DOI 10.7251/VETJEN2401063M **UDK 636.2.09:618.14-006.6**
Original Scientific Paper

CLINICAL ENDOMETRITIS IN COWS: CORRELATION OF BODY CONDITION, QUALITY OF VAGINAL MUCUS, LACTATION, DAILY MILK PRODUCTION AND THE PERCENTAGE OF POLYMORPHONUCLEAR CELLS IN THE CYTOLOGICAL SMEAR OF THE ENDOMETRIUM

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Summary
This study aimed to determine the correlation between body condition score, production parameters (lactation and daily milk production), vaginal mucus quality and the percentage of polymorphonuclear cells from cytological smears originating from the uterus of dairy cows diagnosed with clinical endometritis. The study included 14 Holstein-Friesian cows in the first and second lactation. Assessment of the quality of vaginal mucus was performed using the Metricheck® instrument. Cytological smears from the uterus were sampled using the Cytoprint AI® catheter. Evaluation of the quality of vaginal mucus and sampling of the cytological smear from the uterus was performed on the 25th and 26th days of lactation. The results of this study indicated that there was no significant correlation between the percentage of polymorphonuclear cells and milk yield or lactation. However, a significant negative correlation was found between body condition score and vaginal mucus quality, suggesting that cows with higher body condition scores have a lower risk of developing clinical endometritis. The results of this study are in line with the existing literature suggesting that body condition scores can serve as an indicator of the degree of risk for the development of clinical endometritis.
Key words: daily milk yield, vaginal mucus quality, lactation, dairy cow, body condition score, polymorphonuclear cells.

INTRODUCTION

Changing the gestation-non-lactation phase into the non-gestation-lactation phase represents a high risk factor for the onset of puerperal diseases and is called the transition period (Contreras and Sordillo, 2011). During this period, physiological oscillations occur, primarily in hormonal and metabolic pathways (Maletić et al., 2024). Immediately after calving, homeoretic mechanisms redistribute energy to the

mammary gland, which leads to a negative energy balance (NEB). Systemic inflammation as well as mineral imbalances are also processes that are common during this period. Half of newly calved cows fail to adapt to new challenges, which results in structural and molecular changes at the level of the reproductive organs, primarily at the level of the uterus and ovaries (Serbetci et al., 2023). These changes can be manifested in the form of clinical and subclinical diseases (Maletić et al., 2024). These diseases include puerperal diseases of the uterus, metabolic imbalances and locomotor diseases (Vergara et al., 2014).

The prevalence of puerperal uterine diseases such as puerperal metritis, clinical metritis, clinical endometritis (CE) and subclinical endometritis is 2.8%, 25%, 29% and 26%, respectively (Vallejo-Timaran et al., 2021). Overall, the prevalence of postpartum uterine diseases during the first 60 days after calving is 51.2%. However, the prevalence depends on the type of farm, i.e. the way of keeping, the individual characteristics of the cows, the day of sampling and the applied reference values.

Clinical endometritis is a disease of the uterus that occurs after 21 days after calving (DAC) and is manifested by clinically visible signs of the disease, and therefore does not represent a problem in routine diagnostics (Sheldon et al., 2009). However, the degree of damage or inflammation during CE can vary. Methods to determine the degree of damage/inflammation caused by CE can be quantified using Metricheck® (Pleticha et al., 2009) and by determining the degree of endometrial inflammation using cytological smears (Milovanović et al., 2023).

The aim of this study is to determine whether there is a connection/correlation between individual body factors (body condition score (BCS)), production parameters (lactation and daily milk yield), quality of vaginal mucus and the percentage of polymorphonuclear cells (PMN) in cows suffering from clinical endometritis.

MATERIALS AND METHODS

This experiment included a total of n=14 cows of the Holstein-Friesian breed that were in the first and second lactation. The average body weight of the cows included in the experiment was 550±32.4 kg. All cows were fed an identical diet in the form of a total mixed ration (TMR). Cows were vaccinated with Bovilis BVD® (MSD Animal Health, Wellington, New Zealand), Bovilis IBR Marker live® (MSD Animal Health, Wellington, New Zealand) and Bravoxin 10® (MSD Animal Health, Wellington, New Zealand). Using the DairyComp 305 farm software (Valley Agricultural Software, Tulare, California), data related to the number of lactations, days in lactation and daily milk yield were obtained.

