritis increases the atypical ovarian functioning, in particular the begin and duration of luteal activity. As stated by M. Dickson *et al.* (2020), inflammation in uterus caused changes of mobility and functions of spermatozoa, increase phagocytosis of spermatozoa and zygotes obtained have less possibility to be developed to the stage of blastocyst or even morula.

Negative impact of endometritis on cows' reproductive function causes the necessity of looking for the methods for early diagnosis and treatment of animals. Risks of endometritis occurrence increase under conditions of obstetric care for dystocia, due to increased bacterial contamination, although there is an opinion of I. Sheldon *et al.* (2019) that uterine health depends more on the ability of the endometrium to tolerate pathogens than on the ability to resist bacterial penetration. It was established that uterine microflora of healthy cows and cows with clinical endometritis and sub-clinical endometritis consists of community of 127 different types of Aerobic and Anaerobic bacteria included to 48 families. Quantity of bacteria in the uterine of cows with detected endometritis is essentially lower. Upon the clinical endometritis the level of *Trueperella pyogenes* is increasing. Similar results were obtained in a study by O. Pascottini *et al.* (2020), which noted no significant difference between the microbiome of healthy cows and cows with subclinical endometritis, so, the authors believe that subclinical endometritis is a consequence of dysregulation of inflammation rather than changes in the uterine microbiota. However, in cows with clinical endometritis, a decrease in bacterial diversity in the uterus and the dominance of *Trueperella pyogenes* were found. It should be noted that the presence of *Trueperella pyogenes* in clinical endometritis is associated with a decrease in milk production and fertility after the first insemination, as well as a lengthening of the period from calving to insemination in cows (Paiano *et al.*, 2021). These studies indicate the need to normalise the uterine microflora during clinical endometritis.

The vagina and uterus share a common bacterial community during the postpartum period. In the study of R. Miranda-CasoLuengo *et al.* (2019), changes associated with the development of endometritis were observed on the 7th day after calving, when the vaginal and uterine microbiomes were most similar. The authors of this study identified at least three different types of microbiomes associated with the further development of postpartum endometritis. All of these microbiome types had reduced bacterial diversity. Traditional antimicrobials are used to treat endometritis. Antibiotics are considered to be effective in the treatment of endometritis, but their active use causes the emergence of pathogenic microorganisms' resistance. It has been found by A. Manimaran *et al.* (2019) that *E. coli*, as one of the possible causative agents of endometritis, has a difference in sensitivity to gentamicin and oxytetracycline. Prolonged intrauterine antibiotic therapy for persistent endometritis can lead to fungal endometritis, which significantly complicates treatment and requires specific therapy (Saini *et al.*, 2019). The use of antibiotics can impose restrictions on the use of milk, which leads to additional losses.

To replace antibiotic therapy, the possibility of alternative treatments that involve the use of PGF2α in combination with intrauterine antibacterial drugs, immunomodulators and herbal remedies is being studied (Parmar, 2021). The use of herbal remedies is considered to be one of the priority and affordable methods that have a certain effectiveness. It was found that oral administration of *Aegle marmelos* and *Murraya koenigii* leaf powder reduces bacterial load and inflammation in endometritic cows. *Achyranthes aspera, Azadirachta indica, Ocimum tenuiflorum, Allium sativum, Withania somnifera* and many other plants are also considered effective for solving gynaecological problems in cows (Nikhade *et al.*, 2019). Essential oils of cinnamon, clove, oregano and thyme have been shown to be effective in inhibiting control strains of *Escherichia coli, Fusobacterium necrophorum, Trueperella pyogenes* and *Staphylococcus aureus*, which are considered typical bacteria that cause endometritis. For the prevention of endometritis, positive results were obtained using intrauterine ozone therapy (Escandón *et al.*, 2020). There is evidence found by E. Hussein

& K. Hussein (2022) of positive results in the treatment of bovine metritis and endometritis with biomolecules with silver nanoparticles.

Using all the variety of methods for treating and preventing endometritis, it is important not only to inhibit pathogens in the uterus but also to restore its eubiosis. There is evidence that lactic acid bacteria, which can inhibit pathogen growth, can be used to treat endometritis. Comparison of uterine mucus microbiota profiles made before and after treatment with lactic acid bacteria and antibiotics revealed that lactic acid bacteria can cure endometritis and restore normal physiological state, avoiding the disadvantages of antibiotic treatment, such as a decrease in the amount of beneficial microbiota (Yang *et al.*, 2021).

Among bacterial drugs, probiotics are of traditional interest, as their action is aimed at inhibiting pathogenic microorganisms. Their inclusion in feed and food products helps to inhibit the growth of *Escherichia coli, Listeria monocytogenes*, *Salmonella sp.* and *Bacillus cereus*. According to N. Hashem & A. Gonzalez-Bulnes (2022), the biological activity of probiotics can be a promising alternative to antibiotics for maintaining and restoring eubiosis and reproductive system function, but it should be toned that in-depth studies are needed to determine the mechanisms by which probiotics can affect reproductive processes and to define the specifics of probiotic use during sensitive reproductive periods. The purpose of this study was to investigate the effectiveness of *Lactobacillus acidophilus*-based probiotics for the treatment of cow endometritis and to analyse how they affect the uterine microbiota after administration and what effect this bacterial culture may have on bull germ cells.

