USE OF PROBIOTICS GIPROLAM AND SIMBITER-2 TO CORRECT THE VAGINA BIOCENOSIS IN DOWN-CALVING COWS

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A b s t r a c t

Postpartum endometritis prophylaxis in high producing dairy cows in the intensive animal husbandry is an urgent one because of great economic losses resulted from the productive function disorder, decreased performance and premature culling. Opportunistic pathogenic microflora and disbacteriosis may cause postpartum endometritis along with specific infections. Potentially dangerous pathogenic bacteria from cows with gynecological pathology contaminate the calves at birth, causing gastrointestinal diseases. Various pharmaceuticals and biologicals could be used to prevent postpartum endometritis, including those providing antioxidative effects, immunomodulatory activity, normalization of hormonal and metabolic status in cows’ genitals and uterine involution. Antibiotics are in most common use, though their frequent application is accompanied by microecological disorders. Probiotics are considered as a perspective alternative to antibiotics for correcting genital microflora in calving cows. In the paper we report a study of the impact of probiotics Giprolam and Simbiter-2 on microflora of down-calving cows’ birth canal in view to prevent postpartum endometritis and intestinal disorders in calves. Thirty-six red-and-white cows with milk yield of 5100-5400 kg for previous lactation were chosen. The cows of the group 1 (n = 12) and group 2 (n = 12) have received Giprolam and Simbiter-2, respectively, for 5-7 days prior to calving every 24 hours, 100 cm³ intravaginally. The cows of the group 3 (n = 12) served as a control (no probiotics). Clinical observations were carried out in mother cows for 14 days after calving, and in the calves during colostrum period. Indigenous and opportunistic microflora was studied in the birth canal before and after calving, in colostrum and in large intestine in the calves. Bacteriologic examination of cervical mucus, reproductive tract discharge, colostrum, excrement, as well as cultural, morphological and biochemical study were performed traditionally. The efficacy of probiotic treatment has been stated. The experiments showed that Giprolam and Simbiter-2 in 71.4 % and 85.8 % cases, respectively, could effectively provide a physiological level of postpartum indigenous microflora, prevent colonization of the reproductive tract by opportunistic and pathogenic microflora and restrict postpartum endometritis. The mother cow treatment with Giprolam and Simbiter-2 could also prevent gastrointestinal diseases in 50.0 % and 41.7 % of the calves, respectively. The high potency of these probiotics is due to lactic acid bacteria capable of genital tract colonization, providing optimal indigenous microflora level and the resistance of the genital tract to harmful microflora.

Keywords: cows, microflora of maternal passages, microflora of colostrum, microflora of intestine in calves, probiotics, postpartum endometritis, prophylaxis

High incidence of acute postpartum endometritis in cows results in significant economic loss due to reproduction malfunction, milk production losses, and anticipated culling of animals [1-5]. Along with agents of infectious diseases [6-8], opportunistic pathogenic microbial flora and disbacteriosis manifested by persistent quantitative and qualitative changes in bacterial community of normal microbial flora plays an important role in the etiology and development of postpartum endometritis [9-11]. At gynecologic pathology in cows, the possible pathogenic bacteria may infect calves at birth causing gastrointestinal diseases [12].
Selenium medicines with antioxidant and immunomodulatory effect [13, 14], medicines from placenta normalizing postpartum hormonal-metabolic and involution processes in genitals [15, 16], preparations intensifying the uterine activity [17-19], and antimicrobial medicines [20-22] are recommended for use to prevent development of postpartum endometritis in cows. One of the main postpartum endometritis preventative measures is use of means eliminating inflammations [23, 24]. Use of antibacterial medicines during 3 days postpartum with preventative purpose does not have significant effect on vaginal microbiocenosis. At the same time, long-term treatment course decreases quantitative and qualitative characteristics of normoflora, increases pH of vaginal secret that prevents recovery of vaginal microbiocenosis postpartum and creates conditions for propagation of opportunistic pathogenic microbial flora [25, 26]. Number of lactobacillus and bifidobacterium is significantly decreased, and opportunistic pathogenic microbial flora increases in the genital tract postpartum causing postpartum purulent-septic diseases due to washing out of microorganisms from vagina by amniotic fluid and blood, traumatization of birth canal, and contamination of vagina by intestinal microflora [27, 28]. Recovery of protective microbial flora at disbiotic disorders without the use of biotherapeutic medicines is challenged as confirmed by disease recurrences [29-33].

