



## Physicochemical and Microbiological Properties of Cervicovaginal Mucus in Dairy Cow Health Monitoring

Isatay Jakupov<sup>1,\*</sup>, Zhongliang Jiang<sup>2</sup>, Alexander Shevtsov<sup>3</sup>, Gulzhan Yeszhanova<sup>1</sup>, Dinara Birimzhanova<sup>4,\*</sup>, Aida Abultdinova<sup>1</sup>, Gulnur Mamytbekova<sup>1</sup> and Albert Zabrodin<sup>1</sup>

<sup>1</sup>S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan

<sup>2</sup>Northwest Agriculture and Forestry University, Xianyang, China

<sup>3</sup>National Center for Biotechnology, Astana, Kazakhstan

<sup>4</sup>L.N. Gumilyov Eurasian National University, Astana, Kazakhstan

\*Corresponding author: [isatay.jakupov@gmail.com](mailto:isatay.jakupov@gmail.com) (IJ); [dinarabirimzhananova@gmail.com](mailto:dinarabirimzhananova@gmail.com) (DB)

**Article History:** 25-216 Received: 07-Aug-25 Revised: 03-Sep-25 Accepted: 16-Sep-25 Online First: 23-Oct-25

### ABSTRACT

Cervicovaginal mucus (CVM) is a hydrogel whose composition and consistency vary with the estrous cycle in cows. The current investigation was designed to study the physicochemical and microbiological characteristics and enzymatic composition of cervicovaginal mucus, as well as their diagnostic significance in diseases of the uterus in cows, and to determine a biomarker based on them. The study was conducted on Holstein-Friesian cows from dairy farms in North Kazakhstan, using cervicovaginal mucus collected from both clinically healthy animals and those with uterine diseases. The physicochemical properties of cervicovaginal mucus were studied using infrared spectroscopy, test strips, and qualitative reactions to enzymes. Analysis of the physicochemical and microbiological properties of cervicovaginal mucus in cows showed that using infrared spectroscopy, carboxyl (C=O), hydroxyl (O-H), and C-H bonds were detected, indicating the presence of mucin. The pH of mucus in clinically healthy cows was 0.85% higher than in sick cows, indicating a change in the acid-base balance associated with uterine pathologies. Metagenomic sequencing of 16S rRNA determined that potentially pathogenic bacteria, such as *Trueperella* (4.56%), *Fusobacterium C* (5.17%), and *Helicococcus* (5.22%), were detected in the microbiota of sick animals. The determination of peroxidase using benzidine and guaiacol samples did not reveal a statistically significant difference between the methods. However, the guaiacol test showed a greater number of positive reactions (81.3% vs 70%). This study showed significant changes in the physicochemical properties, microbiota, and enzymatic profile of cervicovaginal mucus, depending on the uterus condition. The identified biomarkers can be used as diagnostic indicators of inflammatory diseases of the reproductive system in cows.

**Keywords:** Cow health, Uterine pathology, Cervicovaginal mucus, Infrared spectroscopy, Microbiota, Enzyme activity.

### INTRODUCTION

Mucus is synthesized by specialized goblet-shaped cells in the columnar epithelium lining all organs exposed to the external environment (Abrami et al. 2024). It performs many functions, including lubrication for passing objects, maintaining a hydrated layer above the epithelium, a barrier to pathogens and harmful substances, and a permeable gel layer to exchange gases and nutrients with the underlying epithelium (Bansil and Turner 2018; Ardicli et al. 2024).

Cervicovaginal mucus (CVM) is a hydrogel consisting of water and a solid component that forms a three-dimensional network of three or more structural units (García et al. 2024). The amount and consistency of

discharge from the cow's cervix vary depending on the estrous cycle stage (Huang et al. 2024). Important physical characteristics of CVM include color, consistency, pH, spinnbarkeit value, and fern pattern, which are considered effective laboratory tools for detecting infertility in cattle (Hanumant et al. 2019; Maksymyuk et al. 2022; Naliukhin et al. 2024). Mucus also contains a wide range of antimicrobial enzymes, peptides, and immunoglobulins that protect epithelial cells of the respiratory, gastrointestinal, genitourinary, visual, and auditory systems (Sheng and Hasnain 2022; Vllahu et al. 2024). The quality of cervical mucus is a good indicator of a cow's physiological state during estrus, and high-quality mucus leads to high fertility rates (Khanoria et al. 2022; Rhoads 2023).

**Cite This Article as:** Jakupov I, Jiang Z, Shevtsov A, Yeszhanova G, Birimzhanova D, Abultdinova A, Mamytbekova G and Zabrodin A, 2025. Physicochemical and microbiological properties of cervicovaginal mucus in dairy cow health monitoring. International Journal of Veterinary Science 14(6): 1359-1367. <https://doi.org/10.47278/journal.ijvs/2025.128>

Water is the main component of vaginal fluid, and reports on the water content of this secretum show significant differences. However, it is generally assumed that during the follicular phase, under the influence of estrogen, it makes up about 95-99% of the total amount; during the luteal phase, the percentage can decrease to 85%. The main soluble components include sodium chloride, phosphate and bicarbonate salts, soluble proteins, and amino acids. The insoluble fraction consists of macromolecular glycoproteins, which are the main determinants of the physical properties of mucus. These glycoproteins secreted by the cervical epithelium are formed by a polypeptide backbone (20-25%) carrying numerous heterosaccharide side chains (75-80%) and are also known as mucins. Mucins form a three-dimensional network throughout the mass of mucus secretions, giving the vaginal fluid the properties of a gel. The main property of mucins is the ability to bind large volumes of water. As a result, the vaginal fluid behaves like a highly hydrated gel (Rutllant et al. 2005).

Mucus mainly consists of water (~95% by weight), mucins (~0.2-5.0%), globular proteins (~0.5%), salts (~0.5-1.0%), lipids (1-2%), DNA and cells and cellular debris (Boegh et al. 2013; Button et al. 2013; Kavishvar and Ramachandran 2023) and forms a dense, viscoelastic layer on top of epithelial cells, which serves as a selective barrier for drugs and other molecules (Leal et al. 2017).

The rheological properties showed a noticeable difference, as the CVM's branching pattern has a significant relationship with the frequency of conception and can, therefore, be used as a criterion for predicting the correct timing of artificial insemination (Sharma et al. 2013; Verma et al. 2014).

Another research compares the activity of the enzymes alkaline phosphatase (ALP), lactate dehydrogenase (LDH), -amylase, -mannosidase, -N-acetylglucosaminidase, -glucuronidase, and -galactosidase in the cervical mucus of cows during spontaneous and induced estrus and found different activity of most enzymes in the cervical mucus of cows (Tsiligianni et al. 2003).

