

## АКУШЕРСТВО І БІОТЕХНОЛОГІЯ ВІДТВОРЕННЯ

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### Acellular probiotics and asparagic acid in the prevention of postpartum endometritis in cows

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Postpartum endometritis is one of the most widespread pathologies in animal husbandry. They often occur on the background of exposure on animals stress factors, microclimate disorders, complete feeding, which is accompanied by decrease of the resistance, leads to increasing morbidity, etc. The aim of the research is to develop a method of prevention of postpartum endometritis in cows using cell-free probiotics "BaciniL" and "Lactimet" with 4 % suspension of aspartic acid. Complex use of integrated cell-free probiotics "BaciniL" and "Lactimet" with 4% suspension of asparagic acid for the prevention of postpartum endometritis in doses of 7.5 and 10 ml each at 3-fold use once a day for 3 days in a row allowed to obtain 100% preventive efficacy. The mechanism of action of probiotic "BaciniL" is based on the high activity of components of its composition - immunostimulants (lipopolysaccharides), bacteriocins and enzymes, probiotic "Lactimet" due to biosynthetic lactic acid and a complex of fermentation products. Complex application of probiotics and aspartic acid increases their bacteriostatic activity, which allows more actively suppress the proliferation of pathogenic and opportunistic microflora, complicating the flowing of postpartum endometritis; promotes liquefaction of exudate accumulated in the uterine cavity due to enzymes included in probiotic "BaciniL"; leads to activation of local immunity of endometrial tissues due to immunostimulating activity of components of "BaciniL" and "Lactimet" probiotics; creation of low pH level in the uterine cavity due to aspartic acid and biosynthetic lactic acid.

**Key words:** cows, postpartum endometritis, prophylaxis, acellular probiotics, aspartic acid.

**Problem statement and analysis of resent research.** The industrial animal husbandry is accompanied by an increased incidence of mastitis, endometritis, limb diseases, the pathology of respiratory and fermentation organs, etc. They occur on the background of the impact on animals of stress factors, microclimate disorders, full-fledged feeding, which is accompanied by a decrease in resistance, leads to increase in morbidity, etc. [1-4].

As mentioned above, among cow diseases, postpartum endometritis is one of the widespread pathologies. Postpartum endometritis is an inflammation mainly of the lining of the uterus. They lead to significant economic damage to livestock production and are the main reasons for the decrease in animal productivity. Depending on the nature of inflammation, postpartum endometritis is divided

into catarrhal, purulent-catarrhal and fibrinous. It is believed that the main form of postpartum endometritis in cows is purulent-catarrhal endometritis. Clinical signs of the disease appear on 6-7 or 8-10 days after calving. In the first case, endometritis develops, as a rule, on the background of the detention of the seedlings or acute subinvolution of the uterus. From the genital organs of the animal, when lying, straining or massaging through the rectum, a large amount of purulent-mucous, purulent or serous-purulent exudate of liquid consistency, gray-brown or yellow-brown color is released. Postpartum endometritis is recorded in 20-57% of settled cows [5].

Measures for treatment and prevention of postpartum endometritis are labor-intensive, take a long time for veterinary specialists and do not

always provide a positive result. At the same time, often used antibacterial therapy leads to contamination of livestock production with sulfanilamides, nitrofurans, antibiotics, which can lead to the development of toxicoses, allergic reactions and dysbacterioses in humans [5].

At the moment, many different ways have been created and applied to prevent endometritis in cows. Thus, probiotic preparation "Giprolam" is used for prevention of postpartum endometritis in cows by intravaginal daily administration within 5-7 days before calving in dose 100 cm<sup>3</sup>, providing normalization of vaginal microbiocenosis, prevention of postpartum endometritis after calving in 71.0-80.0% of cases [7]. Also, for prevention of endometritis in cows, probiotic bacteria are introduced in the form of suppository containing dry biomass of *B. Licheniformis*, *B. Subtilis* and *Lactobacillus amylovorus* cell cultures in equal proportions, cocoa butter, paraffin, milk fat in the following amount per one suppository weighing 7 g: cocoa butter - 2.45 g, paraffin - 1.4 g, milk fat - 0.91 g with microbial strain powder - 1.75 g and disintegrants: citric acid - 0.14 g, food soda - 0.35 g, at the same time cytogumate is used as a biogenic stimulant, the preparation is introduced daily starting 20 days before calving at a rate of 50 ml per head in a mixture with food, uterotone - once a day in a dose of 10 ml for three consecutive days after calving, and suppositories - once a day on suppository 4 for 3 days after calving [8].