Today, use of probiotics as the most essential competitors of pathogenic and opportunistic pathogenic microflora serves as an alternative to antimicrobial medicines [34-37]. However, their use in the first day postpartum does not always prevent occurrence and development of catarrhal endometritis that is due to disbiotic disorders in vaginal microbiotype before birth. In this regard, it is perspective to use probiotic medicines for correction of microbionenosis of birth canals in cows prior to calving.

In present study, we have for the first time shown the need for intravaginal use of probiotics to down-calving cows for prevention of postpartum endometritis that also promote optimization of intestinal microbiocenosis in newborn calves and their addiction to gastrointestinal diseases.

Purpose of this study is to learn corrective effect of Giprolam and Simbiter-2 probiotics on vaginal microbial flora in down-calving cows.

Techniques. Study was carried out in commercial dairy farm Vysokoye (EcoNivaAgro LLC, Liskinsky District, Voronezh Region) in 36 animals of red and pied breed with milk yield in the past lactation of 5100-5400 kg. During interlactation period cows were kept loose housing at deep litter. Groups were formed accounting for the expected calving term. Animals were moved to pre-calving section and, afterwards, to calving section during 10-15 days. Cows were divided into three groups. Within 5-7 days before calving, animals of trial group I \((n = 12)\) were treated daily (with 24 hour interval) intravaginally by probiotic medicine Giprolam (Biotechagro LLC, Russia) by Janet’s syringe and gynecologic pipette \((100 \text{ cm}^3, 5-7 \text{ injections})\). Multiprobiotic Simbiter-2 (Research Industrial Enterprise O.D. Prolisok, Ukraine) was injected intravaginally in cows of trial group II \((n = 12)\) according to similar scheme [38]. Medicines were not provided to group III (control, \(n = 12\)).

Clinical surveillance was performed over cows within 14 days following calving and over calves in colostral period. State of birth canal microbiocenosis before and after calving, quantitative and qualitative bacterial composition of colostrum and large intestines in calves was assessed by quantity and frequency of occurrence of indigenous and opportunistic pathogenic microbial flora. Bacteriologic tests of cervical mucus, discharges from genital tracts, colostrum, excrements, studying of cultural and morphologic properties of identified microorganisms were carried out by commonly accepted methods [39].
Preventative effectiveness of probiotics was determined by formula:

$$E = 100 \times \frac{(B-A)}{B},$$

where E stands for effectiveness, %; A and B are disease frequency amongst animals treated and not treated with medicine, % [40].

Obtained results are presented as mean values ($M$) and standard errors of the mean ($\pm m$).

**Results.** Giprolam medicine (registration № PVR 1-35.13/02987) is a suspension which contains viable strains of lactobacillus *Lactobacillus fermentum* 44/1 (Russian National Collection of Industrial Microorganisms B-2940) and *Lactococcus lactis* subsp. *lactis* 574 (Russian National Collection of Industrial Microorganisms B-3145), nor less than $1 \times 10^8$ CFU/cm$^3$, and additional substances (water, milk serum, glucose, and yeast extract). Lactobacillus strains are able to succeed in birth canals of cows and have antagonistic effect on opportunistic pathogenic microbial flora penetrating to womb. Multiprobiotic Simbiter-2 — is a multicomponent medicine for normalization of vaginal microflora in female organism. Medicine is based on the key protective microorganisms of urogenital tract, i.e. bacteria *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. gasseri*, *L. brevis*, *Bifidobacterium bifidum*, *B. longum*, *B. breve*, *B. infantis*, *B. adolescentis*, *Propionibacterium freudenreichii* ssp. *shermanii*, *P. acidipropionici*. They actively ferment glycogen to organic acids, synthesize hydrogen peroxide, bacteriocine and lysozyme having high adhesive ability regarding epitheliocytes, produce vitamins and polysaccharides [38].

