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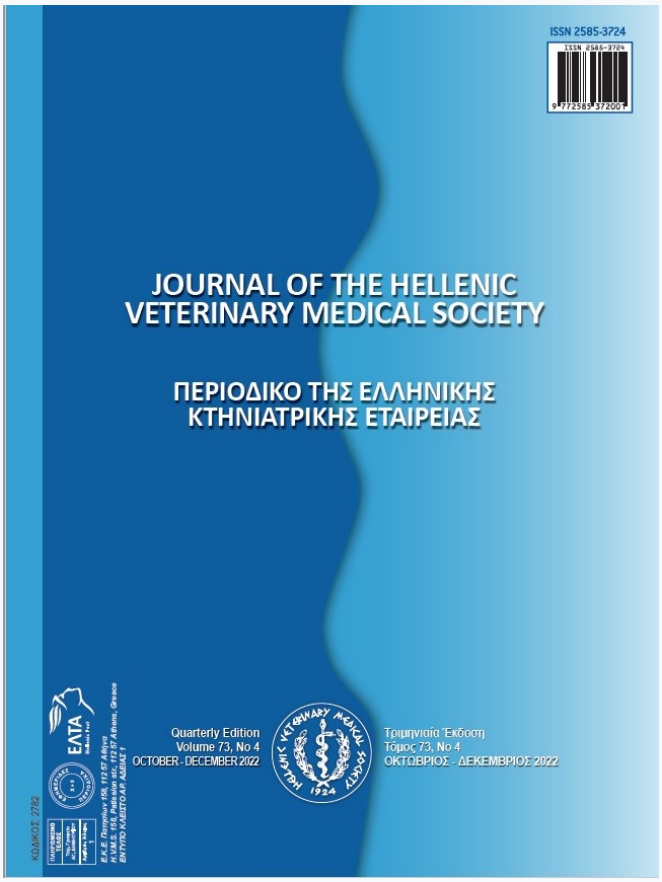


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# Determination of predictive factors for the clinical cure rate of endometritis in Holstein dairy cows

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**ABSTRACT:** Endometritis is one of the most common disorders that negatively influence reproductive performance in dairy cows. The purpose of this study was to determine the predictive factors of the clinical cure of endometritis. A total of 700 Holstein dairy cows were examined for signs of clinical endometritis between 30 and 40 days in milk (the first examination in the time of clean test). A total of 131 cows with clinical endometritis were assigned for treatment. The cows receive suspension of intrauterine infusion. Cytological examination, reagent test strips, and clinical findings of reproductive organs were evaluated at the time of treatment (the first examination) and two weeks after the treatment (the second examination: for evaluation of response to treatment (cows with disappearance of clinical signs) of endometritis). After evaluation of treatment, cows were grouped into the responsive (R) (n=56) and unresponsive (NR) (n=75) to treatment. Percentages of non-degenerated neutrophil (NDN), degenerated neutrophil (DN), total neutrophils and leukocyte esterase activity were significantly higher *(P<0.05)* in the NR cows than those of R ones to treatment at the second examinations. Moreover, the nitrate test was significantly higher (at 5 and 15 min) in the NR group than the responsive group at the first examination. However, protein concentration and percentages of lymphocyte cells were significantly higher in the NR animals than the R cows at the first and second stages of examination *(P<0.05)*. Increasing of lymphocytes percentage, protein concentration, and nitrate test at the first stage of examination in the NR cows showed more severe inflammatory conditions and uterine infection in the NR cows. Evaluation of these factors can predict the clinical cure of endometritis in dairy cows.

**Keywords:** clinical cure; dairy cows; postpartum endometritis

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## INTRODUCTION

Endometritis is an important infection of the uterus in dairy cows. In cattle, clinical endometritis is defined as the presence of a purulent or mucopurulent uterine discharge in the vagina, 21 days or more after the parturition without systemic signs of illness. On the other side, subclinical endometritis lacks purulent material in the vagina but comes with polymorphonuclear leukocytes (PMN) in uterine lavage (Gilbert et al. 2005) or cytobrush (Kasimanickam et al. 2004a), indicating inflammatory reactions in the endometrium, without systemic signs of illness (Cheong et al. 2011; Gilbert et al. 1998; Gilbert et al. 2005; Kasimanickam et al. 2004a; LeBlanc et al. 2002a).