Clinical examinations of the cows were performed on the 25th and 26th days after calving. Immediately before determining the quality of vaginal mucus and sampling the swab from the uterus, a visual inspection of the cows was performed. Only cows with pathological vaginal discharge were selected for the experiment.

The assessment of body condition (BCS) was performed according to the method described by (Bauer, 2021), which consists of a gradation scale of BCS 2-5.
Checking the quality of vaginal mucus was performed using the Metricheck® instrument (Figure 1) (Simcro, New Zealand). Before introducing the instrument into the vagina, the lips were cleaned with a paper towel to prevent contamination. After that, the instrument was introduced into the vagina and led to the fornix vaginae. The handle of the instrument was slightly raised towards the dorsal commissure of the vagina and the instrument retracted caudally in order to collect vaginal mucus from the ventral wall of the vaginal canal (Milovanović et al., 2023). Determination of the quality of vaginal mucus was performed using the visual method described by (Pleticha et al., 2009), as follows: grade 0=clear transparent mucus, grade 1=mucus with patches of pus, grade 2=mucopurulent discharge, grade 3=purulent/ smelly/putrid discharge.

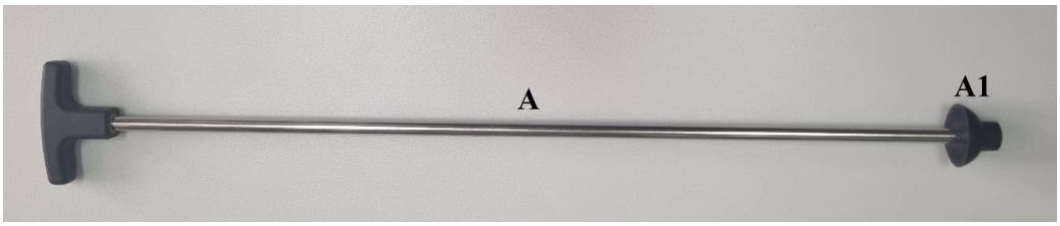

Figure 1 Metrichecka® instrument for qualitative analysis of the quality of vaginal mucus (A-Metrichecka®; A1- vaginal mucus collector)

Sampling of the cytological swab from the uterus was performed using the Cytoprint AI® catheter (Figure 2).

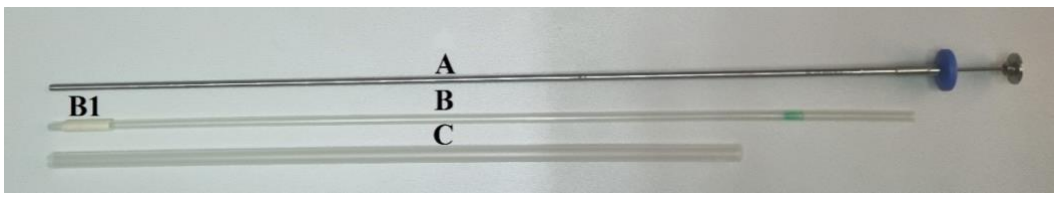

Figure 2 Inseminator and Cytoprint AI® catheter for endometrial cytology swab sampling (A- inseminator for artificial insemination; B-Cytoprint AI® catheter; B1- tip of the Cytoprint AI® catheter for endometrial cytology smear sampling; C- Cytoprint AI® catheter protective tube)

Before sampling the cytological smear, the *ampulla recti* was emptied manually. After that, the lips were wiped with a paper towel. The Cytoprint AI® catheter is placed on the inseminator (gun). Using the bimanual method, with rectal guidance, the Cytoprint AI® catheter was introduced through the cervix into the uterus. The swab is exposed by pulling the protective tube towards the handle of the inseminator. A swab from the dorsal wall of the uterus was sampled by rotating the inseminator with the Cytoprint

AI® catheter in a clockwise direction. After that, the protective tube of the Cytoprint AI® catheter was returned to its initial position cranially in order to protect the cytological swab from contamination when retracing it outside (Milovanović et al., 2023). After the extraction of the instrument outside the vagina, the preparation of the cytological preparation was started. A clean microscope slide (Menzel Gläser, Braunschweig, Germany) was prepared and the tip of the Cytoprint AI® catheter containing cells of endometrial origin was placed on it. The silicone tip of the catheter is rotated along the entire length of the microscope slide in order to spread the cells evenly on the microscope slide. The microscope slide with the cytological smear was then left to dry at room temperature and transported to the laboratory.