### 1. Microbial flora (lg CFU/cm$^3$) of birth canals in red and pied cows at treatment by Giprolam and Simbiter-2 probiotics ($M \pm m$)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em> spp.</td>
<td>7.5 ± 0.59*</td>
<td>7.6 ± 0.83*</td>
<td>6.5 ± 0.71*</td>
</tr>
<tr>
<td></td>
<td>7.6 ± 0.61*</td>
<td>7.5 ± 0.10*</td>
<td>5.6 ± 0.94*</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp.</td>
<td>7.6 ± 0.72*</td>
<td>7.7 ± 0.69*</td>
<td>5.6 ± 0.46*</td>
</tr>
<tr>
<td></td>
<td>7.7 ± 0.86*</td>
<td>7.5 ± 0.73*</td>
<td>5.4 ± 0.68*</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>2.2 ± 0.02*</td>
<td>2.4 ± 0.17*</td>
<td>3.2 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>2.2 ± 0.01*</td>
<td>2.6 ± 0.23*</td>
<td>3.5 ± 0.41*</td>
</tr>
<tr>
<td><em>Staphylococcus saprophyticus</em></td>
<td>5.5 ± 0.49*</td>
<td>4.6 ± 0.16*</td>
<td>5.8 ± 0.12*</td>
</tr>
<tr>
<td></td>
<td>5.7 ± 0.17*</td>
<td>4.5 ± 0.21*</td>
<td>5.6 ± 0.81*</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>3.6 ± 0.02*</td>
<td>4.6 ± 0.61*</td>
<td>5.3 ± 0.25*</td>
</tr>
<tr>
<td></td>
<td>4.5 ± 0.74*</td>
<td>5.5 ± 0.32*</td>
<td>4.3 ± 0.17*</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>not isolated</td>
<td>not isolated</td>
<td>not isolated</td>
</tr>
<tr>
<td></td>
<td>4.3 ± 0.03 (25.0)</td>
<td>4.3 ± 0.03 (25.0)</td>
<td>4.3 ± 0.03 (25.0)</td>
</tr>
<tr>
<td><em>Streptococcus</em> group C</td>
<td>4.5 ± 0.16 (50.0)</td>
<td>4.5 ± 0.69 (25.0)</td>
<td>5.7 ± 0.45 (75.0)</td>
</tr>
<tr>
<td></td>
<td>4.4 ± 0.58 (50.0)</td>
<td>4.5 ± 0.65 (33.3)</td>
<td>6.6 ± 0.81 (75.0)</td>
</tr>
<tr>
<td><em>Enterococcus</em> faecalis</td>
<td>not isolated</td>
<td>3.9 ± 0.01 (16.7)</td>
<td>3.6 ± 0.22 (25.0)</td>
</tr>
<tr>
<td></td>
<td>2.6 ± 0.01 (25.0)</td>
<td>3.4 ± 0.01 (25.0)</td>
<td>4.3 ± 0.85 (75.0)</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp. hemolytic</td>
<td>not isolated</td>
<td>2.5 ± 0.86 (8.3)</td>
<td>5.6 ± 0.38 (25.0)</td>
</tr>
<tr>
<td></td>
<td>3.9 ± 0.01 (25.0)</td>
<td>3.6 ± 0.73 (8.3)</td>
<td>5.3 ± 0.52 (75.0)</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>not isolated</td>
<td>2.7 ± 0.85 (8.3)</td>
<td>not isolated</td>
</tr>
<tr>
<td></td>
<td>2.5 ± 0.85 (8.3)</td>
<td>4.9 ± 0.02 (25.0)</td>
<td>5.7 ± 0.65 (50.0)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>not isolated</td>
<td>not isolated</td>
<td>5.7 ± 0.65 (50.0)</td>
</tr>
</tbody>
</table>