For the prevention of endometritis in cows is also used a preparation called Endosporin, which is a lyophilized biomass of *Bacillus subtilis* 39 and *Bacillus subtilis* 51 in cell ratios 1:1 at the following ratio of components per 1 g of dry preparation: *Bacillus subtilis* 39 strain 4-5x10<sup>11</sup> cells and *Bacillus subtilis* 51 strain 4-5x10<sup>11</sup> cells, sucrose stabilizer 40 mg, gelatin 10 mg, the rest is dry residue of growth medium and metabolites of bacteria. 0.5 g of dry preparation contains at least 400-500 billion cells. [9, 10]. Also used for prevention of endometritis in cows the preparation Vetomgin, which represents the gynecological suppositories weighing 2 g and is immobilized dried spore biomass of bacteria *Bacillus subtilis* BKPM-7048 and *Bacillus licheniformis* BKTIM-7038 on foam basis. The preparation is used by intrauterine administration of 1 suppository 1 or 2 times a day for three days starting from the first hours after calving of 2 or 3 suppositories simultaneously [11].

In order to detoxify the body of cows in the postpartum period, prevent the development of postpartum diseases and restore reproductive function, we use a solution for oral use "Gepavex 200" - 1 liter of the drug contains sorbitol - 200.0 g, magnesium (sulfate) - 10.0 g; carni-

tine - 25.0 g; DL-methionine - 10.0 g; choline (chloride) - 18.75 g. The drug improves appetite, normalizes digestion processes due to increased secretion of the stomach and pancreas, releases the energy of carbohydrates and contributes to a decrease in the level of fatty acids and their metabolism. Protects the liver and prevents its obesity, prevents the lack of magnesium. It promotes and accelerates the release the energy from fatty acids, thus reducing the schedule of basic amino acids and simultaneously producing, due to glucose metabolism, intermediates that are used for the synthesis of amino acids [6].

The material presented above, demonstrated that all the listed methods of preventing endometritis in cows based on probiotic preparations have a high preventive effect in postpartum endometritis in cows. However, their use is not always effective, which is associated with the use of dosage forms with a low titer of bacteria and with suboptimal administration regimens.

At the present stage, the use of cell-free probiotic drugs in combination with organic acids to preserve the correct microbiocenosis of reproductive organs is relevant. Unlike antibiotics, these preparations demonstrated higher antagonistic effects on pathogenic bacteria [12].

**The aim of the research** of the work is to develop a method for the prevention of postpartum endometritis in cows using acellular probiotics Bacinil and Lactimet with a 4% suspension of aspartic acid.

**Material and methods of research.** During the development of schemes of prevention of a postnatal endometritis at cows with use of acellular probiotics of Bacinil and Laktimet in the conditions of KPSUP "The Grodno poultry farm" of the Grodno district, the Grodno region the researches were conducted on clinically healthy calved animals.

Acellular probiotic Batsinil represents the spore-forming bacteria - *Bacillus subtilis* BIM V-497 D, an acellular probiotic of Laktimet - represents metabolites of bifidobacteria of *Bifidobacterium adolescentis* BIM B-456 B and lactic bacteria *Lactobacillus rhamnosus* BIM B-457 D [13, 14].

Aspartic acid - (amino succinic acid, aspartate, aminobutanediic acid) is an aliphatic amino acid, one of the 20 proteinogenic amino acids of the body. It occurs in all organisms in free form and in the composition of proteins. In addition, it plays the role of a neurotransmitter in the central nervous system. The acid itself and its salts are used as components of drugs, activating immunity, and due to a decrease in pH - increases the effectiveness of antibacterial agents [15, 16].

At the same time, the optimal dose and multiplicity of drug administration were worked out. For this, 12 groups of cows of 10 animals in the group were formed.

