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Methods for the Diagnosis and Treatment of Subclinical Endometritis in Holstein Cows

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ABSTRACT

Maintaining the health of dairy cows and ensuring their high fertility are urgent tasks for veterinary gynecology. Among the leading problems are inflammatory and functional gynecological diseases, resulting in long-term infertility. The study aims to compare the hematological parameters of cows during the insemination period and at the time of pregnancy diagnosis and determine the prevalence of subclinical endometritis among Holstein-Friesian cows. The study utilized the methods of endoscopy, ultrasound, transrectal palpation, and an express test, which were performed on Holstein cows at commercial facilities in the Almaty Region (Kazakhstan) and the Department of Obstetrics, Surgery, and Reproductive Biotechnology. Hematological tests were carried out at the Kazakh-Japan Innovation Center. Ultrasounds showed an increased size of the uterine cavity and thickened uterine walls. Research was conducted on cows in three age groups: 2.5–4, 5–7, and over 7 years old. The average prevalence of subclinical endometritis amounted to 8.63%. Throughout treatment, the hematological parameters of diseased cows changed. The study suggests that early diagnosis, effective treatment, and prevention are crucial in maintaining reproductive health and high milk yields considering cattle breed and lifestyle. The study analyzed the indications for the use and effectiveness of drugs, including Ihglyukovit, Tylometrinum, ASD-2F, and Uteroton. Low-level laser therapy was examined in terms of its contribution to the effectiveness of therapy for the disease under study. The benefits of multivitamin supplements were reviewed.

Key words: Holstein breed, Subclinical endometritis, Hematology, Treatment.

INTRODUCTION

The reproductive health of dairy cattle plays a critical role in ensuring the productivity and economic efficiency of livestock farms. A significant factor affecting the reproductive function of cows is subclinical endometritis. This inflammatory uterine disease progresses without pronounced clinical symptoms but leads to reduced fertility and prolonged periods of infertility.

The urgency of this problem is owed to the high prevalence of this disease among dairy cows, especially in the postpartum period (Gayathri et al. 2025). Without timely diagnosis and treatment, the success rate of artificial insemination decreases, the interbreeding period grows longer, and dairy cattle breeding suffers significant economic losses.

Thus, research into the reasons behind reduced reproductive function in highly productive animals and the development of new methods to diagnose the physiological and pathological condition of reproductive organs allows expanding the list of clinical signs and identifying animals that are more susceptible to pathologies and those who recover quickly. The key objectives of dairy cattle breeding are to increase the livestock population, provide them with veterinary treatment, and implement preventive measures. Cows with endometritis do not produce discharge, which leads to prolonged infertility, extending the interbreeding period (Dzhakupov et al. 2020; Pereira et al. 2022).

Uterine diseases in cows are diagnosed using various biophysical methods and parameters. Biophysical diagnostics of reproductive pathologies include transrectal

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ultrasound scanning. Portable, versatile, and cost-effective ultrasound equipment is commercially available and increasingly used in cattle breeding (Fricke 2002; Kasimanickam et al. 2004; Poit et al. 2024).

The effectiveness of laboratory and clinical methods in diagnosing latent types of endometritis was proven by Cheong et al. (2011): laboratory tests showed 37% prevalence, and clinical diagnostic methods were effectively used to detect acute types of pathology. Jakupov et al. (2024) employed the following laboratory tests to diagnose and evaluate the presence and severity of endometritis in cows: the Nagorny-Kalinovsky test, the Whiteside test, the Katerinov test, the Gavrish silver nitrate test, the peroxide test and clinical examinations (rectal and vaginal). These tests were selected specifically to detect inflammatory processes in the uterus, particularly endometritis. Kuzmich et al. (2012) described the following procedure: a large drop of cream taken from the womb is dropped on a clean glass surface, a drop of sugar solution dissolved in 10% water is added to it, and after 1–2min, 2–3 drops of concentrated sulfuric acid are added. If there is an underlying endometritis, the mixture will be colored ink-blue. The reaction is run at room temperature without additional heating. In the absence of inflammation products, the mixture of reagents with cream remains colorless or slightly yellow.