Materials and Methods

The use of a probiotic drugs with lactic acid bacteria for the treatment and prevention of cow endometritis was studied. The research was conducted at the State Enterprise Research Farm "Oleksandrivske" and at the Reproduction Biotechnology laboratory of the Institute of Animals Breeding and Genetics named after M.V. Zubets of the National Academy of Agrarian Science of Ukraine, the results of which were analysed and summarised in 2024. As a source of lactic acid bacteria, probiotic "Bovilact" (developed by the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Agrarian Sciences of Ukraine), which contains strain B-2691 *Lactobacillus acidophilus*, which is stored in the Ukrainian Collection of Microorganisms was used. This medicine is used as a probiotic for the treatment and prevention of calf dysbiosis. The product is a light brown powder that contains 50 billion live cells of lactic acid bacteria per 1g. The drug is known to have an antagonistic activity against a wide range of pathogenic and opportunistic microorganisms due to the synthesis of L-lactic acid. In particular, it is highly effective for the treatment of colibacillosis, toxic dyspepsia, salmonellosis and is used to stabilise the microflora of the digestive tract.

The study was conducted on a population of Ukrainian Black-and-White dairy cows. The cows were kept tethered. The cows were fed three times a day. The cows were milked in stalls using milking equipment with a milk pipeline. Every day, the cows were provided with exercise in the feed and exercise area, straw bedding was brought into the stalls, free access to drinking water was provided, and veterinarians and staff had access to the animals the whole time. The conditions of cows and research design met the requirements of the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty" (2006).

In a dairy farm, cows were examined for endometritis in the period from 7 to 30 days after calving. Cows were examined by clinical examination and rectal examination. As a result of the examination, 20 cows with indicators of endometritis were identified. Confirmation of the diagnosis of endometritis was provided

by studying the reaction of uterine mucus to sulphur-containing amino acids according to the method of G. Kalinovskiy & G. Podoprigora (1987). According to this method, 4 cm^3 of a 0.5% solution of acetic acid lead was added to a test tube, to which a 20% solution of caustic sodium was dropwise added until a precipitate (lead oxide hydrate) was formed. After 15-20 seconds, the caustic sodium solution was added again until the precipitate disappeared. Then, 1.5-2.0 cm^3 of uterine mucus was added to the test tube, shaken gently and heated without boiling. In the case of endometritis, the mixture gets the colour of strongly brewed tea. Mucus samples were taken with a Korczak spoon. The analysis of uterine mucus was carried out under the conditions of the farm veterinary point. Cows with confirmed endometritis were divided into four groups according to the severity of the disease (Table 1).

Table 1. *Grouping of cows by severity of endometritis*

Group	Number of cows	Clinical indicators
1 (sub-clinical)	6	the cervix and uterus are slightly enlarged, located in the pelvic cavity, there is no fluctuation and no exudate is released
2 (with mild course of the disease)	4	the uterus is enlarged, partially in the pelvic cavity, slightly fluctuating, hypotonic, and there is a slight releasing of exudate
3 (with severe course of the disease)	2	the uterus is located entirely in the abdominal cavity, very enlarged, atonic, significant exudation
4 (with morphological changes in uterus)	8	the uterus is located entirely in the abdominal cavity, very enlarged, atonic, there is a thickening of the uterine walls and their fusion with the peritoneum of the pelvic and abdominal cavity, significant exudation, purulent discharge

Source: developed by the authors

The cows were injected with a suspension of the investigational medical product into the uterine cavity using a rectocervical insemination catheter connected to a Janet syringe. The dosage was 500 billion microbial cells of lactic acid bacteria suspended in 100 cm^3 of sodium chloride saline. The frequency of administration was from one to three times with an interval of 6 days. 6 days after each administration of the drug, cows were examined for indicators of endometritis by analysing uterine mucus for the content of sulphur-containing amino acids (Kalinovskiy & Podoprigora, 1987). The recovery of the cows was recorded and, in this case, treatment was stopped. If it was necessary to continue therapy, the drug was re-administered at the specified dose. At the end of the experiment, the proportion of cows in each group that recovered and the average frequency of drug administration to obtain a positive result were determined. In order to study the effect of the probiotic with lactic acid bacteria on the total bacterial contamination of the uterine from 7 to 30 days after calving, 14 cows were selected, of which 6 were healthy and 8 were sick with endometritis. The diagnosis of endometritis was confirmed after clinical examination by determining the reaction of uterine mucus to sulphur-containing amino acids (Kalinovskiy & Podoprigora, 1987). Uterine mucus was collected with a Korczak spoon in compliance with the rules of asepsis. A sterile vaginal mirror was used to prevent contact between the instrument and other parts of the animal's body. At the same time, mucus samples were taken for bacteriological analysis. On the same day, 300 billion cells of lactic acid bacteria in the form of a suspension in 100 cm^3 of sterile sodium chloride solution were injected into the uterus of cows using a catheter for rectocervical insemination attached to a Janet syringe. Bacteriological studies of uterine mucus were carried out by the method of sowing in the

Boryspil District State Laboratory of Veterinary Medicine (Boryspil, Kyiv region, Ukraine). They determined the total level of bacterial contamination and identified the presence of lactic acid bacteria. According to the degree of bacterial contamination, 5 classes were determined (Table 2). The samples were taken again 6 days after the probiotic administration. Bacterial contamination was determined in the samples and lactic acid bacteria were identified.