To carry out the experiments, we prepared a 4% suspension of aspartic acid, which was subsequently introduced to animals.

Various doses of cell-free probiotics were diluted in 100 ml of sterile isotonic sodium chloride solution. All experimental animals were introduced intrauterine starting from day 5 after calving.

Cows of the first control group were given the probiotic preparation "Bacnil" at a dose of 10 ml once a day for 3 consecutive days.

The cows of the second control group were given the probiotic preparation "Lactimet" at a dose of 10 ml once a day for 3 consecutive days.

The cows of the first test group were given the probiotic preparation "Bacnil" at a dose of 7.5 ml once a day for 3 consecutive days.

The cows of the second test group were given the probiotic "Bacnyl" at a dose of 10 ml and 10 ml of 4% aspartic acid once a day for 3 next days.

The cows of the third test group were given the probiotic preparation "Bacnyl" at a dose of 7.5 ml and 15 ml of 4% aspartic acid 1 once a day 3 days in a row

The cows of the fourth test group were given the probiotic preparation "Lactimet" at a dose of 7.5 ml once a day for 3 next days.

The cows of the fifth test group were given the probiotic preparation Lactimet in a dose of 10 ml once a day for 3 next days and 10 ml of 4% aspartic acid once a day for 3 next days.

The sixth test group cows were given the probiotic preparation Lactimet 7.5 ml and 15 ml of 4% aspartic acid once a day 3 next days.

The cows of the seventh test group were given the probiotic preparation "Bacnil" 10 ml with 3 times use once a day 3 days in a row and preparation "Lactimet" at a dose of 10 ml 1 once a day 3 days in a row.

Cows of the eighth trial group were given the probiotic preparation "Bacnil" 7.5 ml with 3 times use once a day 3 days in a row and preparation "Lactimet" 7.5 ml 1 once a day 3 days in a row.

The cows of the ninth test group were given the probiotic preparation "Bacnil" at a dose of 10 ml with 3 times use once a day 3 days in a row, the preparation "Lactimet" 10 ml once a day 3 days in a row and 10 ml 4% aspartic acid once a day 3 days in a row.

The cows of the tenth test group were given the probiotic preparation "Bacnil" 7.5 ml with 3 times use once a day 3 days in a row, preparation "Lactimet" 7.5 ml once a day 3 days in a row and 15 ml 4% aspartic acid once a day 3 days in a row.

**Research results and discussion** Table 1 shows the results of the effectiveness of various regimens for preventing postpartum endometritis in cows.

The results show that the integrated use of cell-free probiotics and aspartic acid increases the effectiveness of prevention of postpartum endometritis. This is due to the fact that there is a synergistic increase of the activity of used components due to a decrease in the pH in the uterine cavity of newfound cows due to asparagric acid, which significantly inhibits the growth of pathogenic and opportunistic microflora, and the use of probiotics also inhibits their growth due to bactericidal and bacteriostatic action.

As a result of the studies, it was found that in cows of control groups, the effectiveness of the cell-free probiotic "Bacnil," used to prevent postpartum endometritis at a dose of 10.0 ml with 3 times use once a day 3 days in a row, was 80%, and the efficiency of the probiotic "Lactimet" in the same dose - 70%.

Reducing the dosage of these preparations to 7.5 ml significantly reduced the efficiency to 50%, which is 20-30% lower than the control.

Complex use of cell-free probiotics "Bacnil" and "Lactimet," used for prevention of postpartum endometritis at a dose of 7.5 ml each with 3-fold use once a day 3 days in a row, made it possible to increase prophylactic efficiency to 80%, which is 30% higher than the use of probiotics separately.

Use of 4% aspartic acid suspension increases prophylactic efficiency of probiotics "Bacnil" and "Lactimet," used in a dose of 7.5 ml by 10-20%, but does not contribute to increase efficiency in a dose of 10.0 ml. At the same time, the efficiency from the use of probiotics in doses of 10.0 ml with aspartic acid did not differ in efficiency from their use without aspartic acid.