Pathogenic or opportunistic bacteria are usually found in the CVM of sick cows, which are associated with inflammatory diseases such as endometritis, vaginitis, or other reproductive tract infections (Adnane et al. 2024). The cow's vagina contains a complex and dynamic microbial community consisting of aerobic, facultatively anaerobic, and anaerobic bacteria. Common inhabitants of the cow's vaginal tract include *Streptococcus* sp., *Staphylococcus* sp., *Enterococci*, and representatives of *Enterobacteriaceae*. The most common phylophiles in the vaginal microbiome of dairy cows are *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* (Laguardia-Nascimento et al. 2015; Nesengani et al. 2017; Giannattasio-Ferraz et al. 2019). Some studies also report the presence of *Tenericutes* and *Fusobacteria* as significant components (Chen et al. 2020; Quadros et al. 2020; Quereda et al. 2020; Moreno et al. 2022).

The cervix is a key anatomical barrier in the reproductive tract and its microbiome plays an important role in maintaining uterine health (Sheldon and Dobson 2004; Thulasiraman et al. 2025; Vorobyov et al. 2025). The abnormalities in the cervix's microbiome, especially an overrepresentation of certain pathogenic bacteria, such as *Staphylococcus aureus*, as well as elevated levels of

*Bacteroidetes* and *Fusobacteria*, are associated with reproductive diseases such as metritis (Zubova et al. 2021). Understanding these changes in microbial composition is crucial for identifying potential biomarkers of reproductive disorders and developing targeted interventions to restore microbial balance (Adnane et al. 2018; Jakupov et al. 2024). The composition of the genital microbiome undergoes significant fluctuations throughout the life of a cow, which are influenced by anatomical structure, age, cyclicality, pH dynamics of the vagina, nutrition and keeping conditions (Adnane and Chapwanya 2024; Gnezdilova et al. 2025).

Thus, the physicochemical and microbiological characteristics of cows' CVM have diagnostic value and are a source of a biomarker of inflammation. Qualitative and quantitative determination of the parameters by which it will be possible to differentiate between sick and clinically healthy animals is needed. This work was aimed to study the physicochemical and microbiological characteristics, and enzymatic composition of CVM, as well as their diagnostic significance in diseases of the uterus in cows, and to determine a biomarker based on them.

## MATERIALS AND METHODS

### Study period and location

CVM samples were collected from October 2023 to October 2024 in agricultural companies in the Akmola and North Kazakhstan regions of the Republic of Kazakhstan.

### Animals and clinical examination

Studies of the physicochemical properties and composition of CVM of 1-5 lactation Holstein-Friesian cows were conducted in the Scientific Laboratory of Veterinary Medicine, Department of Veterinary Medicine, Faculty of Veterinary Medicine and Animal Husbandry Technology, S. Seifullin Kazakh Agrotechnical Research University, the scientific laboratory of the Nazarbayev University Autonomous Educational Organization, and the Laboratory of Applied Genetics, National Center of Biotechnology. Before sampling, the animals were examined for the presence of uterine diseases using clinical methods (medical history, external examination, rectal and vaginal examination) and laboratory methods (Nagorny-Kalinovsky method, Whiteside method, cytological examination).

### Sample collection

The CVM sampling was conducted as follows: the animals were fixed, and the external genitalia, sciatic tubercles, and tail root were cleaned. Then, the technician inserted a hand in a sterile obstetric glove treated with an antiseptic solution into the vagina, advanced to the cervix, and CVM was collected manually. The collected CVM was placed in sterile containers for biological samples with a capacity of 100mL.

### Laboratory analyses

The mucus composition was studied using a Fourier-transform infrared (FTIR) spectrometer (Lab 523/C4). The data obtained was interpreted according to the characteristic frequencies. Mucus samples were examined using UrineRS H11 analytical test strips (High Technology, Inc.) and a Kelilong PH-061 pH meter (Kelilong Electron Co.).

When studying the composition of the microbiota of CVM, the technology of metagenomic DNA sequencing was used, based on the isolation and sequencing of the 16S rRNA gene, which contains both conserved regions (common to all bacteria) and variable regions characteristic of specific species or genera. In addition, metagenomic sequencing provided a quantitative assessment of the proportion of each microorganism in a sample, which allowed us to build a complete picture of the microbiota's composition. The 16S rRNA gene (a unique and highly conserved region of the nucleotide chain of all bacteria, often used to identify bacteria) was isolated from the samples.

The method is considered the "gold standard" for the most complete analysis of the microbiota composition. It allows researchers to analyze which bacteria are present in the body (and in what percentage) and what pathogenetic role bacteria play in various diseases (Verhelst et al. 2004). Conditionally pathogenic microorganisms that cause the development of inflammation (metritis, endometritis) enter the uterine cavity of cows endogenously.

Qualitative reactions for the determination of catalase and xanthine oxidase were used to study the enzymatic composition of cows' CVM, and benzidine and guaiacol samples were used to determine peroxidase.

### Statistical Analysis

Descriptive statistics methods were used for statistical data processing and the  $\chi^2$  criterion and Student's t-test for independent samples were used to verify the significance of differences in results between the methods under consideration.

## RESULTS

### Infrared (IR) spectroscopy

The composition of the mucus was determined by IR spectroscopy of the IR spectra of 7 samples. The IR spectra showed maxima of 3,281.50 and 1,636.58 $\text{cm}^{-1}$ , valence and deformation vibrations of the OH group. The IR spectra of all seven samples were identical, which indicates the presence of water. The samples were then dried. Each sample was applied to a 96-well microplate, six copies per sample, and air-dried for 8-12 hours at room temperature. Next, the IR spectra of the same samples were taken again.

The spectral data confirmed the presence of carboxyl (C=O), hydroxyl (O-H), and C-H functional groups. The corresponding IR absorption bands were observed at 1,650–1,750  $\text{cm}^{-1}$  for C=O, 3,200–3,300  $\text{cm}^{-1}$  for O-H and 2,950–3,050  $\text{cm}^{-1}$  for C-H bonds (Fig. 1). The presence of absorption bands in the IR region of carboxyl, hydroxyl, and C-H bonds in all samples indicates the presence of mucin, which is confirmed by literature data (Mehtio et al. 2004; Johnson et al. 2009; Travo et al. 2010; Denton et al. 2011; Armstrong et al. 2017).

