Praca oryginalna *Original paper*

Evaluation of the effectiveness of treatment of uterine inflammation in mares using hydrogen peroxide, ethacridine lactate in combination with probiotic therapy

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Summary

The aim of the study was to evaluate the usefulness of a minimally invasive therapeutic procedure involving single-uterine irrigation with hydrogen peroxide and ethacridine lactate, combined with oral probiotic therapy, in mares with endometritis. The study involved eight mares aged 3-21 years, for whom the diagnosis was based on clinical, ultrasound and microbiological examinations and an assessment of the pH of uterine mucus. In the baseline examination, microbial growth was detected in 7 of 8 samples (87.5%), whereas in the follow-up examination after treatment, in 3 of 8 samples (37.5%). No microbial growth was noted in the follow-up study in 5 of 8 samples (62.5%). At the same time, the following were observed: a decrease in the amount of fluid in the uterine cavity, an improvement in the ultrasound image of the endometrium, and a decrease in the pH of uterine mucus from 8.5-9.0 to 7.0-7.5. The results obtained showed that the procedure applied may represent a promising supportive method for the treatment of endometritis in mares and require further evaluation in controlled trials. A microbiological examination isolated bacteria of the *Enterobacteriaceae* group, *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas aeruginosa*. After the treatment, a follow-up test showed no *Pseudomonas aeruginosa*, and the number of samples with a positive culture result decreased markedly.

Keywords: endometritis, mare, hydrogen peroxide, ethacridine lactate, probiotics, uterine irrigation

Inflammation of the uterine lining (endometritis) is among the most commonly diagnosed disorders of the reproductive system in mares, and is a major cause of reduced fertility (1, 6, 12, 14, 18, 23, 33). The aetiology of the condition involves multiple factors, including bacterial infections, disorders of the uterine self-cleansing mechanisms, fluid accumulation within the uterine lumen, and an abnormal inflammatory response of the endometrium (6, 7, 12, 18, 19, 23, 33). The most commonly isolated microorganisms include *Streptococcus equi* subsp. *zooepidemicus*, *Staphylococcus* spp., bacteria of the *Enterobacteriaceae* group, and *Pseudomonas aeruginosa* (1, 6, 14, 18, 23).

Consequences of endometritis may include cycle disturbances, failed conceptions, early embryonic loss, and considerable economic losses in horse breeding (6, 12, 18, 23, 33). For this reason, it is important to diagnose the condition early and to select an effective but safe therapeutic approach.

In veterinary practice, the treatment is most commonly based on uterine irrigation and the use of antibacterial medications administered intrauterinely or

systemically (2, 7, 13, 18, 19, 23, 24, 26, 28, 29, 33-35). The effectiveness of such a procedure, however, is often limited, especially in chronic cases where bacterial biofilm is also present and where antibiotic resistance is increasing in the isolated strains (6, 8-10, 15, 23, 27).

Recent years have seen a growing focus on the need to seek non-antibiotic or supportive methods to improve the intrauterine environment and reduce the need for the use of antibiotics (3, 4, 10, 13, 20-22). Such solutions include, e.g. irrigation with antiseptic agents and procedures that support the body's microbial balance (4, 10, 13, 20-22).

The aim of the study was to evaluate the usefulness of a treatment regimen comprising a single uterine irrigation with hydrogen peroxide and ethacridine lactate, accompanied by a 16-day oral probiotic therapy, in mares with endometritis, by assessing changes in ultrasound images, microbiological test results, and uterine mucus pH.

Material and methods

The present study required no approval from the Ethics Committee, as the treatment was carried out with the consent of the animal owners and at their request.

The study involved 8 mares aged 3 warmblood 21 years. Six cold-blooded mares originated from a privately owned free-range breeding system, whereas two warmblood mares of the Małopolska breed were kept under a stabled management system. The mares were included in the study due to difficulties achieving pregnancy.

During the study, all animals had constant access to water, hay and roughage, and the Małopolska mares were additionally fed concentrated feed. Throughout the winter, the animals were administered mineral and vitamin supplements. The mares included in the study exhibited no signs of disease or abnormalities in their general condition.

During the breeding season, fertility disorders were observed in all the mares under study, manifesting as a failure to conceive and an abnormal course of oestrus behaviour. The diagnosis of endometritis was based on clinical and ultrasound examinations of the reproductive system, microbiological test results and an assessment of uterine mucus pH (5, 11, 16, 17, 25, 30, 31, 36).