Table 2. *Classification of samples by the level of bacterial contamination*

Class	Bacterial contamination level, thousand colony forming units per 1 cm^3	Degree of bacterial contamination
1	0	sterile
2	1-100	insignificant
3	101-2,000	weak
4	2,001-50,000	average
5	over 50,000	strong

Note: gradation of bacterial contamination in bacteriological examination reports
Source: developed by the authors

The biological effect of the investigational medicinal product on bull sperm was studied in the laboratory of beef cattle reproduction at the M.V. Zubets Institute of Animal Breeding and Genetics. Frozen bull semen in pellets was thawed in 1 cm^3 of 2.9% sodium citrate solution and added to tubes with different concentrations of lactic acid bacteria. The concentration of lactic acid bacteria in the test samples was 3, 2, 1 and 0.5 billion per 1 cm^3. The medicine was dissolved in sodium citrate at the specified concentration and then 1 cm^3 of the solution was mixed with dissolved semen. Thawed sperm was used as a control. A 1 cm^3 solution of sodium citrate was added to the control.

The samples were kept in a thermostat for 5 hours. The temperature of the thermostat was 38 °C. Sperm survival was assessed by motility (the number of live cells with straightforward motion in points, on a 10-point scale). Sperm motility was assessed before incubation and every one hour using a Biolam microscope at 200× magnification.

The effectiveness of the use of the probiotic for the treatment of endometritis was assessed by the percentage of recovered cows and the average number of injections of the probiotic suspension to obtain a positive result. The bacterial contamination of uterine mucus from healthy and endometritic cows before and after administration of the probiotic was determined by sowing the samples on meat-peptone agar, followed by incubation at an average temperature of 37 °C for 24-48 hours and counting the number of colonies. When assessing the survival of sperm in the medium with lactic acid bacteria, the incubation time was estimated and the number of sperm with straightforward motion was counted.

Results and Discussion

After injecting a probiotic with a culture of *Lactobacillus acidophilus* bacteria into the uterine cavity of cows suffering from endometritis, it was expected that due to its antagonistic effect, the pathogenic microflora should be suppressed, which eventually contribute to the cows' recovery. Since the medicine is intended to normalise the intestinal microflora, it was not known whether it would have a similar positive effect when administered into the uterine cavity. There was also not clear how effective it will be on different severities of endometritis. A positive result of the treatment of cow endometritis with the probiotic was obtained 6 days after the first administration (Table 3).

Table 3. *Efficiency of treatment of cow endometritis with use of the probiotic*

Indicator	Group of cows according to the severity of endometritis			
	1	2	3	4
Number of cows selected into the group, heads	6	4	2	8
Recovered cows after the probiotic injection from 1 to 3 times, heads	6	4	2	6
-- // --, %	100	100	100	73
Average amount of administrations before recover	1.0	1.5	1.5	2.6

Source: developed by the authors

In the first group, with subclinical endometritis, 6 days after the first administration of the suspension, recovering of all cows was confirmed. Also, recovery of some cows was observed after the first administration in groups 2 and 3. After three injections of the lactic acid bacteria suspension into the uterine cavity, 18 out of 20 cows recovered. The overall positive result of the treatment was 90%. For the majority of cows, one or two doses of the probiotic lead to recovery, and the treatment effectiveness for cows with subclinical, mild and severe endometritis was 100%. The exception was the cows in group 4 (with morphological changes in the uterus). In this group, up to 3 injections of the drug were required to ensure a positive result, and the treatment effectiveness reached 73%. Intrauterine administration of the medicine is in fact a direct contamination with lactic acid bacteria and it affects the balance of microflora. The task was to investigate how the number of bacteria in the uterine mucus of cows was changed after the administration of the probiotic. In particular, the authors were interested in how the drug affects the uterine environment not only in cows with endometritis but also in healthy cows. It was also necessary to study whether, after a single administration of the probiotic, lactic acid bacteria will stay (colonised) in the uterus. In two groups of cows, one of which included clinically healthy cows and the other cows diagnosed with endometritis, the total bacterial contamination of uterine mucus was studied (Table 4).