### pH measurement

The analytical test strips were immersed in a sample container for 30 seconds. The color change of the pH paper was compared with the standard color scale of the indicator strip. The pH was also measured with a pH meter. When measuring the pH of CVM, the results were obtained depending on the condition of the uterus in cows (n=30) (Table 1).

**Table 1:** Results of measuring the pH of CVM in cows, depending on the condition of the uterus

Groups	n	The pH value	
		pH meter	Test strips
Sick animals	15	8.15±0.12	8.13±0.13
Clinically healthy animals	15	8.22±0.09	8.27±0.15

Mean pH was slightly higher in clinically healthy cows. With the pH meter, the difference was 0.07 pH units (8.22 vs 8.15), corresponding to an approximately 15% lower hydrogen ion activity in healthy animals. With test strips, the difference was 0.14 pH units (8.27 vs 8.13),  $\approx$  28% lower  $[\text{H}^+]$  in healthy animals.

### Microbiological indicators

Bacteriological examination of CVM from clinically healthy cows and cows with uterine pathologies using 16S rRNA metagenomic sequencing showed the presence of predominantly saprophytic microorganisms. Table 2 shows the summary data on animal diseases and their microbiota.

**Table 2:** Genera of bacteria found in the uterus of healthy cows and cows with uterine pathologies using 16S rRNA metagenomic sequencing

Groups	Microbiota	
	Name	%
Healthy animals (n=8)	Faecousia	7.860
	RUG13077	1.340
	Cryptobacteroides	5.522
	PeH17	0.504
	Phocaeicola A 858004	5.649
	Atopostipes	0.535
	Paraprevotella	2.187
	Streptobacillus 993623	0.787
	Phascolarctobacterium A	0.581
	Corynebacterium	1.753
	Pseudomonas E 647464	0.669
	RF16	1.318
	Actinobacillus B	0.611
	Sick animals (n=8)	Faecousia
RUG13077		0.798
Cryptobacteroides		2.831
PeH17		0.603
Phocaeicola A 858004		3.665
Atopostipes		0.922
Paraprevotella		1.086
Streptobacillus 993623		0.525
Phascolarctobacterium A		0.805
Alloprevotella		1.332
Histophilus		4.342
Ureaplasma		1.634
Helcococcus		5.222
Fusobacterium C		5.177
Bacteroides H	2.046	
Porphyromonas A 859426	1.100	
Odoribacter 865973	0.703	
Porphyromonas A 859424	0.676	
Prevotella	0.608	
Trueperella	4.568	

The bacterial composition of the CVM of healthy cows and cows with uterine pathologies included 24 genera, nine of which were found in both groups of animals. These include *Faecousia*, RUG13077, *Cryptobacteroides*, PeH17, *Phocaeicola A* 858004, *Atopostipes*, *Paraprevotella*, *Streptobacillus* 993623, and *Phascolarctobacterium A*.

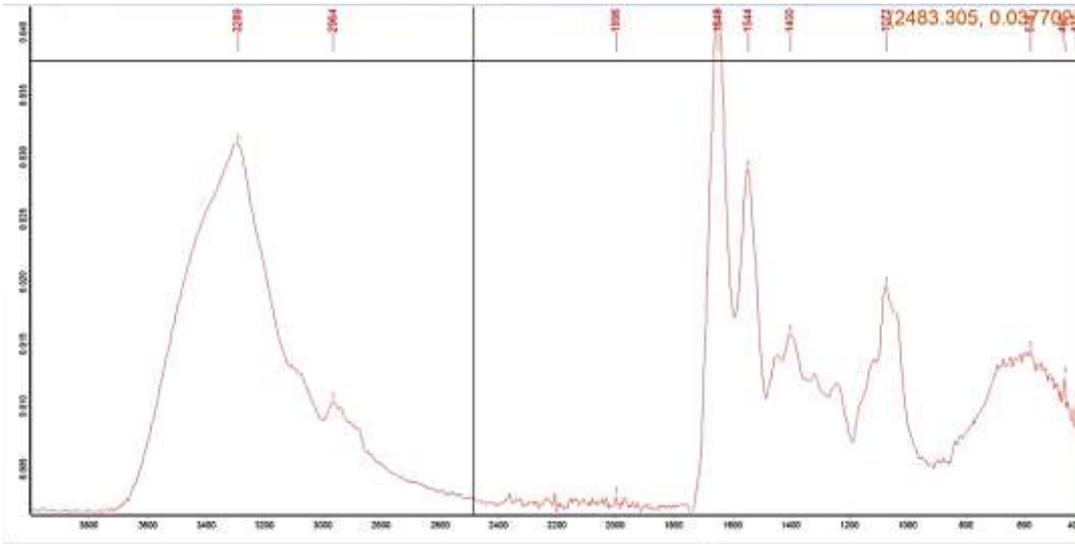


Fig. 1: IR spectrum of a mucus sample after drying.

Table 3: Results of qualitative reactions for the determination of catalase and xanthine oxidase in CVM in cows (n = 15)

Item No.	Course	n	Catalase		Xanthine oxidase			
			Foam formation		Foam formation		Discoloration	
			n	%	n	%	n	%
1	Acute	5	5	100	5	100	5	100
2	Chronic	5	5	100	5	100	5	100
3	Clinically healthy animals	5	1	20	1	20	1	20

Four bacterial genera were identified exclusively in healthy animals: *Corynebacterium* (1.753%), RF16 (1.318%), *Pseudomonas* E 647464 (0.669%) and *Actinobacillus* B (0.611%).

In total, 13 main genera of bacteria were found in healthy animals. The microbiota of healthy animals was dominated by the genera *Faecousia* (7.860%) and *Phocaeicola* A 858004 (5.649%). Other representatives, such as *Cryptobacteroides* (5.522%) and *Paraprevotella* (2.187%), occurred in smaller but noticeable proportions. The remaining bacterial genera were represented in much smaller quantities (less than 2%).

20 main genera of bacteria were identified in sick animals. In the microbiota of sick animals, the most common genera were *Helcococcus* (5.222%), *Fusobacterium* C (5.177%), *Faecousia* (5.135%), and *Trueperella* (4.568%).

According to the results of microbiota studies, RF16, *Actinobacillus* B, *Corynebacterium*, and *Pseudomonas* E 647464 were detected only in healthy animals. *Alloprevotella*, *Trueperella*, *Helcococcus*, *Ureaplasma*, *Fusobacterium* C, *Bacteroides* H, *Porphyromonas* A 859426, *Porphyromonas* A 859424, *Odoribacter* 865973, *Prevotella*, and *Histophilus* were detected in sick animals.