The study was conducted under field conditions at the premises of the animals' owners. Ultrasound assessment was performed using an Aloka 500 system with a linear probe operating at 3-7 MHz. The presence and amount of fluid in the uterine cavity were assessed, as well as endometrial echogenicity.

The history obtained from the animal owners and the results of a detailed clinical examination determined the mare's eligibility for the study.

The material for microbiological testing was collected from the uterus using sterile Kruuse Equivet swabs, which were placed in transport medium. The pH of the uterine mucus was determined by the strip method.

Microbiological testing of the swabs taken was carried out at the Laboratory of the Clinic of Animal Reproduction at the University of Life Sciences in Lublin. Microbiological examination was carried out using enrichment media, including sheep blood agar and Sabouraud agar, to exclude the presence of moulds, yeasts, and dermatophytes. After 24 hours of incubation, the colony morphology was assessed. Next, Edwards, MacConkey, Chapman and Mueller-Hinton media were used to further identify the isolated pathogens and determine their susceptibility. Identification of the species in the laboratory was possible only for *E. coli* and *Pseudomonas aeruginosa*, and was not required for the present study. The priority was to determine drug susceptibility in relation to the bacterial species or family. The breakpoints for drug resistance/drug susceptibility, determined using the disc diffusion method, apply to the entire family. However, breeders have not accepted a detailed species-specific assessment due to the additional costs involved. Antibiotic susceptibility was determined based on penicillin, oxytetracycline, ampicillin, amoxicillin with clavulanic acid, cephalexin, enrofloxacin, gentamicin and marbofloxacin.

The therapeutic procedure involved a single uterine irrigation with 500 mL of a mixture containing hydrogen peroxide and ethacridine lactate, prepared *ex tempore*. It was assumed that this procedure could help clear the uterine cavity and improve the endometrial environment.

For the next 16 days, the following were used orally: a preparation containing *Lactiplantibacillus plantarum* (ATCC 8014, 2×10^{11} CFU/kg), inactivated *Bifidobacterium* spp. and *Lactobacillus plantarum* bacteria (12.1.14), and starch as a filler component (HORSEBIOM probiotic preparation). Upon completion of the treatment, a second sample was collected from the uterus for microbiological testing, and the pH of the uterine mucus was assessed.

The results of the microbiological examination, the pH assessment of uterine mucus, and the ultrasound assessment of the amount of fluid in the uterine cavity are presented in the tables and figures. Fisher's exact test was used to analyse differences in the frequency of positive culture results between the baseline and follow-up examinations, with $p < 0.05$ considered significant.

Results and discussion

Microbiological assessment involved comparing culture results from the baseline and follow-up examinations. The analysis considered microbial growth, the profiles of the isolated bacteria, and the nature of the changes observed in individual mares. Changes in the pH of the uterine mucus were assessed in parallel with ultrasound images of the endometrium and the presence of fluid in the uterine cavity, allowing laboratory parameters to be compared with clinical observations. In the baseline examination (02.06.2025), microbial growth was noted in 7 of 8 mares (87.5%), whereas no growth was noted in 1 of 8 samples (12.5%). Among the isolated bacteria, *Streptococcus* spp., *Staphylococcus* spp., bacteria of the *Enterobacteriaceae* group, *Escherichia coli* and *Pseudomonas aeruginosa* were predominantly found. At the follow-up examination (18.06.2025), upon completion of treatment, microbial growth was observed in 3 of 8 samples (37.5%), whereas no growth was observed in 5 of 8 samples (62.5%). These results

indicate a notable reduction in the frequency of positive culture results following the treatment. Analysis of changes in individual mares showed a reduction in the number of positive cultures and a favourable change in the profile of the isolated microorganisms. It was particularly significant that the follow-up examination detected no presence of *Pseudomonas aeruginosa*. A detailed summary of the results is provided in Table 1 and Figure 1.