But the complex use of probiotics "Bacnil" and "Lactimet" in doses of both 7.5 and 10.0 ml each with 4% aspartic acid suspension allows preventing postpartum endometritis in cows with an efficiency of 100%, which is 20% higher than the use of probiotics without aspartic acid.

Analyzing the results can be concluded that aspartic acid in the complex use of probiotics "Bacnil" and "Lactimet" contributes increasing the efficiency of prevention of postpartum endometritis. It was noted that the complex use of probiotics without aspartic acid made it possible to obtain an efficiency in preventing postpartum endometritis by 10-20% compared to their individual use, but the addition of acid contributed to preventing the disease in 100% of treated cows.

We believe that aspartic acid in the complex use of probiotics due to the different mechanism

Table 1 – Results of research on the development of a method for the prevention of postpartum endometritis of cows

Groups	Application Scheme	Number of animals in the group	Sick, cows/percent	Preventive efficiency, %
1 control	"Bacinyll" 10 ml with 3 times use once a day 3 days in a row. Intrauterine	10	2/20	80
2 control	"Lactimet" 10 ml once a day 3 days in a row. Intrauterine	10	3/30	70
1 experimental	"Bacinyll" 7.5 ml with 3 times use once a day 3 days in a row. Intrauterine	10	5/50	50
2 experimental	"Bacinyll" 10 ml with 3 times use once a day 3 days in a row with 4% aspartic acid. Intrauterine	10	2/20	80
3 experimental	"Bacinyll" 7.5 ml with 3 times use once a day 3 days in a row with 4% aspartic acid.	10	4/40	60
4 experimental	"Lactimet" 7.5 ml 1 once a day 3 days in a row. Intrauterine	10	5/50	50
5 experimental	"Lactimet" 10 ml once a day 3 days in a row with 4% aspartic acid. Intrauterine	10	3/30	70
6 experimental	"Lactimet" 7.5 ml once a day 3 days in a row with 4% aspartic acid. Intrauterine	10	4/40	60
7 experimental	"Bacinyll" 10 ml with 3 times use once a day 3 day in a row + "Lactimet" 10 ml once a day 3 day in a row. Intrauterine	10	1/10	90
8 experimental	"Bacinyll" 7.5 ml with 3 times use 1 once a day 3 day in a row + "Lactimet" 7.5 ml once a day 3 day in a row. Intrauterine	10	2/20	80
9 experimental	"Bacinyll" 10 ml with 3 times use once a day 3 days in a row + "Lactimet" 10 ml once a day 3 days in a row with 4 % aspartic acid. Intrauterine	10	0/0	100
10 experimental	"Bacinyll" 7.5 ml with 3 times use once a day 3 days in a row + "Lactimet" 7.5 ml once a day 3 days in a row with 4% aspartic acid. Intrauterine	10	0/0	100

of their action increases the efficiency of prevention to 100%.

Considering the fact that the action of the probiotic "Baciniil" is based on the high activity of immunostimulants, bacteriocins and enzymes contained in the culture fluid, and the probiotic "Lactimet" due to biosynthetic lactic acid and a complex of protein fermentation products.

The integrated use of probiotics and aspartic acid contributes to an increase of their bacteriostatic activity, which allows more actively suppressing the reproduction of pathogenic and conditionally pathogenic microflora that complicate the course of postpartum endometritis; promotes the thinning of exudate accumulated in the uterine cavity due to enzymes included in the probiotic "Baciniil"; leads to the activation of local immunity of endometrial tissues due to the immunostim-

ulating components of probiotics "Baciniil" and "Lactimet"; reduces pH level in uterine cavity due to aspartic acid and biosynthetic lactic acid.

These components make it possible to obtain high preventive efficiency of the developed method.

**Conclusions.** To prevent postpartum endometritis in cows, they had to use one of the regimens starting from 2-5 days after calving:

Intrauterine co-introducing of the cell-free probiotic "Baciniil" 10 ml with 3 times use once a day 3 days in a row and the cell-free probiotic "Lactimet" at a dose of 10 ml once a day 3 days in a row;

Intrauterine co-introducing of cell-free probiotic "Baciniil" at doses of 7 and 10 ml and cell-free probiotic "Lactimet" 10 ml once a day 3 days in a row and 10 ml 4% aspartic acid once a day 3 days in a row.