Table 4. *Effect of intrauterine administration of the drug on the total bacterial contamination of uterine mucus*

Clinical state of the cow	Inventory number of the cow	Class by bacterial contamination		The presence of lactic acid bacteria	
		prior to the administration of the probiotic	after the administration of the probiotic	prior to the administration of the probiotic	after the administration of the probiotic
Healthy	2997	2	2	-	±
	2928	3	2	-	±
	1009	2	2	-	±
	1169	2	2	-	+
	0240	2	2	-	+
	3139	3	2	-	+
Endometritis	0285	4	2	-	±
	2739	4	2	-	±
	1004	4	2	-	+
	2866	4	2	-	±
	2503	4	2	-	±
	2759	4	2	-	±
	2832	4	2	-	±
	2916	4	2	-	+

Note: "-" lactic acid bacteria are not detected; "±" lactic acid bacteria are detected in minor number; "+" clearly determined presence of lactic acid bacteria

Source: developed by the authors

Prior to the administration of a suspension of lactic acid bacteria into the uterine cavity in clinically healthy cows, the bacterial contamination of uterine mucus was at the level of the 2nd and 3rd classes, which corresponds to up to 100 or up to 2,000 thousand colony-forming units per 1 cm^3. Lactic acid bacteria were detected in the selected samples. In cows with endometritis, the bacterial contamination of uterine mucus was much higher and corresponded to the 4th class of contamination (from 2,000 to 5,000 thousand colony-forming units per 1 cm^3). Lactic acid bacteria were not detected in the uterine mucus samples taken in this group.

The administration of a suspension made from the probiotic helped to stabilise the total bacterial contamination of uterine mucus in both groups of cows. 6 days after the administration of the probiotic with lactic acid bacteria into the uterus of cows, the total level of bacterial contamination of uterine mucus of all cows corresponded to the second class (up to 100 thousand colony-forming units per 1 cm^3). Lactic acid bacteria were found in the bacterial cultures of all animals, indicating that they colonised the uterus. Within the groups, in 50% of healthy cows and in 25% of cows with diagnosed endometritis, lactic acid bacteria were clearly detected in large numbers.

The microflora of the uterus of healthy cows, before the administration of the probiotic, did not include lactic acid bacteria. Since the antagonistic activity of the bacteria included in the probiotic caused by the synthesis of L-lactic acid, it was assumed that changes in the microbiome and the appearance of *Lactobacillus acidophilus* in it could lead to changes in the intrauterine environment. Changes in the uterus can affect the fertility of cows due to the influence of lactic acid bacteria on bull sperm. The survival of bull sperm during incubation in semen solvents with the addition of lactic acid bacteria and comparison with the control without lactic acid bacteria was studied in laboratory conditions (Table 5).

Table 5. *Motility (points) of bull sperm during incubation in medium with lactic acid bacteria*

Incubation hours	Control	Concentration of lactic acid bacteria, billion/cm^3			
		3	2	1	0.5
0	4.5	4.1	4.1	4.1	4.0
1	4.5	D	0.5	2.5	3.9
2	2.5		D	D	3.8
3	2.5				D
4	1.0				
5	D				

Note: D – death of all sperms
Source: developed by the authors

At the beginning of incubation, the control sample at 4.5 points in terms of the number of live sperm with straightforward movement was evaluated. During the first hour of incubation, no significant changes were detected. A decrease in the number of live sperm in the control group was observed after 2 hours of incubation. After 5 hours, no live sperm were detected.

Rapid changes in sperm motility were noticed in the experimental samples. The addition of lactic acid bacteria to the tubes for incubating thawed bull semen immediately led to a decrease in the number of live spermatozoa by 0.4-0.5 points compared to the control. A decrease in the number of sperm with straightforward motion was observed in all experimental samples, regardless of the concentration of lactic acid bacteria. During further incubation in a thermostat, the survival of germ cells was affected by the concentration of lactic acid bacteria. In the sample with a concentration of 3 billion/cm^3 of lactic acid bacteria, no live sperm

were detected after one hour of incubation. At concentrations of lactic acid bacteria of 2 and 1 billion/cm^3, the death of all sperm was observed at the second hour of incubation, while in the sample with a concentration of lactic acid bacteria of 0.5 billion/cm^3, sperm activity was completely lost after two hours of incubation. The rate of change in sperm motility in the experimental samples was inversely proportional to the concentration of lactic acid bacteria.

The results confirmed the possible risks of bull sperm death under the influence of lactic acid bacteria that colonised in the uterine environment. However, during the experiment, the number of lactic acid bacteria was in a therapeutic dose and significantly (in the smallest sample, more than 5,000 times) exceeded the number of microorganisms in the uterus of cows, which was detected 6 days after the administration of the drug. Since a decrease in the concentration of lactic acid bacteria is associated with a prolongation of sperm survival during incubation, the negative impact of the probiotic on fertilisation of treated and recovered cows is likely to be negligible.