Tab. 1. Results of microbiological testing of samples taken from the mare's uterus before and after the treatment

Mare	Baseline examination	Follow-up examination after 16 days	Nature of the change
1	*Streptococcus* spp., *Pseudomonas aeruginosa*	*Enterobacteriaceae*	No *Pseudomonas aeruginosa* was detected; a change in the family of isolated microorganisms
2	No growth	No growth	Negative result maintained
3	*Streptococcus* spp., *Escherichia coli*	No growth	No growth in follow-up examination
4	*Enterobacteriaceae*	No growth	No growth in follow-up examination
5	*Escherichia coli*, *Staphylococcus* spp.	*Enterobacteriaceae*	A change in the family of isolated microorganisms
6	*Enterobacteriaceae*	*Staphylococcus* spp.	A change in the family of isolated microorganisms
7	*Enterobacteriaceae*	No growth	No growth in follow-up examination
8	*Enterobacteriaceae*	No growth	No growth in follow-up examination

In the baseline examination, microbial growth was observed in 7 of 8 samples (87.5%), whereas in the follow-up examination, it was observed in 3 of 8 samples (37.5%). The difference was assessed by Fisher's exact test.

Analysis of the pH and the relationship with the clinical picture

Baseline examination showed an alkaline pH of uterine mucus ranging from 8.5 to 9.0 in all the mares (Fig. 2). These results were accompanied by fluid in the uterine cavity and abnormal endometrial echogenicity (23, 25, 26, 31, 36).

In the follow-up examination, the pH values decreased to 7.0-7.5. At the same time, a reduction in the amount of fluid in the uterine cavity was observed, along with an improvement in endometrial echogenicity towards a pattern more closely resembling the physiological state.

The resulting changes were accompanied by improved uterine clinical condition, reduced fluid volume within its lumen, and fewer positive microbiological results. A return of pH to values close to physiological levels may indicate an improvement in the uterine environment, potentially more conducive to normal reproductive function.

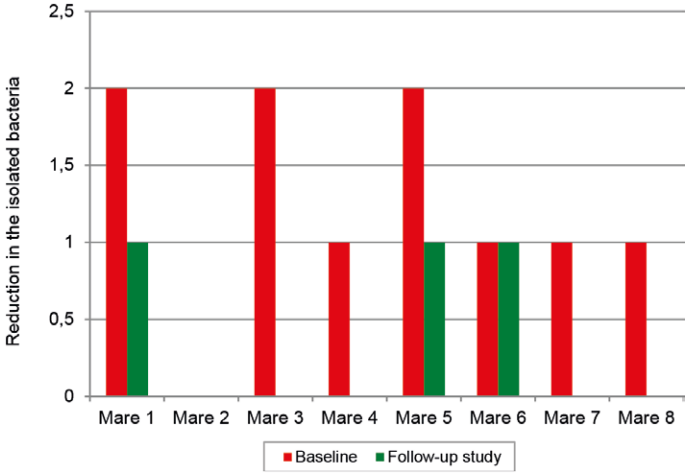

Fig. 1. A graphical summary; a reduction in the number of positive microbiological results for individual mares in the baseline and follow-up examinations

The literature indicates that in chronic or recurrent endometritis, the efficacy of conventional antibiotic therapy may be limited, especially in the presence of bacterial biofilm and increasing microbial resistance (6, 8-10, 15, 23, 27). For this reason, non-antibiotic and supportive methods are gaining increasing interest (3, 4, 10, 13, 20-22).

Tab. 2. Assessment of the amount of fluid in the uterine cavity in the baseline and follow-up examinations

Mare	Baseline examination (cm)	Follow-up examination (cm)	Drop (cm)	Drop (%)
1	3.5	1.0	2.5	71.4
2	2.9	1.5	1.4	48.3
3	3.0	0.8	2.2	73.3
4	2.9	1.4	1.5	51.7
5	3.0	1.8	1.2	40.0
6	2.7	2.2	0.5	18.5
7	2.4	1.8	0.6	25.0
8	2.8	1.4	1.4	50.0