Studies also report the results of hematologic, biochemical, and immunologic blood tests in cows with follicular ovarian cysts and subclinical endometritis. Cows with subclinical endometritis and follicular cysts of the ovaries show significantly lower levels of triglycerides, cholesterol, band neutrophils, lymphocytes and monocytes. The number of phagocytic cells and phagocytic capacity are decreased, and fat and protein metabolism is impaired. Weakened phagocytosis and monocytopenia contribute to the development of latent endometritis against the background of follicular cysts (Nazifi et al. 2008; Heidarpour et al. 2014; Semivolos and Zemliankin 2016).

Mucus in the vagina is a clear sign of uterine inflammation, although it is important to consider the day of postpartum and the type of mucus observed. Mucus can be collected by palpating the cervix from the vaginal side and the lateral, dorsal, and ventral vaginal walls while wearing sterile obstetric gloves. After removing the gloves, the sticky mucus is examined. The advantage of this method is its simplicity and evaluation based on the presence of a specific odor (Bonnet et al. 1993; Pereira et al. 2023).

Heidarpour et al. (2014) discovered treatment-related changes in hematological parameters among dairy cows with clinical and subclinical endometritis. The findings confirm that endometritis causes changes in hematological parameters, which improve after treatment. LeBlanc (2008) noted that after an acute form of endometritis is treated, if the treatment is not stopped due to clinical symptoms, the disease usually goes into a latent form. Studies report a general rise in the latent form from 19.86 to 30.0%, including among cows with prolonged infertility (from 57.5 to 76.7%). However, little is known about hematological changes in dairy cows during the postpartum period.

The current diagnosis can be established based on uterine cytology or blood samples by diagnostic blood biomarkers. Subclinical endometritis suggests a link between disease-related systemic changes and endometrial immune responses, despite the apparent restriction of the endometrium (Cengiz et al. 2021; Diaz-Lundahl et al. 2022).

Delayed ovarian cyclicity, metritis, and endometritis in dairy cows after calving are often associated with the state of immune function during pregnancy and transition into the post-calving period. Delayed ovarian cyclicity (5–10%) is associated with a higher risk of metritis and endometritis (Paiano et al. 2023; Barański et al. 2024).

The prevalence of subclinical endometritis was also assessed in clinically treated cows post-treatment. The treatment involved cephapirin infusions. The study was conducted on 222 Polish Holstein cows diagnosed using vaginoscopy and ultrasonography (Senosy et al. 2011).

Subclinical endometritis is an inflammatory process in the endometrium that proceeds without pronounced clinical signs and in the absence of pathologic discharge from the genital organs between heat periods. The cause is, as a rule, imperfect or incomplete treatment of cows with acute endometritis and a decline in nonspecific immune response after clinical recovery, leading to latent endometritis as a complication. Clinically, this disease is manifested in prolonged or permanent infertility with repeated insemination without fertilization. Rectal examination reveals decreased uterine tone. Irregular thickening of the uterine walls may also be observed. Subclinical endometritis is highly prevalent in dairy cows and has a negative impact on reproductive and economic performance. Considering the stability and welfare of livestock, the genetic selection of animals with high resistance to uterine disease should be prioritized. Diaz-Lundahl et al. (2022) assessed the heritability of subclinical endometritis in Norwegian Red cows. Samples were collected at the time of artificial insemination using cytotape. The percentage of polymorphonuclear cells (PMN) in each sample was determined through cytology by counting 300 PMN and epithelial cells. The average share of PMN amounted to 5%.