Traditional treatment of bovine endometritis is complicated by the emergence of antibiotic resistance of common pathogens. In particular, the analysis of *E. coli,* isolated from endometritis of cows revealed its resistance to most antibiotics, so effective treatment of animals required the use of new complex drugs (Shafique *et al.*, 2021; Karatieieva *et al.*, 2024). The use of probiotics for therapeutic purposes allows to avoid further development of pathogen resistance to antimicrobial drugs. In the current study, the probiotic with *Lactobacillus acidophilus* was highly effective. Recovery was observed in 100% of cows in case of disease without morphological changes in the uterus and at the level of 73% among cows with morphological changes in the uterus. The potential of using lactic acid bacteria for the health of the genital organs of cows is of interest in dairy farming. In particular, it was found by S. Peter *et al.* (2018) that *Lactobacillus buchneri* had a positive effect on the health of the uterus, which led to an improvement in the reproductive function of cows. And the presence of *Lactobacillus acidophilus* in vaginal samples led to the suppression of a number of pathogens (*Staphylococcus* spp., *Pseudomonas* spp., *Kocurea* spp. and *Granulicatella* spp.), according to L. Khamees *et al.* (2022). The practice of using lactic acid bacteria can be an effective alternative to antimicrobials, but there are certain caveats. In experiments on the uterine microbiota conducted by M. Wang *et al.* (2018), *Lactobacillus* and *Acinetobacter* were found in dairy cows with subclinical endometritis. The probiotic "Bovilact" is advisable to use for the treatment of cows with endometritis, it can replace antimicrobial drugs, both in conventional and organic livestock, but the study of uterine microbiomes of cows with the inclusion of *Lactobacillus acidophilus* should be continued.

The use of lactic acid bacteria for the treatment of bovine endometritis directly affects other uterine bacteria. There are research results in the literature that indicate the inhibition of certain pathogenic cultures by lactic acid bacteria (Peter *et al.*, 2018). There are also reports of differences in the uterine microbiomes of healthy cows and cows with clinical and subclinical endometritis. As stated by R. Paiano *et al.* (2022), healthy cows have a greater diversity of bacterials, and endometritis diseases significantly reduces it. In modern studies, less attention is paid to changes in the total bacterial colonisation of uterine mucus and the effect of individual bacteria on this indicator. The authors of the current study have found that intrauterine administration of a probiotic with *Lactobacillus acidophilus* led to stabilisation of the total number of bacteria in uterine mucus. In healthy cows, as well as in the case of endometritis, the total bacterial colonisation after 6 days did not exceed 100 thousand colony-forming units per 1 cm^3 and in some cows causes its significant decrease.

It was not possible to evaluate the effect of probiotics with *Lactobacillus acidophilus* on the number of bacteria in the uterine mucus of cows. But there is more information about the effect of such probiotics on the intestinal microflora. In particular, the inclusion of *Lactobacillus acidophilus* in cat food led to decrease in the total number of anaerobes in the faeces and to significant decrease in certain groups of microorganisms. Also, a tendency to a slight decrease in pH and restoration of the typical microbiome after the end of probiotic administration was observed (Marshall-Jones *et al.*, 2006). Earlier studies on humans by A. Lidbeck *et al.* (1987) also showed changes in the total number of bacteria in the intestines and oropharynx and a decrease in the number of *E. coli* during probiotic administration. These studies revealed a decrease in the number of *Lactobacillus acidophilus* after the end of the probiotic administration to the initial level.

Thus, the changes caused by the probiotic after injection into the uterine cavity led to inhibition of pathogenic microflora and normalisation of the number of bacteria in mucus at the level typical for healthy cows. This had a therapeutic effect on endometritis and did not affect negatively healthy cows. The presence of lactic acid bacteria in the uterine mucus 6 days after administration of the probiotic was probably a temporary phenomenon and will not affect the reproductive use of cows.

Endometritis is often observed at the end of the voluntary waiting period. The clinical endometritis reduces the efficiency of cow fertilisation using artificial insemination, although no correlation between cow fertilisation and the presence of certain bacterial species has been found (Ballas *et al.*, 2021). The absence of a confirmed effect of bacteria on cow fertility does not mean that the use of a probiotic with *Lactobacillus acidophilus* will not have negative consequences. The risks that may arise are directly related to the effect of lactobacilli on the viability of germ cells. In the current study, no lactic acid bacteria were observed in the uterine microflora of clinically healthy cows and cows with endometritis. Thus, *Lactobacillus acidophilus* is not a typical representative of the uterine microbiome, so their presence, due to the influence on the pH of uterus and because of other factors, can negatively affect the fertilisation of cows due to sperm death. In particular, a decrease in pH in the uterus as a possible cause of lowering of fertility in cows during high nitrogen concentrations in the blood plasma was reported by M. Rhoads *et al.* (2004). According to S. Hugentobler *et al.* (2004), in cattle, the pH of the oviducts was 7.60 and the uterus – 6.96 and remained stable regardless of the day of the cycle. Optimal level of pH for maintaining the vital activity of sperm is 7...7.5, but changes below 6.5 and above 8 leads to a deterioration in most parameters (Contri *et al.*, 2013). Since the use of "Bovilact" causes lowering of pH, there are risks to the viability of sperm introduced into the uterus during insemination.