Various techniques are available to assess the endometritis, including transrectal palpation of the uterus (Heuwieser et al. 2000), vaginal examination, ultrasonography, biopsy, pathological and bacteriological examinations (Youngquist and Threlfall 2006). The subclinical endometritis can mainly be diagnosed by endometrial cytology with cytobrush and low volume uterine lavage (Gilbert et al. 2005; Kasimanickam et al. 2004a). However, within the past few years, a straightforward creative cow-side test, named leukocyte esterase (LE) activity technique has been proposed for the diagnosis of subclinical endometritis (Arango-Sabogal et al. 2019; Barakat et al. 2020; Cheong et al. 2012; Couto et al. 2013; Helfrich et al. 2020; Santos 2006). The technique assesses LE activity in the uterine lavage fluid, uterine and cervical cytobrush samples using reagent strips similar to those used for urinalysis. Moreover, the assessment of protein content and pH values of uterine discharge facilitates the diagnosis of endometritis (Arango-Sabogal et al. 2019; Barakat et al. 2020; Cheong et al. 2012; Couto et al. 2013; Helfrich et al. 2020; Santos 2006).

Endometritis reduces reproductive performance in dairy cows and different management programs have been implemented to improve and control the endometritis such as intramuscular injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and intrauterine infusion of antibiotics (Dubuc et al. 2021; LeBlanc et al. 2002b; Wang et al. 2021). Response to the treatment of clinical endometritis and the subsequent improvement of the reproductive performance varies among reports. Some studies reported that the routine infusion of antibiotics might not be efficacious for improving clinical endometritis and reproductive performance (Ahmadi et al. 2019; Ahmadi et al. 2018; Cohen et al. 1995; Feldmann and Hoedemaker 2005; Malinowski et al. 2011; Okawa et al. 2017; Thurmond et al. 1993); however, other studies found that the intrauterine administration of antibiotics could improve the reproductive performance (LeBlanc et al. 2002b; Runciman et al. 2008; Sheldon and Noakes 1998; Tison et al. 2017). The efficacy of $PGF_{2\alpha}$ or synthetic analogs for the treatment of the endometritis also varies in different studies (Ahmadi et al. 2018; Haimerl et al. 2018; LeBlanc et al. 2002b; Sheldon and Noakes 1998), which can be due to the multifactorial nature of the disease and individual differences of cows (Lefebvre and Stock 2012). What's moreover, there is scarcity of information about the condition of the uterine cavity at the time of treatment and regarding, factors that can be used to predict the unresponsive cows to treatment of clinical endometritis. Therefore, the aims of the study were: 1) to compare certain parameters of uterine discharge, characteristics of ovaries, uterine horn at the time of treatment and two weeks after the treatment between the responsive (R) and unresponsive (NR) to treatment cows 2) to assess reproductive indices and factors that can be used to predict clinical cure rate of endometritis and reproductive performance in dairy cows.

## MATERIALS AND METHODS

### Animals, evaluation of clinical endometritis and study design

The present study was conducted on a commercial dairy farm with 2000 lactating dairy cows. The average (±SEM) daily yield of cows was 37.17±0.42. The average days to 1st service, days open (DO) and first service conception rate (FSCR) for the farm was 83.09±1.57, 132.7±5.7 day and 42.4%, respectively. All cows were fed the same total mixed ration, and housed in open shed barns. The animals were milked three times a day. The animals were milked three times a day. Body condition scores (BCS) of the cows was recorded based on a 1 to 5 score at the time of treatment (Ferguson et al. 1994).