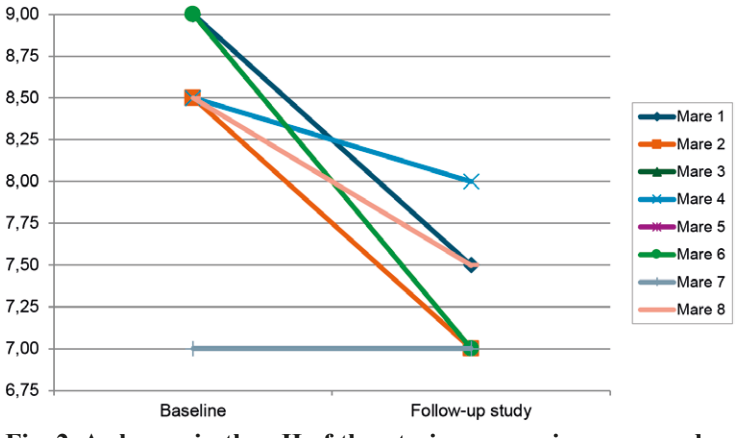

Fig. 2. A change in the pH of the uterine mucus in mares undergoing treatment

It is noteworthy that the microbiological assessment of resistance showed complete resistance to penicillin in the mares under study. One hundred per cent sensitivity was demonstrated for marbofloxacin and enrofloxacin. However, neither of these fluoroquinolones is authorised for use in horses in Poland. Oxytetracycline showed moderate susceptibility in three mares, and resistance in the rest, although the antibiotic is authorised for the treatment of horses. The remaining antibiotics exhibit sensitivity, moderate sensitivity, or resistance; however, as with marbofloxacin and enrofloxacin, they are not authorised for use in horses.

Microbiological analysis revealed a change in the family of the bacteria isolated from three mares. This may be due to contamination of the sample during collection in the stable, under unsterile conditions. The echogenicity of the uterus and the pH of the cervical mucus returned to normal physiological levels; consequently, this finding was not clinically significant.

The present study showed a clear reduction in the frequency of positive culture results, normalisation of the pH of uterine mucus, and improvement in the ultrasound image. These results are consistent with the notion that improving environmental conditions within the uterus may help reduce the inflammatory process (4, 7, 10, 13, 20, 26, 33, 34).

It should be stressed, however, that this was a pilot study involving only a small number of mares. Limitations of the study include the absence of a control group and the lack of assessment of long-term reproductive indicators, i.e., conception rates. For this reason, the results should not be interpreted as evidence that the method applied is superior to standard antibiotic therapy. It is more appropriate to conclude that the procedure described may represent a promising supportive method in the treatment of endometritis in mares and deserves further evaluation in studies involving a larger group of animals. The reduction in the number of positive cultures, the improvement in the ultrasound image, and the decrease in the pH of uterine mucus indicate that the protocol used may be of practical value, particularly under field conditions, as part of a supportive procedure (4, 13, 15, 20). The results clearly demonstrate the effectiveness of the applied method. The complete eradication of *Pseudomonas aeruginosa* is the most significant therapeutic success, given its drug resistance and biofilm-forming ability. The combination of a live strain of lactic acid bacteria with inactivated microorganisms enables the simultaneous delivery of active bacteria, structural proteins, cell wall fragments and immunomodulatory metabolites from the inactivated bacteria. This composition promotes the maintenance of microbial balance in the digestive tract and supports immunomodulatory activity within the gut-vagina axis.

Furthermore, the presence of starch may additionally promote the stabilisation of the intestinal microbiota, providing probiotic bacteria with the appropriate metabolic conditions for the production of beneficial bioactive compounds.

Probiotic bacteria, such as *Lactobacillus plantarum* and *Bifidobacterium* spp., serve a key role in maintaining the microbiological and immunological homeostasis of the mare's body.

A live *L. plantarum* strain can compete with pathogenic bacteria in the intestines by limiting their growth, adherence to the epithelium and toxin production, thereby indirectly reducing their adverse impact on the entire body.

However, the actions of probiotic bacteria and their metabolites extend far beyond the digestive tract, including the reproductive system via the so-called gut-vagina axis. This mechanism is based on immunometabolic communication between the intestinal microbiota and the mucous membranes of the reproductive tract. Metabolites produced by probiotic bacteria, such as short-chain fatty acids (SCFAs), lactic acid, and bioactive peptides, have antibacterial, antifungal, and antiviral effects and modulate the activity of immune system cells, influencing the cytokine profile, regulating the inflammatory response, and maintaining epithelial integrity. Consequently, this indirectly strengthens the mucosal immune system and improves the microenvironment of the reproductive tract, potentially reducing the risk of infection and supporting proper reproductive function in mares.

Streptococcus spp. were detected in a single sample, as these strains are increasingly resistant to penicillins and tetracyclines (*Staphylococcus* spp. were found sporadically, and their presence after therapy was clinically insignificant).