There are few data in the literature on the interaction of *Lactobacillus acidophilus* with bull sperm, although there is information on the interaction with other bacteria of the genus *Lactobacillus*. In particular, the addition of a probiotic containing *Lactobacillus crispatus, Lactobacillus gasseri* and *Lactobacillus brevis* to diluted bull semen is recommended as a means of inhibiting *Mycoplasma bovis* and reducing the risk of its transmission during artificial insemination (García-Galán *et al.*, 2020). This study found that after 15 hours of incubation of diluted semen with a bacterial concentration of 3.24...324 million CFU, the pH decreased from 6.93 to 6.73, but did not show how this affected sperm survival. Another study by M. Lenický *et al.* (2022) found that short-term coincubation of dilute sperm with *L. curvatus* and *L. hilgardii* had a beneficial effect on sperm motility, mitochondrial activity, and antioxidant properties. In the current study, coincubation of thawed sperm in a medium with a therapeutic concentration of *Lactobacillus acidophilus* during the first two hours led to sperm

death. It should be noted that the concentration of bacteria in the incubation medium in this case significantly exceeded the number of bacteria registered in other studies, like the one by Z. Mohammed *et al.* (2019), in which conclusions were made. The decrease in the concentration of bacteria in the solution is associated with a lengthening of the period during which the presence of live sperm was observed. Due to the fact that the therapeutic concentration of lactic acid bacteria in the uterus after administration of the investigational probiotic did not last long, and after 6 days the total level of bacterial contamination did not exceed 100 thousand CFU, per 1 cm^3, the authors of the current study believe that the risk of sperm death due to the presence of lactobacilli in the uterus is insignificant. Although the fertility of cows treated for endometritis with the probiotic requires additional analysis.

Conclusions

The use of probiotics with lactic acid bacteria may be an option for alternative treatment of cow endometritis. The introduction into the uterine cavity of a suspension of the probiotic based on the strain B-2691 *Lactobacillus acidophilus* with a therapeutic concentration of 500 billion microbial cells allowed to effectively treat clinical endometritis of mild and severe level. In the case of severe endometritis with morphological changes in the uterus, the effectiveness of three times application of probiotic remained high with the recovery rate of more than 70%.

The introduction of probiotics with lactic acid bacteria into the uterine cavity allowed to reduce the total number of bacteria in the mucus of endometritis and clinically healthy cows in 6 days and stabilise it at a level under 100 thousand CFU per 1 cm^3. In 6 days after the probiotic administration, lactic acid bacteria were detected in the uterus, which indicates their ability to remain viable in the intrauterine environment for a certain period of time.

The presence of *Lactobacillus acidophilus* in therapeutic concentration negative affected the activity of sperm and the duration of time during which they remain viable during joint incubation. So, insemination of cows during intrauterine administration of the probiotic may not be effective. It is advisable to inseminate cows after complete recovery, 6 days after the last administration of the probiotic, when the total number of bacteria in the uterine mucus decreases to the levels typical for clinically healthy cows. In this case the risks of reducing the viability of sperm during insemination is insignificant. Further research should be directed to identifying other cultures and strains of lactic acid bacteria effective while treating the endometritis of cows. Additionally, it is necessary to determine the optimal interval between the last introduction of a suspension with live bacterial culture and the admission of a cow for insemination.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

References

[1] Ballas, P., Reinländer, U., Schlegl, R., Ehling-Schulz, M., Drillich, M., & Wagener, K. (2021). Characterization of intrauterine cultivable aerobic microbiota at the time of insemination in dairy cows with and without mild endometritis. *Theriogenology*, 159, 28-34. doi: 10.1016/j.theriogenology.2020.10.018.

[2] Contri, A., Gloria, A., Robbe, D., Valorz, C., Wegher, L., & Carluccio, A. (2013). Kinematic study on the effect of pH on bull sperm function. *Animal Reproduction Science*, 136(4), 252-259. doi: 10.1016/j.anireprosci.2012.11.008.

[3] Dickson, M.J., *et al.* (2020). Experimentally induced endometritis impairs the developmental capacity of bovine oocytes. *Biology of Reproduction*, 103(3), 508-520. doi: 10.1093/biolre/ioaa069.

[4] Escandón, B.M., Espinoza, J.S., Perea, F.P., Quito, F., Ochoa, R., López, G.E., Galarza, D.A., & Garzón, J.P. (2020). Intrauterine therapy with ozone reduces subclinical endometritis and improves reproductive performance in postpartum dairy cows managed in pasture-based systems. *Tropical Animal Health and Production*, 52(5), 2523-2528. doi: 10.1007/s11250-020-02298-3.

[5] García-Galán, A., Gómez-Martín, Á., Bataller, E., Gomis, J., Sánchez, A., Gadea, J., Vieira, L.A., García-Roselló, E., & De la Fe, C. (2020). The addition of *Lactobacillus* spp., enrofloxacin or doxycycline negatively affects the viability of *Mycoplasma bovis* in diluted bovine semen. *Animals*, 10(5), article number 837. doi: 10.3390/ani10050837.

[6] Hashem, N.M., & Gonzalez-Bulnes, A. (2022). The use of probiotics for management and improvement of reproductive eubiosis and function. *Nutrients*, 14(4), article number 902. doi: 10.3390/nu14040902.

[7] Hugentobler, S., Morris, D.G., Kane, M.T., & Sreenan, J.M. (2004). In situ oviduct and uterine pH in cattle. *Theriogenology*, 61(7-8), 1419-1427. doi: 10.1016/j.theriogenology.2003.08.008.