Early diagnosis, effective treatment, and prevention are crucial in maintaining the reproductive health and high milk yields of cattle considering their breed and lifestyle. Ihglyukovit is a drug used to stimulate the immune system in animals. It helps to increase the overall resistance, improving adaptation to stress and disease. Tylometrinum is an antibiotic that can be administered intrauterinely to treat infectious endometritis, an inflammation of the uterine mucosa in cows. This drug helps to eliminate infectious processes and improve uterine health. ASD-2F is a medication used to restore the immune system, improve metabolism, and reduce inflammation. It is often used in veterinary practice to treat diseases in animals. Uteroton is a drug that stimulates uterine contractions. It improves uterine tone and contractions after delivery or when the uterus is hypotonic. Uteroton is especially useful in the postpartum recovery of the uterus or the treatment of postpartum complications. Low-level laser therapy (LLLT) is a physiotherapy method to improve microcirculation and accelerate tissue repair processes. It helps to speed up wound healing and reduce inflammation. Multivitamins are supplements that support normal metabolism, help strengthen the body and maintain its normal function. They are used to prevent vitamin deficiencies and improve the overall health of animals. Each treatment option has a different purpose in veterinary practice and must be used according to specialist recommendations.

Thus, the study aims to assess the impact of subclinical endometritis on the reproductive function of Holstein cows and to develop effective methods for its diagnosis and treatment.

MATERIALS AND METHODS

We employed comprehensive methods to diagnose and treat subclinical endometritis in Holstein cows. The research was conducted at commercial facilities in the Almaty Region (Kazakhstan) and the Department of Obstetrics, Surgery and Reproductive Biotechnology. Hematologic tests were performed at the Kazakh-Japan Innovation Center.

Diagnostic methods

Subclinical endometritis was diagnosed in cows using the following methods:

1. Transrectal palpation — to assess the uterus and its tone and detect any pathological changes.
2. Ultrasonography (ultrasound) — to assess the structure of the endometrium, uterine wall thickness, the presence of exudate and the degree of inflammatory changes, if any. Ultrasound, endoscopy, and an express test were performed on Holstein Friesian cows between 21 and 28 days postpartum. All cows were clinically examined three times at 2-week intervals.
3. Endoscopy — to visually assess the uterine mucosa and diagnose inflammatory processes.
4. Express test with Lugol's solution — to detect pathologically changed uterine epithelium. Iodine stains healthy cells brown, while pathological areas remain light or colorless.
5. Mirror pelvic examination — to assess the condition of the vaginal mucosa, detect inflammatory processes, and determine the type of discharge.
6. Hematology tests — to determine blood parameters including erythrocytes, leukocytes, platelets, hemoglobin, and the hematocrit formula. Blood was collected from the tail vein using Monovette vacuum systems.

Clinical examinations were performed on all cows three times at 2-week intervals. Subclinical endometritis was diagnosed in 67 cows. Twenty-two postpartum cows were selected for hematologic examination. Cows were housed according to the results of clinical examinations, which involved analyzing veterinary and zootechnical documentation and rectal and vaginal examination. Experimental and control groups were created using the analogy principle.

Research methods

Ultrasounds were performed with a PU-2200 VET Doppler scanner and a Mindray Z5 VET system.

Lugol's solution was prepared with the following ingredients (per 100mL of solution): 10g of potassium iodide, 5g of iodine crystals, and 150mL of room-temperature distilled water. Blood samples for hematology were taken from the tail vein with Monovette vacuum systems.

Subclinical endometritis was detected with an express test. In a positive test, the cervix was uniformly stained brown. If pathological foci were present, they were not stained and the sample was considered negative or iodine-negative.

Lugol's solution showed areas of pathologically altered epithelium, virtually indistinguishable from healthy epithelium in color and relief upon visual examination, as they did not rise above the surface of the surrounding tissues.

Normally, epithelial cells absorb the substance and acquire a characteristic coloration after some time. In contrast, pathological tissues experience a lack of glycogen, responsible for iodine absorption. Thus, iodine stained healthy cells brown, while abnormal cells remained uncolored, usually appearing white or yellow.

Cervical mucus was removed with a tampon soaked in a weak solution of acetic acid, which caused the cervical vessels to constrict and allowed a better view of the cervix. Pathological foci turned white when treated with a 1% acetic acid solution.

The procedure typically takes 5–10 minutes, but a longer examination is possible.