Despite numerous publications on the diagnosis and treatment of endometritis in mares, little attention has been paid to alternative treatment methods, and the mechanism by which oral probiotic therapy affects the function of the uterine microbiome in mares remains poorly understood. The authors have found no parallel concept in the available literature. In a described clinical study, Mahal et al. (20) used a hydrogen peroxide and povidone-iodine solution for uterine irrigation, without focusing on bacteriology or the microbiome. Recent research suggests that, in addition to eliminating pathogenic bacteria, it is necessary to provide an appropriate environment for symbiotic bacteria. A publication by Silva J. A. et al. provides important information relevant to veterinary practice (32). These authors identified over 250 bacterial strains isolated from more than 70 healthy mares. Nearly 90% of isolated strains are lactic acid bacteria that act as antagonists to pathogenic bacteria. This conclusion suggests that oral or topical administration of appropriate *Lactobacillus* strains may be effective for treating uterine inflammation.

The method employed in the present study works in multiple ways: hydrogen peroxide mechanically removes biofilm and lowers the pH of the uterus; rivanol

has antiseptic and astringent properties; and probiotics stabilise the environment by colonising it with beneficial microflora. This resulted in an effect superior to that achieved with antibiotic therapy alone, whose effectiveness in chronic uterine inflammation is estimated at 30-50%. What is important, this method does not generate the risk of increasing antibiotic resistance, giving it an advantage and making it a real competitor to antibiotics in the treatment of endometritis. The literature highlights the growing problem of antibiotic resistance in bacteria that cause endometritis in mares.

Previously sensitive to penicillin, *Streptococcus* spp. are now increasingly resistant to β-lactams and tetracyclines. Albihn et al. (2003) reported that over 30% of *Streptococcus zooepidemicus* strains isolated from mares were tetracycline-resistant. Certain strains of *Staphylococcus* spp. have resistance mechanisms typical of MRSA (methicillin-resistant *Staphylococcus aureus*) and the ability to form biofilms, which hinders the penetration of antibiotics. Even if *Staphylococcus* spp. are present in limited numbers in the uterus, their potential resistance may make standard treatment ineffective. *Pseudomonas aeruginosa* is a bacterium considered to be one of the most difficult pathogens to treat in veterinary medicine. It is naturally resistant to penicillins, cephalosporins and tetracyclines, and has a high biofilm-forming ability. Antibiotic therapy often shows limited efficacy against *Pseudomonas* infections, which frequently evolve into chronic conditions.

Based on the results presented above, it can be concluded that uterine irrigation with a hydrogen peroxide and rivanol solution, combined with probiotic therapy, appears to be exceptionally effective.

The mechanism of action of this method is not dependent on classic bacterial targets (enzymes, ribosomes or membrane proteins), to which bacteria develop resistance. Hydrogen peroxide acts through non-specific oxidation and destruction of membranes and DNA, rivanol exhibits antiseptic and astringent properties, and probiotics compete with pathogens for colonisation sites, which, taken together, limit the ability of microorganisms to persist in the uterine environment.

As a result, the method remains largely unaffected by the growing problem of antibiotic resistance and can be regarded as a viable alternative to antibiotic therapy for the treatment of endometritis in mares.

From a practical perspective, it was also found that a high mucus pH (8.5-9.0) correlated with the presence of infection and endometrial inflammation, whereas normalisation of the pH (7.0-7.5) was accompanied by clinical improvement, including a reduction in fluid, a normal ultrasound image, and a reduced number of infected samples. Therefore, the pH of mucus may be a useful and rapid marker of the effectiveness of endometritis therapy in mares, as it directly reflects the conditions of the uterine environment, and the results obtained indicate a clear trend towards recovery, since the improvement in the pH occurred in parallel with microbiological improvement and clinical picture. In summary, after the treatment, the percentage of positive culture results dropped from 87.5% to 37.5%. Furthermore, follow-up examination found no presence of *Pseudomonas aeruginosa*. Moreover, a reduction in the pH of uterine mucus and a decrease in the volume of the uterine cavity fluid were observed in the mares under study. The authors believe that the procedure applied may represent a promising supportive treatment for endometritis in mares, although it requires confirmation in controlled studies involving a larger number of mares.

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