**Determination of enzymes**

The catalytic activity of catalase is independent of pH changes in the range of 5-10.5. To determine catalase, 1mL of cervical mucus is poured into a test tube, and 1 ml of 3% hydrogen peroxide is added. Rapid release of oxygen bubbles is observed, indicating the catalase enzyme's presence (Zhang et al. 2019).

To determine xanthine oxidase, 1mL of mucus is poured into test tubes: the first one contains the mucus of a sick animal, and the second one contains the mucus of a healthy animal. One drop of 0.01% methylene blue solution

and 1mL of 3% hydrogen peroxide solution are added to both tubes. After some time, the liquid discolors in the first tube, and the foam appears 1-1.5cm high. In the second tube, the liquid's color does not change, and foam formation is less than 0.5cm high. The test results are shown in Table 3.

In all cows with an acute course (n = 5), a 100% positive reaction to catalase (foam formation) was observed in the CVM, indicating this enzyme's active presence. Similarly, xanthine oxidase was detected in all animals (100%) by both foam formation and discoloration. It confirms the pronounced enzymatic activity associated with acute inflammation.

The results of the chronic course were identical to the acute course: 100% positive reactions to catalase and xanthine oxidase. This may indicate an ongoing inflammatory process in which the enzymes remain active in the mucosa. In the group of clinically healthy animals, only one cow out of five (20%) showed weak catalase and xanthine oxidase activity, reflecting low or absent enzymatic activity, which is typical for healthy mucosa without signs of inflammation.

A high activity of catalase and xanthine oxidase was established, correlating with an inflammatory process. The activity of these enzymes was significantly lower in the clinically healthy cows, which confirms the absence of inflammatory changes in the mucosa.

Qualitative reactions to enzymes can be used as a diagnostic criterion for differentiating inflammatory conditions (acute or chronic) and determining the clinical health of animals. A reaction with benzidine and guaiacol was used to determine peroxidase. For the benzidine test, a 0.5% benzidine solution with 50% acetic acid was prepared in a volume of 5mL per 1 sample of CVM. The resulting solution was mixed with an equal amount of 3% hydrogen peroxide solution, and the resulting reagent was added to

1mL of mucus in a test tube. The reaction was considered positive if the contents of the tube were blue or green. The guaiacol sample was prepared by mixing a 1% guaiacol solution in distilled water and benzidine peroxide (a few drops of 30% hydrogen peroxide were added to the benzidine solution). Next, 1mL of mucus was added to the tube, 0.5mL of 1% guaiacol solution, and five drops of benzidine peroxide solution were alternately added to the mucus, after which the sample and reagents were mixed. The reaction was considered positive if the contents of the tube were colored red or burgundy (Table 4).

**Table 4:** Results of qualitative reactions for the determination of peroxidase in CVM in cows

Item No.	Test name	n	Positive samples		Negative samples	
			n	%	n	%
1	Benzidine sample	10	7	70	3	30
2	Guaiacol test	16	13	81.3	3	18.7

For a critical value of  $\chi^2$  with a significance level of 0.05 and a degree of freedom of 1 (df = 1), the threshold value is 3.841. Since the calculated value of  $\chi^2=0.439$  is less than the threshold value, the null hypothesis is not rejected. There was no statistically significant difference between the results of the benzidine and guaiacol tests. Both methods showed similar effectiveness in determining peroxidase, although the guaiacol test had 11.3% more positive reactions.

Thus, the physicochemical and microbiological parameters and the enzymatic composition of CVM in cows were determined depending on the condition of the uterus.

## DISCUSSION

In the present study, cervicovaginal mucus (CVM) was investigated through IR spectroscopy in order to identify its major functional groups. The analysis showed clear absorption bands of carboxyl (C=O) and hydroxyl (O-H) groups, recorded at 1,724.0; 1,687.3  $\text{cm}^{-1}$  and 3,484.7; 3,286.0; 3,158.7  $\text{cm}^{-1}$ . These results are in line with the findings of Armstrong et al. (2017), who reported almost identical IR spectra for D-glucaric acid. Similarly, Denton et al. (2011) described peaks for the same groups in their work, which further supports our findings. Mehtio et al. (2004) also mentioned a strong carbonyl peak at 1,725  $\text{cm}^{-1}$  in galactaric acid, falling within the same range as observed in our samples. Another study by Travo et al. (2010) focused on the range of 950–1,750  $\text{cm}^{-1}$  and highlighted bands belonging to protein amides I and II, as well as glycosylation peaks. These observations are in line with our results and suggest that the spectral profile of CVM is largely determined by its glycopeptide nature. Interestingly, in our samples the glycosylation bands also showed variations in intensity, which might be related to differences in carbohydrate content among individual animals.

The properties of mucin were also investigated by Johnson et al. (2009). The peak at 1,630 $\text{cm}^{-1}$  belongs to the carbonyl group. The peak at 2,990 $\text{cm}^{-1}$  belongs to the aliphatic stretching of C-H due to the connecting methylene groups. The region between 2,500 and 3,700 $\text{cm}^{-1}$  is wide due to the overlap of the carboxyl group, the -OH group

from the alcohol group and the NH group from the amide group. In addition, a very wide peak in this range indicates strong hydrogen bond interactions due to the -OH groups.

When studying the pH of cows' CVMs, the average values were found in animals with uterine diseases in the range of 8.13-8.15, whereas in clinically healthy animals, they were 8.22-8.27. For the pH meter,  $p > 0.05$  means no statistically significant differences between the groups of sick and clinically healthy animals. However, for the test strips,  $p < 0.05$  indicates statistically significant differences in pH between the groups. Thus, the test strips recorded a significant increase in pH in clinically healthy animals, while the pH meter did not show a significant difference.

According to the published literature, the pH of CVM in clinically healthy cows capable of reproduction ranges from 7.0 to 8.0 (Siregar et al. 2019). There are still no established pH parameters of mucus in animals with uterine diseases: for example, there is evidence that the pH of mucus in animals with endometritis was  $7.95 \pm 0.096$  (Palanisamy et al. 2014) and in animals with subclinical endometritis was  $7.44 \pm 0.21$  (Raval et al. 2018). There were no significant statistical differences between the pH values of sick and healthy animals, which correlates with the findings of current investigation. However, this issue requires further study to establish diagnostic parameters for animals with uterine diseases.