A total of 700 Holstein dairy cows were examined for signs of clinical endometritis between 30 and 40 days in milk (DIM) (the first scheduled examination). Clinical endometritis was evaluated by manual vaginal examination, transrectal palpation and ultrasonography. The manual vaginal examination was carried out according to the previously described procedure (Ahmadi et al. 2018) and four grades of clinical endometritis were determined based on the signs and symptoms. Briefly, score 0: clear discharge; score 1:

mucus containing flecks of white or off-white pus; score 2: discharge containing approximately 50% pus, 50% mucus material; and score 3: discharge containing > 50% purulent material. The cows with mucopurulent (approximately 50% pus, 50% mucus) or purulent (>50% pus) discharge in the vagina, 21 days or more postpartum, and is not accompanied systemic signs were assumed to have clinical endometritis (McDougall et al. 2007; Sheldon et al. 2006). Transrectal evaluation of the cervix and uterus involves estimation of size, symmetry, and consistency. However, cervical diameter >7.5 cm, 21 days or more post partum was an acceptable alternative for definition of endometritis (Barlund et al. 2008). Ultrasonography (A B-mode, real-time scanner ultrasound, SIUI 800V, chine) was used for evaluation of uterine fluid, mucus and pus within the uterine lumen. Cows with echogenic fluid at uterine lumen in ultrasonographic examination presumed to have clinical endometritis (Barlund et al. 2008; Kasimanickam et al. 2004b).

A total of 131 Holstein dairy cows with clinical endometritis were diagnosed and treated in the first examination. The animals were treated with a 50-ml suspension of intrauterine procaine penicillin and dihydrostreptomycin sulphate (200 + 250 mg $mL^{-1}$, Pen Strep, Norbrook laboratories, Newry, Northern Ireland), *Zataria multiflora* extract (60 mL; 918 mg $mL^{-1}$) and placebo at the first examination. The second examination was performed on days 44 to 54 DIM (two weeks after the first examination) to assess the clinical cure (clinical resolution) of endometritis after treatment. Cows were grouped into the R (n=56) and NR (n=75) to treatment after the assessment of clinical cure of endometritis at second examination (after treatment). R to treatment group was defined as cows with disappearance of clinical signs (clinical resolution) of endometritis after treatment at second examination. The responsive cows had no purulent or mucopurulent discharges and cervical diameter was less than 7.5 cm at the second examination. NR to treatment group was defined as cows with not disappearance of clinical signs (clinical resolution) of endometritis after treatment at second examination.

**Cytological examination**

Cytological examination was performed just before the treatment (first examination) and 14 days after the treatment (second examination). The vulva was cleaned with water and antiseptic materials. Then, cervical and uterine discharge samples for cytological examination were collected as previously described (Ahmadi et al. 2005; Yavari et al. 2009). The specimen was smeared on a clean glass microscope slide and fixed with ethanol for 10 min and stained with a Giemsa stain for 20 min at the first and second examination. Percentages of neutrophils, lymphocytes, epithelial and plasma cells were determined on cytological examination at the first and second examinations. Cytological assessment was determined based on the percentage of cells by counting 100-200 cells at 400 × magnification in 20 microscopic field that were randomly selected. Types of neutrophils were classified into two subtypes based on degenerative status: non-degenerate neutrophil (NDN) and degenerate neutrophil (DN) as described previously (Brownlow 1983).

**Reagent test strips**

The reagent test strips were added to the cervical and uterine discharge samples (Analyticon Biotechnologies AG D-35104 Lichtenfels, Germany, Combi screen 11 sys plus). The pH, protein, nitrate and specific gravity test on the reagent test strips were evaluated after 1, 5, 10 and 15 min. LE activity was evaluated after 2, 5, 10, and 15 min.

Nitrate, LE and protein results were categorized in several categories based on the color of the reagent area. The nitrate categorization included: code 0: negative, code 1: +, code 2: + + based on the color change, The LE categorization included code 0: negative; code 1: 25; code 2: 75 and code 3: 500 leukocytes per μL. The protein categorization included: code 0: negative, code 1: trace, code 2: 30, code 3: 100, code 4: 500 mg/dl

**Examination of reproductive organs**

The diameters of left and right uterine horns, cervix, and structures on the ovaries were evaluated by rectal palpation and ultrasonography at the first and second examination.

All parameters of cytological examination, reagent test strips, and characteristics of reproductive organs were evaluated at the first and second examination in dairy cows.

**Evaluation of reproductive performance**

All cows followed the normal herd reproductive management protocol. For the first AI, all cows received the same presynchronization programs that include administering PGF2α injections 14 d apart with the second injection 12 d before the first GnRH of

Ovsynch timed AI program. All cows received timed AI 16 h after the final GnRH of Ovsynch. Pregnancy was diagnosed using ultrasonography between 32±5 and 110±10 days after AI. Reproductive performance such as calving to first insemination interval, SPC, DO and FSCR were recorded and evaluated. These parameters were compared between the R and NR cows after the treatment.