**Note:** See description of groups in section "Methodology". Figures in parenthesis show frequency of isolation, %; microorganisms marked by star were identified in 100% of animals. Values prior calving and after calving are above and under bar, respectively.

Microflora of birth canals before administration of medicines (Table 1) and in control group within 5–7 days before calving did not significantly differ. *Lactobacillus*, bifidobacteria, *Corynebacterium* spp., *Staphylococcus saprophyticus* and *S. epidermidis* were found in all specimens, while *Streptococcus* group C and *Escherichia coli* were rare. Besides, hemolytic streptococci *Enterococcus faecalis* were found prior to administration of Simbiter-2 in group II and in control animals, and *Enterobacter* spp. — prior to treatment with the probiotic (see Table 1).

During the period before calving, due to Giprolam, positive dynamics in lactobacillus (growth by 1.5%), bifidobacteria (by 1.4%), *Staphylococcus saprophyt-
cus (by 4.2 %), and epidermal staphylococcus (by 19.9 %) counts occurs in birth canal, with notable trend towards reduction of the number of streptococcus group C (by 1.6 %). In 25.0 % cases, Enterococcus faecalis and hemolytic streptococci but no Escherichia were isolated (see Table 1). Prior to administration of Simbiter-2, number of lactobacillus and bifidobacteria was optimal, number of epidermal staphylococcus grew by 17.4 %, hemolytic streptococci grew by 29.7 %, and number of Enterococcus faecalis decreased by 11.7 %. Escherichia and Enterobacter spp. were not found (see Table 1).

After calving, abundance of lactobacillus (by 13.9 %), bifidobacterium (by 15.9 %) was reduced in the control group with increase of the number of opportunistic pathogenic microflora: streptococci group C — by 14.2 %, Enterococcus faecalis — by 15.7 %, Escherichia — by 20.7 %. Frequency of isolation of Enterococcus faecalis, hemolytic streptococci and Escherichia increased by 50.0; 50.0 and 25.0 %, receptively, besides, at frequency of 25.0 %, staphylococci aureus and Enterobacter spp. were isolated (see Table 1). In group I, number of lactobacilli and bifidobacteria was 26.2 and 29.4 % higher than in control, while abundance and frequency of streptococci group C was 33.3 and 25.0 % lower than in control, Enterococcus faecalis — by 39.6 and 50.0 %, hemolytic streptococci — by 27.5 and 50.0 % lower. Staphylococci aureus, Escherichia and Enterobacter spp. were not found. Number of lactobacilli in group II as compared to control was 25.6 % higher, of bifidobacteria — 27.5 % higher, count of streptococci group C was 32.1 % lower, of Enterococcus faecalis — 32.5 % lower, hemolytic streptococci — 19.8 % lower. Staphylococci aureus, Escherichia and Enterobacter spp. were not found. In animals treated by Simbiter-2 frequency of hemolytic streptococci and streptococci group C was 16.7 % lower than in animals treated with Giprolam.

2. Preventative effectiveness of use of Giprolam and Simbiter-2 probiotics in red and pied cows

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Delivery, min (M±m)</td>
<td>60±10</td>
<td>30±10</td>
<td>120±30</td>
</tr>
<tr>
<td>Postpartum endometritis, number (%)</td>
<td>2 (16.7)</td>
<td>1 (8.3)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>Preventative effectiveness, %</td>
<td>71.4</td>
<td>85.8</td>
<td></td>
</tr>
</tbody>
</table>

Note: See description of groups in section “Methodology”.