The bacteria associated with uterine diseases are classified as pathogens, potential pathogens, or opportunistic microorganisms. Recognized lost pathogens associated with severe endometrial inflammation and clinical endometritis include *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *Prevotella melaninogenica*, and representatives of the genus *Proteus* (Williams et al. 2005). Williams et al. (2007) and Wang et al. (2013) suggested that a high level of *E. coli* cells could be the main factor in the onset of uterine infection.

In our study, several bacterial genera were identified exclusively in the CVM of cows with uterine pathologies, including *Trueperella*, *Fusobacterium*, *Prevotella*, *Porphyromonas*, and *Ureaplasma*. These findings are consistent with previous reports. For instance, *Trueperella pyogenes* is recognized as one of the primary pathogens of purulent inflammation, commonly linked with endometritis and purulent vaginal discharge (Swartz et al. 2014; Liu et al. 2024). Similarly, *Fusobacterium necrophorum* has been described as a gram-negative anaerobe frequently associated with necrotic and purulent inflammations, and often found together with *T. pyogenes*, thereby amplifying disease severity (Srinivasan et al. 2021; Beneduzi 2025). Pathogenic strains of *Prevotella* spp. have been linked with chronic inflammatory processes such as endometritis and vaginitis (Adnane and Chapwanya 2024), while *Porphyromonas* spp. are implicated in purulent reproductive tract infections (Moore et al. 2023). Our detection of *Ureaplasma* in sick animals also aligns with earlier studies highlighting its role in inflammatory conditions of the vaginal and uterine mucosa. Interestingly, while previous reports have described *Mycoplasma* and *Actinomyces* spp. as contributors to uterine inflammation (Srinivasan et al. 2021; Moore et al. 2023), these genera were not detected in our samples. In contrast, we observed *Corynebacterium* exclusively in healthy animals, whereas

literature suggests that certain pathogenic strains of this genus can be involved in vaginitis and other reproductive tract inflammations (Moore et al. 2023).

Enterotoxigenic and adhesive strains of *Escherichia coli* can cause inflammation of the vagina and uterus, while opportunistic strains of *Bacteroides spp.* participate in polymicrobial infections and secrete enzymes capable of destroying tissues and increasing inflammation (Alimbekova et al. 2013; Beloborodova et al. 2023).

In our study, anaerobic gram-negative rod-shaped bacteria from the Prevotellaceae family were found in mucus samples collected from cows with chronic catarrhal endometritis. These bacteria are well known as opportunistic pathogens that may worsen inflammatory processes when the host immunity is compromised.

Bacteroides were mostly identified in the CVM of sick cows. Normally, these bacteria inhabit the distal part of the small intestine, but they can also occur in the natural microflora of the female reproductive tract. When the balance of microflora is disturbed, pathogenic strains of Bacteroides can take advantage and cause infections such as cervicitis, endometritis, or even ovarian inflammation.

On the other hand, Corynebacteria (lactobacilli) were found only in mucus from healthy cows. Members of this genus are important because they help maintain an acidic vaginal environment, which plays a key role in suppressing the growth of harmful microorganisms. A decline in such protective bacteria is usually linked with bacterial vaginosis (Goodfellow et al. 2012; Ma et al. 2012).

So, overall, the microbiota composition of cows' CVM can be described as normal and conditionally pathogenic, including *Corynebacteria*, *E. coli*, anaerobes, and others. However, in reproductively active cows, lactic acid bacteria (mainly lactobacilli) dominate and make up around 95–98% of the whole vaginal microflora. By producing lactic acid and hydrogen peroxide, these bacteria restrict the multiplication of pathogens and help maintain a slightly acidic environment (pH 4.5–5.8). When the number of lactobacilli decreases, the balance of microflora is disturbed, which then allows opportunistic organisms such as streptococci, staphylococci, or *E. coli* to increase. This imbalance is one of the major reasons behind postpartum uterine inflammation in cows.

Previous report by Moore et al. (2023) showed that bacterial microflora produces a broad variety of metabolites, including different enzymes that are part of their normal activity. In our work, we confirmed the presence of several enzymes i.e., catalase, xanthine oxidase, and peroxidase, within the CVM of cows. Interestingly, catalase and xanthine oxidase activity were higher in cows suffering from uterine diseases compared with healthy ones. A similar observation was reported by Ningwal et al. (2018), who described the biochemical profile of CVM and found enzymes such as proteases,  $\beta$ -galactosidase, peroxidase, arginase, and catalase.

Moreover, Voelz et al. (2017) pointed out that microbial enzymes and proteins might even serve as potential markers for predicting metritis risk. Following this idea, we suggest that analyzing enzyme activity could also help in diagnosing uterine disorders in cows. Based on our results, peroxidase activity, in particular, may have diagnostic significance, although more research is needed to confirm this.

## Conclusion

Infrared spectroscopy of mucus revealed the presence of carboxyl (C=O), hydroxyl (O-H), and C-H groups, indicating the presence of mucin. In clinically healthy animals, the pH of mucus was, on average, 0.85% higher than in sick animals, which may indicate disruptions of the acid-base balance in uterine pathologies. Metagenomic sequencing of 16S rRNA revealed that *Faecocisia* (7.860%) and *Phocaeicola A* (5.649%) dominated the CVM in healthy cows, and potentially pathogenic microorganisms were found in sick cows, including *Trueperella* (4.568%), *Fusobacterium C* (5.177%), and *Helcococcus* (5.222%). In cows with inflammatory processes in the uterus, catalase and xanthine oxidase are 100% active, whereas in clinically healthy animals, their activity is minimal (20%). The determination of peroxidase using benzidine and guaiacol samples did not reveal a statistically significant difference between the methods, but the guaiacol test gave more positive reactions (81.3% versus 70%). The identified biomarkers can be used in the diagnosis of inflammatory diseases of the uterus in animals.

## DECLARATIONS

**Funding:** This study was conducted within the framework of the state budget-funded program 217, "Science development", under project No. AP19678293 "Development of a test for visual diagnosis of uterine diseases in cows" of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

**Conflict of Interest:** The authors declare that they have no competing interests regarding the publication of this study.

**Data Availability:** The data available is available from the corresponding author upon reasonable request.

**Ethics Statement:** All procedures were approved by the Animal Ethics Committee of the S. Seifullin Kazakh Agrotechnical Research University (Protocol No. 3 of the meeting of the local Ethical Commission on Biological and Medical Ethics of Animal Research dated November 3, 2022).

**Author's Contribution:** IJ: Conceptualization, methodology, writing of the paper (preparation of the original manuscript). ZJ: Observation, validation. AS: Conceptualization, methodology. GY: Observation, validation, manuscript writing. DB: Conceptualization, methodology, manuscript writing. AA: Data curation, formal analysis, visualization. GM: Research, data collection, data interpretation. AZ: Software, data processing, manuscript writing.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

**Publisher's Note:** All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by

its manufacturer is not guaranteed or endorsed by the publisher/editors.