The preparation of the cytological preparation was carried out by staining the cytological smear using MGG Quick Stain (Bio Optica, Milano, Italy) according to the manufacturer's instructions. The cytological preparations were then air-dried. Microscopic evaluation of the preparation was performed by counting a minimum of 200 cells at 400× magnification. Determination of the percentage of polymorphonuclear cells (PMN) was performed by comparing the ratio of endometrial and PMN cells (Figure 3).

Statistical data processing was done in the Prism ver.8 software system (Graphpad, USA), and the results were presented through tables and graphs in the MS Office Package software (Microsoft, USA).

RESULTS

Based on the results obtained in this study, it was observed that the daily milk yield of the examined cows varied from 13.00 L to 29.70 L of milk, on average 22.39 L. The quality of vaginal mucus was in the range between 1 and 3, on average 2.88. The body condition score ranged between 2.5 and 3.25, with an average of 2.85, while the % of PMN in uterine cytological smears was in the range of 5.39-82.92%. Detailed results of these parameters for each cow are shown in Table 1.

Table 1 Results of milk production, percentage of polymorphonuclear cells, body condition score and quality of vaginal mucus shown in individual values for each tested individual

Cow	Lactation / group	Daily milk production in liters	% PMN in a cytological smear	BCS	Quality of vaginal mucus
1.	1	29.7	29.16	2.75	2
2.	2	27.1	61.88	3.25	1
3.	1	27.6	82.92	2.75	3
4.	2	22	50.08	2.75	1
5.	2	19	5.39	3	1

6.	2	26	41.83	2.75	1
7.	2	19	35.23	2.75	2
8.	1	13	69.9	2.75	3
9.	2	21	66.81	3	2
10.	2	23	42.51	3	1
11.	1	25	50.61	2.5	3
12.	1	22	80	3	1
13.	1	16	65.06	3	1
14.	1	23	35.89	3	1

The results of the statistical analysis are shown in Table 2.

Table 2 Correlations established between the examined parameters

Parameters	Lactation / group	Daily milk production in liters	% PMN	BCS	Metricheck®
Lactation	1.00	-0.09	-0.37	0.25	-0.40
Daily milk production in liters	-0.09	1.00	-0.08	-0.15	0.06
% PMN	-0.37	-0.08	1.00	0.06	0.31
BCS	0.25	-0.15	0.06	1.00	-0.66*
Metrichecka®	-0.40	0.06	0.31	-0.66*	1.00

* statistical significance determined at the $p<0.05$ level

A statistically significant correlation was observed in the relationship between BCS and Metricheck® parameters, where it was observed that cows with a high Metricheck® score had a lower BCS value. The relationship between Metricheck® and BCS results is shown in Figure 3.

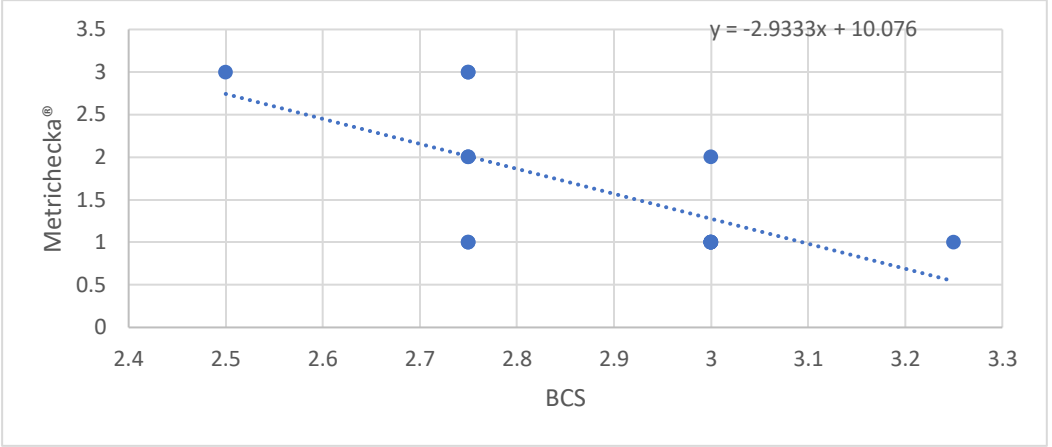

Figure 3 Correlative analysis of Metriheck® and BCS parameter results

DISCUSSION

After calving, cows adapt to the changes caused by transition from the pregnant-non-lactating phase to the non-pregnant-lactating phase. In this period, systemic inflammation occurs, which aims to prepare the reproductive organs for new fertilization and pregnancy. Clinical endometritis occurs if uterine inflammation is prolonged for more than 21 days after calving with the presence of pathological vaginal discharge and increased %PMN (Pascottini et al., 2022). The diagnosis of CE is carried out using the Metricheck® device or manually (Pascottini et al., 2020).