[8] Hussein, E.K, & Hussein, K.A. (2022). Assess the efficiency of silver nanoparticles for treatment of endometritis in Iraqi breed cows. *Egyptian Journal of Veterinary Sciences*, 53(2), 263-271. doi: 10.21608/ejvs.2022.106646.1315.

[9] Kalinovskiy, G.N., & Podoprigora, G.I. (1987). *Express method for diagnosing cow endometritis*. Kyiv: Informatsionnoe pismo.

[10] Karatieieva, O., Posukhin, V., & Borusiewicz, A. (2024). Sanitary and hygienic assessment of the welfare of Ukrainian Black-and-White cattle breed. *Ukrainian Black Sea Region Agrarian Science*, 28(3), 32-40. doi: 10.56407/bs.agrarian/2.2024.32.

[11] Khamees, L.H., Algburi, A.R., & Mohammed, Z.I. (2022). The role of Lactic acid bacteria in combination with conventional antibiotics against bacterial species isolated from genital tract of cattle. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 21(2), 48-55.

[12] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[13] Lenický, M., Kačániová, M., Ďuračka, M., Zajacová, Ž., Muráňová, K., & Tvrdá, E. (2022). Bovine spermatozoa and Lactobacilli interactions: Deterioration or improvement of the sperm vitality? *Animal Reproduction Science*, 247, article number 107131. doi: 10.1016/j.anireprosci.2022.107131.

[14] Lidbeck, A., Gustafsson, J., & Nord, C.E. (1987). Impact of *Lactobacillus acidophilus* supplements on the human oropharyngeal and intestinal microflora. *Scandinavian Journal of Infectious Diseases*, 19(5), 531-537. doi: 10.3109/00365548709032419.

[15] Manimaran, A., Raghu, H.V., Kumaresan, A., Sreela, L., Yadav, A., Layek, S.S., Mooventhan, P., Chand, S., Sarkar, S.N., & Sivaram, M. (2019). Oxytetracycline is more suitable antibiotic for clinical endometritis cows. *Indian Journal of Animal Sciences*, 89(5), 501-505. doi: 10.56093/ijans.v89i5.90013.

[16] Marshall-Jones, Z.V., Baillon, M.L.A., Croft, J.M., & Butterwick, R.F. (2006). Effects of *Lactobacillus acidophilus* DSM13241 as a probiotic in healthy adult cats. *American Journal of Veterinary Research*, 67(6), 1005-1012. doi: 10.2460/ajvr.67.6.1005.

[17] Miranda-CasoLuengo, R., Lu, J., Williams, E.J., Miranda-CasoLuengo, A.A., Carrington, S.D., Evans, A.C.O., & Meijer, W.G. (2019). Delayed differentiation of vaginal and uterine microbiomes in dairy cows developing postpartum endometritis. *PLoS One*, 14(1), article number e0200974. doi: 10.1371/journal.pone.0200974.

[18] Mohammed, Z.A., Mann, G.E., & Robinson, R.S. (2019). Impact of endometritis on postpartum ovarian cyclicity in dairy cows. *The Veterinary Journal*, 248, 8-13. doi: 10.1016/j.tvjl.2019.03.008.

[19] Nikhade, C.T., Deshmukh, S.G., Kuralkar, S.V., Ratnaparkhi, A.R., Chepte, S.D., & Bankar, P.S. (2019). Comparative efficacy of different herbal extract on subclinical endometritis in postpartum cows. *Journal of Entomology and Zoology Studies*, 7(4), 1422-1426.

[20] Paiano, R.B., Bonilla, J., Moreno, A.M., & Baruselli, P.S. (2021). Clinical endometritis with *Trueperella pyogenes* reduces reproductive performance and milk production in dairy cows. *Reproduction in Domestic Animals*, 56(12), 1536-1542. doi: 10.1111/rda.14017.

[21] Paiano, R.B., Moreno, L.Z., Gomes, V.T.M., Parra, B.M., Barbosa, M.R., Sato, M.I.Z., Bonilla, J., Pugliesi, G., Baruselli, P.S., & Moreno, A.M. (2022). Assessment of the main pathogens associated with clinical and subclinical endometritis in cows by culture and MALDI-TOF mass spectrometry identification. *Journal of Dairy Science*, 105(4), 3367-3376. doi: 10.3168/jds.2021-20642.

[22] Parmar, K.H. (2021). Endometritis in bovine: A review. *Agricultural Reviews*, 42(3), 342-347. doi: 10.18805/ag.R-2038.

[23] Pascottini, O.B., Van Schyndel, S.J., Spricigo, J.F.W., Rousseau, J., Weese, J.S., & LeBlanc, S.J. (2020). Dynamics of uterine microbiota in postpartum dairy cows with clinical or subclinical endometritis. *Scientific Reports*, 10(1), article number 12353. doi: 10.1038/s41598-020-69317-z.

[24] Peter, S., Gärtner, M.A., Michel, G., Ibrahim, M., Klopfleisch, R., Lübke-Becker, A., Jung, M., Einspanier, R., & Gabler, C. (2018). Influence of intrauterine administration of *Lactobacillus buchneri* on reproductive performance and pro-inflammatory endometrial mRNA expression of cows with subclinical endometritis. *Scientific Reports*, 8(1), article number 5473. doi: 10.17169/refubium-23786.