## REFERENCES

- Abrami M, Biasin A, Tescione F, Tierno D, Dapas B, Carbone A, Grassi G, Conese M, Di Gioia S, Larobina D and Grassi M, 2024. Mucus Structure, Viscoelastic Properties, and Composition in Chronic Respiratory Diseases. *International Journal of Molecular Sciences* 25(3): 1933. <https://doi.org/10.3390/ijms25031933>
- Adnane M and Chapwanya A, 2024. Microbial gatekeepers of fertility in the female reproductive microbiome of cattle. *International Journal of Molecular Sciences* 25(20): 10923. <https://doi.org/10.3390/ijms252010923>
- Adnane M, Meade KG and O'Farrelly C, 2018. Cervico-vaginal mucus (CVM) – An accessible source of immunologically informative biomolecules. *Veterinary Research Communications* 42: 255-263. <https://doi.org/10.1007/s11259-018-9734-0>
- Adnane M, Whiston R, Tasara T, Bleul U and Chapwanya A, 2024. Harnessing vaginal probiotics for enhanced management of uterine disease and reproductive performance in dairy cows: A conceptual review. *Animals* 14(7): 1073. <https://doi.org/10.3390/ani14071073>
- Alimbekova M, Julanova N, Julanov M, Kasenova G and Myrzaliyev A, 2013. Microflora of the mare vaginæ in norm and abnormal. *Journal of Animal and Veterinary Advances* 12(3): 344-351. <https://doi.org/10.3923/javaa.2013.344-351>
- Ardicli S, Ardicli O, Yazici D, Pat Y, Babayev H, Xiong P, Zeyneloglu C, Garcia-Sanchez A, Shi LL, Viscardi OG, Skolnick S, Ogulur I, Dhir R, Jutel M, Agache I, Janda J, Pali-Schöll I, Nadeau KC, Akdis M and Akdis CA, 2024. Epithelial barrier dysfunction and associated diseases in companion animals: Differences and similarities between humans and animals and research needs. *Allergy* 79(12): 3238–3268. <https://doi.org/10.1111/all.16343>
- Armstrong RD, Kariuki BM, Knight DW and Hitchings GJ, 2017. How to synthesise high purity, crystalline D-glucaric acid selectively. *European Journal of Organic Chemistry* 2017(45): 6811-6814. <https://doi.org/10.1002/ejoc.201701343>
- Bansil R and Turner BS, 2018. The biology of mucus: Composition, synthesis, and organization. *Advanced Drug Delivery Reviews* 124: 3-15.
- Beloborodova N, Fadeev R and Fedotcheva N, 2023. Influence of microbiota-related metabolites associated with inflammation and sepsis on the peroxidase activity of cyclooxygenase in healthy human monocytes and acute monocytic leukemia cells. *International Journal of Molecular Sciences* 24(22): 16244. <https://doi.org/10.3390/ijms242216244>
- Beneduzi N, 2025. Immunological Mechanisms, Cellular Processes, and Hindgut Interactions in the Development of Liver Abscesses in Cattle: A Molecular and Cellular Analysis. PhD thesis, Texas Tech University.
- Boegh M, Foged C, Mullertz A and Nielsen HM, 2013. Mucosal drug delivery: Barriers, in vitro models and formulation strategies. *Journal of Drug Delivery Science and Technology* 23(4): 383-391. [https://doi.org/10.1016/s1773-2247\(13\)50055-4](https://doi.org/10.1016/s1773-2247(13)50055-4)
- Button B, Okada SF, Frederick CB, Thelin WR and Boucher RC, 2013. Mechanosensitive ATP release maintains proper mucus hydration of airways. *Science Signaling* 6(279): ra46. <https://doi.org/10.1126/scisignal.2003755>
- Chen SY, Deng F, Zhang M, Jia X and Lai SJ, 2020. Characterization of vaginal microbiota associated with pregnancy outcomes of artificial insemination in dairy cows. *Journal of Microbiology and Biotechnology* 30(6): 804-810. <https://doi.org/10.4014/jmb.2002.02010>
- Denton TT, Hardcastle KI, Dowd MK and Kiely DE, 2011. Characterization of D-glucaric acid using NMR, X-ray crystal structure, and mm3 molecular modeling analyses. *Carbohydrate Research* 346(16): 2551-2557. <https://doi.org/10.1016/j.carres.2011.08.016>
- García JA, Farace PD, Gioffre AK, Romeo F, Verna A, Mendez MA, Morsella C, Aller JF, Signorini M and Paolicchi FA, 2024. Bovine campylobacteriosis in heifer: pathogenesis study and insights in the conventional and molecular diagnosis in an experimental bovine model and field cases. *Veterinary Research Communications* 48(1): 113–124. <https://doi.org/10.1007/s11259-023-10193-z>
- Giannattasio-Ferraz S, Laguardia-Nascimento M, Gasparini MR, Leite LR, Araujo FMG, de Matos Salim AC, de Oliveira AP, Nicoli JR, de Oliveira GC, da Fonseca FG and Barbosa-Stancioli EF, 2019. A common vaginal microbiota composition among breeds of *Bos Taurus indicus* (Gyr and Nelore). *Brazilian Journal of Microbiology* 50: 1115-1124. <https://doi.org/10.1007/s42770-019-00120-3>
- Gnezdilova L, Kruglova Y, Muradyan Zh and Rozinsky S, 2025. Assessing the Ecological Impact Of Betulin-Containing Feed Additives: Insights From Biochemical Parameters In Breeding Calves And Dairy Cows. *International Journal of Ecosystems and Ecology Science (IJEES)* 15(1): 103-112. <https://doi.org/10.31407/ijeec15.1>
- Goodfellow M, Jones AL and Order V, 2012. *Corynebacteriales* Ord. Nov. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W and Whitman WB (eds), *Bergey's Manual of Systematic Bacteriology*, Springer Verlag, New York, NY, USA, Vol 5, 2nd Ed., pp: 232-243.
- Hanumant D, Tiwari RP, Chaturvedani AK, Paikra D, Chandrakar Ch and Rathe P, 2019. Analysis of corporeal characteristics of cervico-vaginal mucus in cows. *The Pharma Innovation Journal* 8(3): 261-264.
- Huang F, Zhang LL, Niu P, Li XP, Wang XY, Wang J, Wang JR, Suo JJ, Fang D and Gao QH, 2024. An observation of the microstructure of cervical mucus in cows during the proestrus, estrus, and metestrus stages and the impact on sperm penetration ability. *Veterinary Sciences* 11(9): 391. <https://doi.org/10.3390/vetsci11090391>
- Jakupov I, Wehrend A, Abultdinova A, Mamytbekova G, Zharkimbaeva Zh and Zabrodina A, 2024. Development of a rapid test to determine endometritis of cows after calving. *Veterinary World* 17(9): 2028-2035. <https://www.doi.org/10.14202/vetworld.2024.2028-2035>
- Johnson KT, Fath KR, Henricus MM and Banerjee IA, 2009. Self-assembly and growth of smart cell-adhesive mucin-bound microtubes. *Soft Materials* 7(1): 21-36. <https://doi.org/10.1080/15394450802693969>
- Kavishvar D and Ramachandran A, 2023. The yielding behaviour of human mucus. *Advances in Colloid and Interface Science* 322. <https://doi.org/10.1016/j.cis.2023.103049>
- Khanoria N, Singh M and Sharma A, 2022. Association of Ovarian Structures, Uterine Characteristics and Cervico-vaginal Mucus Attributes with Conception in Dairy Cows. *The Indian Journal of Animal Reproduction* 43(2): 1-4. <https://www.doi.org/10.48165/ijar.2022.43.2.1>
- Laguardia-Nascimento M, Branco KM, Gasparini MR, Giannattasio-Ferraz S, Leite LR, Araujo FM, Salim AC, Nicoli JR, de Oliveira GC and Barbosa-Stancioli EF, 2015. Vaginal microbiome characterization of Nelore cattle using metagenomics analysis. *PLOS One* 10: e0143294. <https://doi.org/10.1371/journal.pone.0143294>
- Leal J, Smyth HDC and Ghosh D, 2017. Physicochemical properties of mucus and their impact on transmucosal drug delivery. *International Journal of Pharmaceutics* 532(1): 555-572. <https://doi.org/10.1016/j.ijpharm.2017.09.018>
- Liu N, Shan Q, Wu X, Xu L, Li Y, Wang J, Wang X and Zhu Y, 2024. Phenotypic characteristics, antimicrobial susceptibility and virulence genotype features of *Trueperella*