The express test was employed to detect subclinical endometritis in dairy cows. In postpartum dairy cows, subclinical endometritis is characterized by persistent inflammation of the endometrium, which has a profound detrimental impact on subsequent reproductive performance. Our experiment aimed to evaluate the effectiveness of the express test as a method to detect subclinical endometritis.

Transrectal palpation of the uterus is the most commonly used method of diagnosing subclinical endometritis. However, it is also the most subjective method, because the criteria for a positive diagnosis tend to differ. Examination with a vaginal speculum can detect inflammation of the vaginal walls and the presence of mucus. However, if the cervix is closed, there is no way to determine the presence of discharge with this method.

Thus, the known diagnostic methods have several disadvantages, such as labor intensity, subjectivity, and economic inefficiency in large-scale use in veterinary practice.

Given this, there was a need to expand the list of parameters to detect pathologies, establish new indicators, and identify symptoms to create effective methods for diagnosing obstetric diseases in cows.

Hematological parameters were used to assess the dynamics of cows' condition after treatment. The tests were performed before and after therapy.

Statistical analysis

All data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for hematological parameters and prevalence rates and are reported as mean ± SD. Paired-sample t-tests were used to compare pre-treatment and post-treatment hematological values within the experimental group. A one-way ANOVA was conducted to assess differences in hematological parameters among age groups (2.5–4, 5–7, and over 7 years). Additionally, the Chi-square (χ^2) test was used to compare the prevalence of subclinical endometritis across the three age groups. For each Chi-square test, the degrees of freedom and P-values were reported. A P-value of less than 0.05 was considered statistically significant.

For all statistical tests, a P<0.05 was considered statistically significant. Results were visualized in tables with statistically significant differences denoted using superscript letters or asterisks where applicable.

RESULTS

Ultrasound examinations showed an increased size of the uterine cavity and thickened uterine walls. The mucous layer had a heterogeneous structure. The edges of the uterus were clear and smooth, and the wall was thickened. Thus, subclinical endometritis was confirmed by a positive ultrasound diagnosis.

The performed express test and ultrasound showed subclinical endometritis in 8.0% of cows aged 2.5–4 years and 9.1% of cows aged 5–7 years. The total number of cows included in further research was 67 with 8.8% aged over 7 years. Chi-square analysis revealed no statistically significant difference in the prevalence of subclinical endometritis across age groups (χ^2 = 0.36, P=0.838; Table 1).

Table 1: Results of the express test and ultrasound for subclinical endometritis in Holstein cows by age groups

Age (years)	Subclinical endometritis	
	N	%
2.5–4	23	8.0
5–7	27	9.1
>7	17	8.8
Total	67	8.63±0.40

Differences in prevalence among age groups were analyzed using the Chi-square test: χ^2 = 1.726, df = 2, P = 0.422. No statistically significant differences were observed

During treatment, cows with subclinical endometritis exhibited changes in hematological parameters. Initially, erythrocytes, hemoglobin (101.7), and platelets (277.2) were lower in this group compared to healthy cows. After complete treatment, erythrocyte count increased by 7.1, hemoglobin by 110.4, and platelets by 294. Before treatment, the diagnosed cows had significantly higher levels of leukocytes (10.9), neutrophils (2.5), lymphocytes (60.8), and monocytes (2.6). Post-treatment, leukocytes (9.2), neutrophils (1.9), lymphocytes (54.8) and monocytes (2) significantly decreased. Thus, pre-treatment levels confirm that treatment affected hematological parameters.

Treatment of subclinical endometritis is aimed at eliminating inflammation and restoring reproductive function. Antibiotics are used to eliminate bacterial infections. However, the choice of drugs should be substantiated by bacteriologic analysis. Antibiotics can be administered intravaginally, intrauterinally, or as injections.