**Statistical analysis**

The data were analyzed using the SPSS statistical software (Version 15.0, SPSS Inc, Chicago, Illinois). The effects of treatment protocols, studied groups and their interactions on all parameters of cytological examination, reagent test strips, and characteristics of reproductive organs were analyzed using the Random Intercept Mixed Model. First service conception rate of the animals was compared between studied groups by Chi-square test. The percentage of neutrophils, LE activity, pH, protein, nitrate test, specific gravity and the diameters of cervix and uterine horns were analyzed at first and second examination and between studied groups by t-test. Calving to first service interval, days open and service per conception were analyzed between studied groups using the independent samples t-test. Structures on the ovaries were compared between studied groups using the Chi-square test. Data were presented as the percentage or mean (± SEM) and differences at $P \leq 0.05$ were considered significant.

## RESULTS

In this study, cows with clinical endometritis were 18.7%. In this study, prevalence of dairy cows with clinical endometritis of score 1, 2 and 3 were 53.45% (n=70), 33.58% (n=44) and 12.97% (17), respectively. The 56 (42.7%) of 131 cows with clinical endometritis recovered in 14 days after the treatment (second examination); however, 75 cows (57.3%) did not respond to the treatment. The 32 (57.1%), 17 (30.4%) and 7 (12.5%) of 56 R cows were cows with clinical endometritis of score 1, 2 and 3, respectively. The 38 (50.7%), 27 (36.0%) and 10 (13.3%) of 75 NR cows were cows with clinical endometritis of score 1, 2 and 3, respectively. BCS of the cows was 2.75 to 3.75.

According to the results of Random Intercept Mixed Model, the effect of treatment protocols on parameters of cytological examination, reagent test strips, and characteristics of reproductive organs was not significant and eliminated. Therefore, the final model for the parameters of cytological examination, reagent test strips, and characteristics of reproductive organs included studied groups (R and NR).

**Cytological examination**

Table 1 shows the percentages of NDN, DN and neutrophils cells, lymphocyte, epithelial and plasma cell in both groups of responsive and unresponsive to treatment cows at first and second examination. As presented, the percentages of NDN, DN and neutrophils are significantly higher (P<0.05) in the NR cows compared to the R cows at the second examination. There was a significant difference in the percentage of lymphocyte cells between the two groups at the first and second examination. Additionally, the percentage of epithelial cells was significantly lower in the NR cows than those of R animals at both examinations (Table 1).

**Reagent test strips**

The LE activity was no significant difference between studied groups at the first examination (p> 0.05) (Table 2). The LE activity was significantly higher (P<0.05) in the NR cows compared to those of R at the time points of 2, 5, 10 and 15 minutes at the second examination (Table 2). The protein concentration was significantly higher in the NR cows than the R animals at both examinations (Table 2). Nitrate was significantly higher (P<0.05) in the NR cows com-

**Table 1.** Mean (±SEM) percentage of neutrophils, NDN, DN, Epithelial, lymphocyte and plasma cells between responsive to treatment for endometritis cows and unresponsive cows at the first (30-40 DIM) and second examination (44-54 DIM)

| Exam | Groups | Neutrophil % | NDN % | DN % | Epithelial cells % | Lymphocyte cells % | plasma cells % |
|---|---|---|---|---|---|---|---|
| First | responsive | 73.07± 4.3 | 38.33± 4.3 | 34.67± 3.4 | 25.30± 4.35 [a] | 1.43± 0.7 [a] | 0.02±0.01 |
| | unresponsive | 77.98±3.4 | 43.73± 3.4 | 34.25±2.4 | 14.56± 2.7 [b] | 6.58± 1.8 [b] | 0.04±0.03 |
| Second | responsive | 35.43± 4.3 [a] | 27.08 ±3.5 [a] | 8.32± 1.0 [a] | 61.92± 4.3 [a] | 1.17± 0.4 [a] | 0.00±0.00 |
| | unresponsive | 68.20±3.9 [b] | 40.14± 3.6 [b] | 29.6±2.5 [b] | 24.20±3.3 [b] | 6.09± 1.7 [b] | 0.09±0.00 |