- pyogenes associated with Endometritis of dairy cows. *International Journal of Molecular Sciences* 25(7): 3974. <https://doi.org/10.3390/ijms25073974>
- Ma B, Forney LJ and Ravel J, 2012. Vaginal microbiome: Rethinking health and disease. *Annual Review of Microbiology* 66(1): 371-389. <https://doi.org/10.1146/annurev-micro-092611-150157>
- Maksymyuk HV, Maksymyuk VM, Sedilo HM, Stadnytska OI, Onufrovykh OK, Vorobets ZD and Gutyj BV, 2022. Peculiarities of physico-chemical condition of uterine vaginal mucus during estral cycle. *Ukrainian Journal of Veterinary and Agricultural Sciences* 5(2): 37-42. <https://doi.org/10.32718/ujvas5-2.06>
- Mehtio T, Nurmi L, Ramo V, Mikkonen H and Harlin A, 2004. Synthesis and characterization of copolyanhydrides of carbohydrate-based galactaric acid and adipic acid. *Carbohydrate Research* 402: 102-110. <http://dx.doi.org/10.1016/j.carres.2014.07.009>
- Moore S, Feehily C, Doyle R, Buckley F, Lonergan P, Cotter P and Butler S, 2023. Associations between the postpartum uterine and vaginal microbiota and the subsequent development of purulent vaginal discharge varies with dairy cow breed and parity. *Journal of Dairy Science* 106(11): 8133-8151. <https://doi.org/10.3168/jds.2022-22720>
- Moreno CG, Luque AT, Galvao KN and Otero MC, 2022. Bacterial communities from vagina of dairy healthy heifers and cows with impaired reproductive performance. *Research in Veterinary Science* 142: 15-23. <https://doi.org/10.1016/j.rvsc.2021.11.007>
- Naliukhin AN, Kozlov AV, Eregin AV, Guseva YuE and Kuzina NI, 2024. Responses of soil physico-chemical properties, structure of the microbial community and crop yields to different fertilization practices in Russia's conventional farming system. *Brazilian Journal of Biology* 84: e282493. <https://doi.org/10.1590/1519-6984.282493>
- Nesengani LT, Wang J, Yang Y, Yang L and Lu W, 2017. Unravelling vaginal microbial genetic diversity and abundance between Holstein and Fleckvieh cattle. *RSC Advances* 7: 56137-56143. <https://doi.org/10.1039/C7RA10553C>
- Ningwal D, Nema SP, Kumar S, Kushwah A, Shivhare M and Aich R, 2018. Biochemical profile of cervico-vaginal mucus in relation to fertility in crossbred cows and heifers. *Journal of Animal Research* 8(2): 331-334. <https://doi.org/10.30954/2277-940X.04.2018.25>
- Palanisamy M, Napoleon R, Selvaraju M, Balasubramanian G, Krishnakumar K and Manokaran S, 2014. Nature of genital discharge and pH of cervical mucus and uterine flushing before and after treatment in endometritis affected cows. *International Journal of Livestock Research* 4: 19-24. <http://dx.doi.org/10.5455/ijlr.20141006071857>
- Quadros DL, Zanella R, Bondan C, Zanella GC, Facioli FL, da Silva AN and Zanella EL, 2020. Study of vaginal microbiota of Holstein cows submitted to an estrus synchronization protocol with the use of intravaginal progesterone device. *Research in Veterinary Science* 131: 1-6. <https://doi.org/10.1016/j.rvsc.2020.03.027>
- Quereda JJ, Barba M, Moce ML, Gomis J, Jimenez-Trigos E, Garcia-Munoz A, Gomez-Martin A, Gonzalez-Torres P, Carbonetto B and Garcia-Rosello E, 2020. Vaginal microbiota changes during estrous cycle in dairy heifers. *Frontiers in Veterinary Science* 7: 371. <https://doi.org/10.3389/fvets.2020.00371>
- Raval S, Panchal M, Dhami A and Parmar S, 2018. Endometrial cytology and cervical mucus characteristics of repeat breeding crossbred cows in relation to post-treatment fertility. *The Indian Journal of Veterinary Sciences and Biotechnology* 13: 32-39. <https://doi.org/10.21887/ijvsbt.v13i4.11554>
- Rhoads ML, 2023. Review: Reproductive consequences of whole-body adaptations of dairy cattle to heat stress. *Animal* 17. <https://doi.org/10.1016/j.animal.2023.100847>
- Rutllant J, Lopez-Bejar M and Lopez-Gatius F, 2005. Ultrastructural and rheological properties of bovine vaginal fluid and its relation to sperm motility and fertilization: A review. *Reproduction in Domestic Animals* 40(2): 79-86. <https://doi.org/10.1111/j.1439-0531.2004.00510.x>
- Sharma V, Prasad S and Gupta HP, 2013. Studies on physical and rheological properties of cervico-vaginal mucus during early pregnancy in buffaloes (*Bubalus bubalis*). *Veterinary World* 6(8): 508-551. <https://doi.org/10.5455/vetworld.2013.508-511>
- Sheldon IM and Dobson H, 2004. Postpartum uterine health in cattle. *Animal Reproduction Science* 82-83: 295-306. <https://doi.org/10.1016/j.anireprosci.2004.04.006>
- Sheng YH and Hasnain SZ, 2022. Mucus and Mucins: The Underappreciated Host Defence System. *Frontiers in cellular and infection microbiology* 12: 856962. <https://doi.org/10.3389/fcimb.2022.856962>
- Siregar TN, Armansyah T, Panjaitan B, Gholib G, Herrialfian HA, Sutriana A, Abidin Z, Reynaldi MA, Razak F, Artaliani Y and Yuswar Y, 2019. Changes in cervical mucus as an indicator of fertility in aceh cattle. *Advances in Animal and Veterinary Sciences* 7(4): 306-314. <http://dx.doi.org/10.17582/journal.aavs/2019/7.4.306.314>
- Srinivasan M, Adnane M and Archunan G, 2021. Significance of cervico-vaginal microbes in bovine reproduction and pheromone production - A hypothetical review. *Research in Veterinary Science* 135: 66-71. <https://doi.org/10.1016/j.rvsc.2021.01.003>
- Swartz J, Lachman M, Westveer K, O'Neill T, Geary T, Kott R, Berardinelli J, Hatfield P, Thomson J, Roberts A and Yeoman C, 2014. Characterization of the vaginal microbiota of ewes and cows reveals a unique microbiota with low levels of lactobacilli and near-neutral pH. *Frontiers in Veterinary Science* 1: 19. <https://doi.org/10.3389/fvets.2014.00019>
- Thulasiraman S, Rasool A, Narayanasamy A and Gupta C, 2025. *Clinical Physiology of Female Reproductive Disorders*. In: Rana T (eds), *Fundamentals of Veterinary Pathophysiology*, CRC Press, pp: 223-228.
- Travo A, Piot O, Wolthuis R, Gobinet C, Manfait M, Bara J, Forgue-Lafitte M-E and Jeannesson P, 2010. IR spectral imaging of secreted mucus: a promising new tool for the histopathological recognition of human colonic adenocarcinomas. *Histopathology* 56(7): 921-931. <https://doi.org/10.1111/j.1365-2559.2010.03563.x>
- Tsiligianni Th, Karagiannidis A, Saratsis Ph and Brikas P, 2003. Enzyme activity in bovine cervical mucus during spontaneous and induced estrus. *The Canadian Journal of Veterinary Research* 67(3): 189-193.
- Verhelst R, Verstraelen H, Claeys G, Verschraegen G, Delanghe J, Van Simaey L, De Ganck C, Temmerman M and Vanechoutte M, 2004. Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between *Atopobium vaginae*, *Gardnerella vaginalis* and bacterial vaginosis. *BMC Microbiology* 4(1): 16. <https://doi.org/10.1186/1471-2180-4-16>
- Verma K, Prasad S, Kumaresan A, Mohanty TK, Layek SS, Patbandha TK and Chand S, 2014. Characterization of physico-chemical properties of cervical mucus in relation to parity and conception rate in Murrah buffaloes. *Veterinary World* 7(7): 467-471. <https://doi.org/10.14202/vetworld.2014.467-471>
- Vllahu M, Voli A, Licursi V, Zagami C, D'Amore A, Traulsen J, Woelffling S, Schmid M, Crickley R, Lisle R, Link A, Tosco A, Meyer TF and Boccellato F, 2024. Inflammation promotes stomach epithelial defense by stimulating the secretion of antimicrobial peptides in the mucus. *Gut*