Drugs to restore uterine microflora: Ihglyukovit is used to stimulate the immune system and improve general health. Tylometrinum is administered intrauterinally to treat infectious endometritis and improve uterine health. ASD-2F is used to restore the immune system, improve metabolism, and reduce inflammation. Uteroton promotes uterine contractions and restores uterine tone. LLLT is applied to improve microcirculation and accelerate tissue repair. Multivitamins help support normal metabolism and general health. Treatment is carried out in several stages following the specified days of estrous synchronization to maximize the effectiveness of the therapy.

The ultrasounds showed an increased size of the uterine cavity and thickened uterine walls. The mucous layer demonstrated a heterogeneous structure. The edges of the uterus were clear and smooth, and the wall was thickened. Thus, positive ultrasounds confirmed the presence of subclinical endometritis (Table 2). As a result of the express test and ultrasound, subclinical endometritis was diagnosed in 8.0% of cows aged 2.5–4 years and 9.1% of cows aged 5–7 years. The total number of cows included in the study was 67, of which 8.8% were aged over 7 years. After treatment, the levels of leukocytes (9.2), neutrophils (1.9), lymphocytes (54.8), and monocytes (2) significantly decreased. Thus, pre-treatment blood tests confirm that the administered treatment caused changes in hematological parameters (Table 3).

Table 2: Treatment regimens for cows diagnosed with subclinical endometritis

Therapy	Dose	Administration	Estrous synchronization days							
			1	2	3	4	5	6	7	8
Ihglyukovit	30mL	Intramuscular	+	–	+	–	+	–	+	–
Tylometrinum	100mL	Intrauterine	+	–	+	–	–	–	–	–
ASD-2F	20mL	Subcutaneous	–	+	–	+	–	+	–	+
Uteroton	10mL	Intramuscular	–	+	–	+	–	+	–	–
LLLT	4min	On biologically active points on the sacrum and croup	+	+	+	+	+	–	–	–
Multivitamins	25mL	Intramuscular	+	–	–	–	–	–	–	+

Table 3: Hematological parameters in cows with diagnosed subclinical endometritis

Parameters	Units	Normal	Experimental (n=22)		
			Pre-treatment	Post-treatment	P-value
Leukocytes	n/mm^3	4.0–15.0	10.9±1.7	9.2±1.7*	<0.01
Lymphocytes	%	45.0–80.0	60.8±6.0	54.8±6.0*	<0.05
Monocytes	%	1.0–5.0	2.6±0.6	2.0±0.6*	<0.05
Neutrophils	%	1.0–30.0	2.5±0.6	1.9±0.6*	<0.05
Erythrocytes	n/mm^3	6.0–11.0	5.5±1.6	7.1±1.6*	<0.01
Hemoglobin	g/L	80.0–150.0	101.7±8.7	110.4±8.7*	<0.05
Platelets	n/mm^3	100–800	277.2±16.8	294±16.8*	<0.05

Values (Mean±SD) marked with an asterisk (*) indicate statistically significant differences between pre-treatment and post-treatment values (P<0.05).

DISCUSSION

The study compares the hematological parameters of cows during the insemination period and at the time of pregnancy so as to diagnose and determine the prevalence of subclinical endometritis among Holstein-Friesian cows. The findings of this study underscore the significant impact of subclinical endometritis (SE) on the reproductive health of Holstein cows and highlight the efficacy of a multimodal diagnostic and therapeutic approach.

This study aligns with recent studies focused on understanding and advancing the diagnostic process of subclinical endometritis (Yáñez et al. 2022; Parikh et al. 2022; Pascottini et al. 2023). The ultrasound examination results align with the studies of Ghasemzadeh-Nava et al. (2025) and Barański et al. (2022), who observed the presence of pus and fluid in the uterus, which are generally associated with decreased reproductive performance and increasing odds of diagnosis of endometritis. The ultrasound results indicate a difference in subclinical endometritis expression at different ages, highlighting a relationship between the age of dairy cows and susceptibility to subclinical endometritis. This relationship is supported by the studies of Nanda et al. (2024), who concluded that the incidence of endometritis was higher in 3–5-year-old age groups, followed by 5–7-year-old age groups. In contrast, Guruvandana et al. (2025) concluded that there was no significant difference in the occurrence of subclinical endometritis in two different age groups. This gap calls for more research in understanding the relationship between age and disease prevalence and the specificity of this age-disease susceptibility relationship.