[a, b] Different superscript in columns indicate significant difference (P<0.05).
Neutrophils = Percentage of total neutrophils, NDN= Non degenerative neutrophils, DN= Degenerative neutrophils.

pared to the R animals on 5 and 15 minutes at the first examination and it was tended to increase on 10 minutes at the first examination (P=0.08) (Table 2), but there was no significant difference at the second examination. The pH of discharge and specific gravity test were statistically the same in the two groups at both examinations. The pH and specific gravity of discharge in the R cows and NR ones were ≥ 7.0 ± 0.10 and < 1.010 ± 0.12 respectively, at both examinations. The LE activity and protein concentration were significantly higher at first examination compared to second examination in the R cows but they were not significant difference between the first and second examination in NR cows (P<0.05). The nitrate, pH and specific gravity of discharge were no significant difference at first examination compared to second examination in the R and NR cows (p> 0.05) (Table 2).

**Examination of reproductive organs**

There was no significant difference between the two groups in cervical and uterine horns diameter in both examinations (P > 0.05). The mean cervical diameter was 3.32±0.08 and 3.05±0.09 cm in the first and second examination, respectively. The mean diameter of right and left uterine horns were 3.7±0.16 and 3.45±0.14 cm in the first and 3.35±0.14 and 3.15±0.16 cm in the second examination, respectively. Similarly, no significant difference was observed in ovarian activity between the two groups (Table 3).

**Table 2.** Comparison of reagent strip test between responsive to treatment for endometritis cows and unresponsive cows at different times (1, or 2, 5, 10, 15 minutes); at the first (30-40 DIM) and second examination (44-54 DIM)

| | Examination | | | |
|---|---|---|---|---|
| | First | | Second | |
| Test | R cows | NR cows | R cows | NR cows |
| Nit 1 | 0.05±0.02 | 0.09±0.02 | 0.05±0.01 | 0.06±0.03 |
| Nit 5 | 0.15±0.04 $^{a}$ | 0.30±0.05 $^{b}$ | 0.16±0.04 | 0.17±0.04 |
| Nit 10 | 0.40±0.07 | 0.60±0.07 | 0.30±0.07 | 0.38±0.06 |
| Nit 15 | 0.51±0.080 $^{a}$ | 0.77±0.08 $^{b}$ | 0.48±0.09 | 0.60±0.08 |
| LE 2 | 0.94±0.13 $^{\phi}$ | 1.24±0.13 | 0.14±0.04 $^{A}$ * | 1.01±0.04 $^{B}$ |
| LE 5 | 1.70±0.15 $^{\phi}$ | 2.02±0.14 | 0.28±0.08 $^{A}$ * | 1.70±0.13 $^{B}$ |
| LE 10 | 2.39±0.17 $^{\phi}$ | 2.67±0.14 | 0.81±0.20 $^{A}$ * | 2.40±0.14 $^{B}$ |
| LE 15 | 2.92±0.19 $^{\phi}$ | 3.16±0.14 | 1.20±0.15 $^{A}$ * | 2.90±0.15 $^{B}$ |
| Pro 1 | 3.40±0.06 $^{a\ \phi}$ | 3.60±0.06 $^{b}$ | 2.90±0.07 $^{A}$ * | 3.50±0.04 $^{B}$ |
| Pro 5 | 3.70±0.05 $^{a\ \phi}$ | 3.90±0.05 $^{b}$ | 3.40±0.07 $^{A}$ * | 3.80±0.04 $^{B}$ |
| Pro 10 | 3.90±0.04 $^{a\ \phi}$ | 4.10±0.01 $^{b}$ | 3.60±0.06 $^{A}$ * | 4.10±0.04 $^{B}$ |
| Pro 15 | 4.06±0.05 $^{a\ \phi}$ | 4.20±0.06 $^{b}$ | 3.80±0.05 $^{A}$ * | 4.20±0.05 $^{B}$ |

Nit 1, 5, 10 and 15= nitrate results of strip test at time of 1, 5, 10 and 15 minutes, LE 2, 5, 10 and 15= esterase activity of strip test at time of 2, 5, 10 and 15 minutes and Pro 1, 5, 10 and 15= protein concentration at time of 1, 5, 10 and 15 minutes.