- microbes 16(1): 2390680.  
<https://doi.org/10.1080/19490976.2024.2390680>
- Voelz BE, Kalubowilage M, Bossmann SH, Troyer DL, Chebel RC and Mendonca LGD, 2017. Associations between activity of arginase or matrix metalloproteinase-8 (MMP-8) and metritis in periparturient dairy cattle. *Theriogenology* 97: 83-88.  
<https://doi.org/10.1016/j.theriogenology.2017.04.025>
- Vorobyov N, Selina M and Guselnikova A, 2025. Neural Network Visualization of Stochastic Dependence of Weight Gain Processes on Dairy Productivity of Cows. *Journal of Global Innovations in Agricultural Sciences* 13:691-697.  
<https://doi.org/10.22194/JGIAS/13.1599>
- Wang Y, Ametaj BN, Ambrose DJ and Ganzle MG, 2013. Characterisation of the bacterial microbiota of the vagina of dairy cows and isolation of pediocin-producing *Pediococcus acidilactici*. *BMC Microbiology* 13: 19.  
<https://doi.org/10.1186/1471-2180-13-19>
- Williams EJ, Fischer DP, Noakes DE, England GCW, Rycroft A, Dobson H and Sheldon IM, 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. *Theriogenology* 68(4): 549-559.  
<https://doi.org/10.1016/j.theriogenology.2007.04.056>
- Williams EJ, Fischer DP, Pfeiffer DU, England GCW, Noakes DE, Dobson H and Sheldon IM, 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 63(1): 102-117.  
<https://doi.org/10.1016/j.theriogenology.2004.03.017>
- Zhang M, Yang N, Liu Y and Tang J, 2019. Synthesis of catalase-inorganic hybrid nanoflowers via sonication for colorimetric detection of hydrogen peroxide. *Enzyme and Microbial Technology* 128: 22-25.  
<https://doi.org/10.1016/j.enzmictec.2019.04.016>
- Zubova TV, Pleshkov VA, Smolovskaya OV, Mironov AN, Korobeynikova LN, 2021. The use of carotene-containing preparation in cows for the prevention of postpartum complications, *Veterinary World* 14(5): 1059-1066.  
<https://doi.org/10.14202/vetworld.2021.1059-1066>