[25] Rhoads, M.L., Gilbert, R.O., Lucy, M.C., & Butler, W.R. (2004). Effects of urea infusion on the uterine luminal environment of dairy cows. *Journal of Dairy Science*, 87(9), 2896-2901. doi: 10.3168/jds.S0022-0302(04)73420-7.

[26] Saini, P., Singh, M., & Kumar, P. (2019). Fungal endometritis in bovines. *Open Veterinary Journal*, 9(1), 94-98. doi: 10.4314/ovj.v9i1.16.

[27] Shafique, L., *et al.* (2021). Evidence-based tracking of MDR *E. coli* from bovine endometritis and its elimination by effective novel therapeutics. *Antibiotics*, 10(8), article number 997. doi: 10.3390/antibiotics10080997.

[28] Sheldon, I.M., Cronin, J.G., & Bromfield, J.J. (2019). Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. *Annual Review of Animal Biosciences*, 7, 361-384. doi: 10.1146/annurev-animal-020518-115227.

[29] Ugnivenko, A.N., Demchuk, S.Y, Nosevych, D.K., & Bondarenko, G.P. (2020). Duration of calving stages in beef cows under different housing systems. *Indian Journal of Animal Sciences*, 90(11), 1531-1534. doi: 10.56093/ijans.v90i11.111566.

[30] Wang, M.L., Liu, M.C., Xu, J., An, L.G., Wang, J.F., & Zhu, Y.H. (2018). Uterine microbiota of dairy cows with clinical and subclinical endometritis. *Frontiers in Microbiology*, 9, article number 2691. doi: 10.3389/fmicb.2018.02691.

[31] Yang, L., Huang, W., Yang, C., Ma, T., Hou, Q., Sun, Z., & Zhang, H. (2021). Using PacBio sequencing to investigate the effects of treatment with lactic acid bacteria or antibiotics on cow endometritis. *Electronic Journal of Biotechnology*, 51, 67-78. doi: 10.1016/j.ejbt.2021.02.004.

Ефективність використання молочнокислих бактерій для лікування ендометритів у корів

Анатолій Угнівенко

Доктор сільськогосподарських наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0001-6278-8399

Степан Демчук

Кандидат ветеринарних наук, старший науковий співробітник
Інститут розведення і генетики тварин ім. М.В. Зубця
Національної академії аграрних наук України
08321, вул. Погребняка, 1, с. Чубинське, Україна
https://orcid.org/0000-0002-4708-0955

Дмитро Носевич

Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-2495-2084

Андрій Гетя

Доктор сільськогосподарських наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-4747-9261

Олександр Колісник

Доктор сільськогосподарських наук, старший науковий співробітник
Інститут тваринництва Національної академії аграрних наук України
61026, вул. Тваринників, 1-А, м. Харків, Україна
https://orcid.org/0000-0002-0602-3575

Анотація. Актуальною проблемою є пошук альтернативних методів лікування ендометриту корів, які б не мали недоліків традиційних антимікробних препаратів, зокрема виникнення резистентних форм бактерій та обмеження споживання молока. Метою дослідження було визначити можливість лікування ендометриту суспензією пробіотика, до складу якого входить штам В-2691 *Lactobacillus acidophilus*. Коровам української чорно-рябої молочної породи з підтвердженим ендометритом вводили в порожнину матки суспензію препарату (500 млрд мікробних клітин молочнокислих бактерій у 100 см^3) від 1 до 3 разів з інтервалом 6 діб. Загальна ефективність лікування корів усіх груп після трикратного введення препарату становила 90 %. Спостерігалося 100 % одужання корів із субклінічним, легким та важким клінічним ендометритом, а також 73 % одужання корів із морфологічними змінами в матці. Внутрішньоутробне введення препарату (300 млрд клітин на 100 см^3 фізіологічного розчину) призводило до стабілізації кількості бактерій у матковому слизу. На шосту добу після введення кількість бактерій у матковому слизу хворих на ендометрит і деяких клінічно здорових корів зменшувалась, тому у всіх тварин вона не перевищувала 100 тис.

колонієутворюючих одиниць на 1 см^3. У всіх корів виявлено наявність молочнокислих бактерій. Терапевтичні концентрації *Lactobacillus acidophilus* від 0,5 до 3 млрд/см^3 негативно впливали на активність сперми бугая під час інкубації в термостаті. При максимальній концентрації лактобактерій загибель сперматозоїдів відбувалася впродовж першої години інкубації, а при мінімальній концентрації – впродовж двох годин. У контрольному зразку живі сперматозоїди були виявлені після 4 годин інкубації. Пробіотик доцільно використовувати як альтернативний метод лікування та профілактики ендометриту корів, але через негативний вплив терапевтичних концентрацій *Lactobacillus acidophilus* на виживання сперматозоїдів осіменіння слід проводити не раніше ніж через 6 днів після введення препарату, коли підтверджується зниження кількості бактерій в маткової слизу

Ключові слова: велика рогата худоба; *Lactobacillus acidophilus*; матковий слиз; активність сперматозоїдів; колонієутворюючі одиниці