Calving in cows treated with probiotics was without obstetric aid, while frequency of purulent-catarhal endometritis did not exceed 16.7 % (Table 2). In control in 50.0 % cases, cows were rendered with obstetric aid, fetal removal was 2 and 4 times longer than in groups I and II, one cow and its calf died, and acute purulent-catarhal endometritis was in over half of animals (see Table 2).

Intravaginal injection of Giprolam and Simbiter-2 in down-calving cows promotes postpartum indigenous microflora at physiological level, prevents colonization of birth canals by opportunistic pathogenic microflora and occurrence of postpartum endometritis in 71.4 and 85.8 % cases.

Correction of birth canal biocenosis in cows was accompanied by optimization of quantitative and qualitative composition of colostrum microflora. In day 1 postpartum, in colostrum of animals treated with Giprolam the number of lactobacilli and bifidobacteria was 9.8 and 29.2 times higher than in control, Staphylococcus epidermidis was 6.6 times less; Staphylococcus aureus, streptococci group D and Escherichia were not found. Colostrum of cows from group II contained 6.7 and 17.5 times more lactobacilli and bifidobacteria, 1.5 times less Staphylococcus epidermidis. Staphylococcus aureus was 2.3 times less frequent at 11 times lower count; streptococci group D and Escherichia found in the control with frequencies of 25.0 and 8.3 % were not isolated. Comparison of Giprolam
and Simbiter-2 effects shows that Giprolam increases number of lactobacilli and bifidobacteria 1.5- and 1.7-fold. *Staphylococcus epidermidis* was 4.3 times less, and *Staphylococcus aureus* was not found.

Intravaginal administration of probiotics had positive effect on formation of gastrointestinal normoflora in calves, formation of which starts from fetal movement through the mother's birth canals and directly depends of the sanitary quality and timely production of colostrum (milk), being the lactobacillus and bifidobacterium source. In the day 1 of life, quantity of lactobacillus in the large intestines of calves from cows treated with Giprolam, as compared to control, was 237.4 times higher, bifidobacteria — 38.9 times higher, and lactose positive *Escherichia* — 2.2 times higher than in control, ratio of the latter and lactose negative *E. coli* increased 17.7 times; quantity of *Enterobacter* and *Citrobacter* genera was 3.5 and 10.7 times less; staphylococci and protei were not found. On day 7, quantity of lactobacilli and bifidobacteria was 165.5 and 131.3 times higher, saprophyte staphylococci was 8.3 times higher; opportunistic pathogenic microorganisms *Enterococcus faecalis* was 21.7 times less, *Enterococcus faecium* — 25.3 times less, lactose negative *Escherichia* — 8.7 times less, *Citrobacter* and *Enterobacter* genera — 10.9 and 18.5 times less. On day 1, quantity of lactobacilli was 19.7 times higher than in the control, bifidobacteria — 15.6 times higher, lactose positive *Escherichia* — 16.3 times higher; opportunistic pathogenic *Citrobacter* and *Enterobacter* bacteria were 29.7 and 7.4 times less; *Staphylococcus aureus* and protein were not found on day 1 in the large intestines of calves from cows treated with Simbiter-2. Optimization of normoflora in calves during colostral period due to microecologic effects of Giprolam and Simbiter-2 prevents gastrointestinal diseases in calves in 50.0 and 41.7% cases, respectively.

Therefore, correction of vaginal biocenosis in down-calving cows by Giprolam and Simbiter-2 to a significant degree prevents development of acute postpartum endometritis. High effectiveness of use of such probiotics is due to ability of lactobacilli to colonize birth canals of mother-cows and maintain the optimal composition of indigenous microflora to prevent infection by pathogenic microorganisms and excessive colonization of birth canals by opportunistic pathogenic bacteria. Such treatment also optimizes quantitative and qualitative microflora composition of colostrum and intestines of calves that prevents their gastrointestinal diseases.

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