The hematological results show an increase in hematological parameters in cows with subclinical endometritis, indicating underlying inflammation and activation of the immune system. This aligns with studies that focused on the immune system response to subclinical endometritis (Ji et al. 2024). The hematological results obtained also align with the studies of Valdmann et al. (2022), who observed an increase in white blood cells from cows with endometritis. A systemic resolution of inflammation is reflected in the hematological improvements following treatment, specifically an increase in erythrocytes, hemoglobin, and platelets and a decrease in leukocytes, neutrophils and monocytes.

A limitation of this study is the focus on blood parameters, ignoring other compounds that factor in subclinical endometritis. Further studies focused on glucose levels, blood serums, and protein levels are essential to consolidate the hematological results (Yazlik et al. 2021; Hedia et al. 2021). The study highlights antibiotic therapy as an optimal way of treating subclinical endometritis, and this aligns with recent agricultural and veterinary studies (Paiano et al. 2022). To counteract subsequent antibiotic resistance, alternative methods of treating subclinical endometritis can be explored, such as essential oils of cinnamon, clove, oregano, and thyme, or combining antibiotic treatment with herbal preparations (Paiano 2021; Menoud et al. 2024). Another limitation of this study is the lack of molecular and genetic foundation in understanding heritable traits that may contribute to subclinical endometritis (Diaz-Lundahl et al. 2022). Future research is needed to explore these areas. Longitudinal studies are also needed to evaluate long-term reproductive outcomes, such as conception rates and calving intervals, rather than relying solely on hematological or imaging biomarkers. Furthermore, exploring the role of metabolic stressors such as negative energy balance in high-yielding cows in subclinical endometritis pathogenesis could reveal preventive measures.

To lessen the economic impact of subclinical endometritis, the study recommends early, comprehensive intervention. However, there might be difficulties putting such protocols into place in environments with limited resources. Important first steps include providing access to treatments like LLLT and educating veterinarians on advanced diagnostics (like portable ultrasound). Farmers may benefit from simplified screening tools, such as the express test to enable timely referrals (Jakupov et al. 2024).

By highlighting the benefits of integrated diagnostics and therapies, this study contributes to our understanding of subclinical endometritis management in Holsteins. Despite certain drawbacks, the results highlight the necessity of breed-specific, microbiologically informed, and financially viable methods in dairy reproduction and offer a basis for improving protocols. Filling in these gaps in future research will improve farm profitability and animal welfare.

Conclusion

Our study demonstrates that subclinical endometritis significantly impacts the reproductive function of Holstein cows and highlights the effectiveness of various diagnostic and treatment methods. We conclude that a comprehensive approach to diagnostics including ultrasound, endoscopy, the express test with Lugol's solution, and hematology allows detecting subclinical endometritis promptly to minimize its negative impact on reproductive performance.

A focused treatment regimen with antibiotics, immunomodulators, physiotherapy, and hormonal medications significantly improves cattle health, reduces inflammation, and increases the chances of successful insemination. Hematological tests show higher levels of erythrocytes, hemoglobin, and platelets and lower counts of leukocytes, neutrophils, and monocytes post-treatment, supporting the effectiveness of the chosen treatments. However, limitation of our study is its focus on Holstein cows, which limits opportunities for extrapolation to other cattle breeds. Nevertheless, our findings emphasize the importance of a comprehensive approach to the diagnosis and treatment of subclinical endometritis and point to the need for continued research in this area to improve reproductive performance in dairy cattle breeding.

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Data Availability: Data is available upon request.

Ethics Statement: All procedures involving animals were carried out in accordance with national and institutional guidelines for the care and use of animals in research. Ethical approval was granted by the Ethics Committee of the West Kazakhstan Agrarian Technical University.

Author's Contribution: The researchers who conducted this study declared that they contributed equally to the study. All authors read and approved of the final manuscript.

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