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#### **Безклітинні пробіотики і аспарагінова кислота в профілактиці післяпологового ендометриту у корів** **Красочко П.А., Снітко Т.В.**

Післяпологові ендометрити є одними з широко поширених патологій у тваринництві. Часто вони виникають на тлі впливу на тварин стрес-факторів, порушень мікроклімату, повноцінного годування, що супроводжується зниженням резистентності, призводить до підвищення захворюваності тощо. Мета досліджень – розробити спосіб профілактики післяпологового ендометриту у корів з використанням безклітинних пробіотиків Бациніл і Лактимет з 4 % суспензією аспарагі-

нової кислоти. Комплексне використання безклітинних пробіотиків Бациніл і Лактимет з 4 % суспензією аспарагінової кислоти для профілактики післяпологового ендометриту в дозах 7,5 і 10 мл кожного за 3-кратного використання 1 раз на добу 3 дні поспіль дозволило отримати профілактичну ефективність 100 %. Механізм дії пробіотика Бациніл заснований на високій активності компонентів, що входять до його складу – імуностимуляторів (ліпополісахаридів), бактериоцинів і ферментів, пробіотика Лактимет – за рахунок біосинтетичної молочної кислоти і комплексу продуктів ферментації білків. Комплексне застосування пробіотиків та аспарагінової кислоти сприяє підвищенню їх бактериостатичної активності, що дозволяє більш активно стримувати розмноження патогенної та умовно-патогенної мікрофлори, що ускладнюють перебіг післяпологового ендометриту; сприяє розрідженню ексудату, що накопичився в порожнині матки за рахунок ферментів, що входять до складу пробіотика Бациніл, приводить до активізації місцевого імунітету тканин ендометрія за рахунок імуностимулюючих компонентів пробіотиків Бациніл і Лактимет; створення низького рівня рН в порожнині матки за рахунок аспарагінової кислоти і біосинтетичної молочної кислоти.

**Ключові слова:** корови, післяпологовий ендометрит, профілактика, безклітинні пробіотики, аспарагінова кислота.

#### **Бесклеточные пробиотики и аспарагиновая кислота в профилактике послеродового эндометрита у коров**

**Красочко П.А., Снитко Т.В.**

Послеродовые эндометриты являются одними из широко распространенных патологий в животноводстве. Часто они возникают на фоне воздействия на животных стресс-факторов, нарушений микроклимата, полноценного кормления, что сопровождается снижением резистентности, приводит к повышению заболеваемости и т.д. Цель исследований – разработать способ профилактики послеродового эндометрита у коров с использованием бесклеточных пробиотиков Бацинил и Лактимет с 4 % суспензией аспарагиновой кислоты. Комплексное использование бесклеточных пробиотиков Бацинил и Лактимет с 4 % суспензией аспарагиновой кислоты для профилактики послеродового эндометрита в дозах 7,5 и 10 мл каждого при 3-кратном использовании 1 раз в сутки 3 дня подряд позволило получить профилактическую эффективность 100 %. Механизм действия пробиотика Бацинил основан на высокой активности компонентов входящих в его состав – иммуностимуляторов (липополисахаридов), бактериоцинов и ферментов, пробиотика Лактимет – за счет биосинтетической молочной кислоты и комплекса продуктов ферментации белков. Комплексное применение пробиотиков и аспарагиновой кислоты способствует повышению их бактериостатической активности, позволяющей более активно подавлять размножение патогенной и ус-

ловно-патогенной микрофлоры, осложняющих течение послеродового эндометрита; способствует разжижению экссудата, накопившегося в полости матки за счет ферментов, входящих в состав пробиотика Бацинил; приводит к активизации местного иммунитета тканей эндометрия за счет иммуностимулирующих компонен-

тов пробиотиков Бацинил и Лактимет; создание низкого уровня рН в полости матки за счет аспарагиновой кислоты и биосинтетической молочной кислоты.

**Ключевые слова:** коровы, послеродовой эндометрит, профилактика, бесклеточные пробиотики, аспарагиновая кислота.



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