Based on the obtained results, it can be observed that in theory any increase in BCS by the point 1 would lead to a decrease in the Metricheck® value by 2.933 times, that is, that cows with a lower body condition score have a higher risk factor for the occurrence of CE. In practice, this does not mean that cows should be overfed, but in adequate physical condition according to the productive and reproductive phase of lactation. The results obtained in this study correspond with the results obtained in studies that aimed to determine the relationship between these two observed parameters (Bogado Pascottini and LeBlanc, 2020; Kadivar et al., 2014; Wathes et al., 2009).

Body condition score is a method that can indirectly assess the energy status of cows (Rearte et al., 2023). It was found that in cows with a high energy deficit, uterine inflammation was prolonged and persisted for 2 weeks after calving, unlike cows with a moderate energy deficit (Wathes et al., 2009). Energy deficit indicates maladaptation to the postpartum period with the consequent emergence of metabolic diseases characterized by glucose deficiency (Sordillo and Raphael, 2013). Examination of biochemical parameters from the serum of cows revealed that the levels of non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHB) are significantly higher in cows with CE, in contrast to healthy cows (Bogado Pascottini and LeBlanc, 2020). High levels of NEFA and BHB indicate metabolic maladaptation to the postpartum

period. As a result, polymorphonuclear cells become dysfunctional even while they are in the bloodstream, that is, even before they enter the uterus. Also, by analyzing the complete blood count, it was determined that cows with a high energy deficit have fewer leukocytes than cows with a moderate energy deficit (Wathes et al., 2009). On the other hand, a study (Bartolome et al., 2014) aimed at comparing protocols for CE therapy concluded that cows with a higher BCS respond significantly better to therapy in contrast to cows with low BCS (Bartolome et al., 2014). Therefore, the correlation between BCS and Metricheck® score (vaginal mucus quality) obtained in our study indicates that these parameters can be a useful tool for assessing the risk of CE.

The correlation between PMN % and lactation was not established in our study. Some studies suggest that there is a difference between %PMN according to the number of lactations, i.e. that primiparous heifers have a significantly higher % PMN than multiparous heifers (Mehrzad et al., 2002), i.e. a higher number of circulating eosinophils and immature leukocytes (Mehrzad et al., 2002). The response of the uterus to inflammation depends on the % of PMN. However, if the cow is in a state of energy deficit, % PMN may not be a representative indicator of uterine health, because the functional ability of PMN in that case is impaired by the lack of glucose. Also, in order to establish the relationship between %PMN, production parameters, it may be necessary to include in the analysis cows with more than two lactations, as well as larger groups of animals than is the case in our study.

The correlation between % PMN and daily milk yield was not established in our study. In a study that aimed to determine the relationship between production parameters and subclinical endometritis, namely % PMN and daily milk yield, a positive correlation was found between these two parameters (Barrio et al., 2015). The study by Barrio et al. (2015) was conducted in Spain on a larger number of cows. Also, Spain has a warmer climate, and some studies have shown that % PMN also depends on climatic conditions (Olde Riekerink et al., 2007).

CONCLUSION

A negative correlation was found between BCS and Metricheck® score (vaginal mucus quality). More precisely, each increase in BCS by 1 point led to a decrease in Metricheck® value by 2.933 times. That is, cows with a higher BCS score have a lower risk of developing clinical endometritis. However, this does not mean that cows should be overfed, but that they should be in adequate condition according to the productive and reproductive phase of lactation. Correlation between other observed parameters % PMN and production parameters (lactation and daily milk yield) was not established.

Acknowledgment:The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contracts No. 451-03-66/2024-03/200030 and 451-03-66/2024-03/200143).

Conflict of interest statement: The authors declare that there is no conflict of interest.

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