The nitrate categorization included: code 0: negative, code 1:+, code 2:+ + based on the color change, The LE categorization included code 0: negative; code 1: 25; code 2: 75 and code 3: 500 leukocytes per μL. The protein categorization included: code 0: negative, code 1: trace, code 2: 30, code 3: 100, code 4: 500 mg/dl.

$^{a, b}$ Different letters indicate significant difference at the first examination between responsive to treatment for endometritis cows and unresponsive cows (P<0.05).

$^{A, B}$ Different letters indicate significant difference at the second examination between responsive to treatment for endometritis cows and unresponsive cows (P<0.05).

$^{*, \phi}$ Different symbols indicate significant difference between first and second examination at different times in the responsive to treatment for endometritis cows' group

**Table 3.** Percentage of ovarian activity between responsive to treatment for endometritis cows and unresponsive cows

| | Ovarian activity | |
|---|---|---|
| | Static % | Active % |
| responsive cows | 7.8 | 92.2 |
| unresponsive cows | 6.8 | 93.2 |

Static: absence of a corpus luteum (CL) in both examination (as long as they were 14 days apart) on the ovaries. Active: The presence of a CL on the ovaries.

**Table 4.** Reproductive indices (Mean ± SEM) of studied cows in responsive to treatment for endometritis and unresponsive cows

| Groups | Calving to 1st service | Days open | Service per conception | First service conception rate % |
|---|---|---|---|---|
| responsive cows | 84.4 ±3.0 [a] | 124.1 ± 8.6 | 1.8 ± 0.2 | 45.8 |
| unresponsive cows | 97.6 ± 3.0 [b] | 141.1 ± 8.4 | 2.2 ± 0.2 | 54.2 |

[a, b] Different superscript in column indicate significant difference ($P < 0.05$)

**Reproductive performance**

Table 4 shows the reproductive performance of both groups. As can be seen, calving to first service interval of the R cows was significantly lower compared to the NR cows. Moreover, SPC and DO of the unresponsive cows were higher than those of responsive ones; however, this difference was not significant ($P > 0.05$). Furthermore, first service conception rate was not significantly different between the two groups.

## DISCUSSION

In the present study, several parameters of uterine discharge in cows with clinical endometritis were evaluated and compared between the R and NR to treatment cows. For this purpose, reagent test strips, cytological examination of cervical and uterine discharge, characteristics of ovaries at the time of treatment and two weeks later and reproductive indices were evaluated.

According to the results, the overall clinical cure rate was 42.7%, while in other studies range from 59.5% (Feldmann and Hoedemaker 2005), to 68% (Sheldon et al. 1998) or 77% (LeBlanc et al. 2002b). The differences in the cure rate can be attributed to the multifactorial nature of the disease and individual differences (Lefebvre and Stock 2012).

Neutrophils are in the first line of defense against bacterial infection of uterus (Galvão 2018; Hussain 1989) and undergo traumatic and degenerative changes characterized by distorted lobulated and segmented appearance, rupture of the nuclear membrane and diffusion of chromatin materials due to their exposure to toxic condition (Brownlow 1983). The percentage of DN related with the degree and severity of endometritis in dairy cows. The percentage of DN increased by increasing degree and severity of endometritis in dairy cows (Hajibemani et al. 2016). The results of this study showed that in the unresponsive cows the percentages of NDN, DN and neutrophils in the cytological examination at the second examination were significantly higher ($P<0.05$) than those in responsive cows which can be considered as an alternative approach for evaluation of clinical cure rate of cows with endometritis.

The increase in lymphocytes is indicative of infection (Duncan and Prasse 1986). The present study showed that the percentage of lymphocytes was significantly higher in the NR animals than the R cows at both examinations. The severity of the infection is proportional to the percentage of lymphocytes (Duncan and Prasse 1986). Probably, the severity of endometritis was higher in NR cows.

In the previous studies, LE activity was associated with endometritis and it was used for evaluation of cows suffering from endometritis (Arango-Sabogal et al. 2019; Barakat et al. 2020; Cheong et al. 2012; Couto et al. 2013; Helfrich et al. 2020; Ricci et al. 2017; Santos 2006). In the present study, the LE activity was significantly higher *($P<0.05$)* at the second examination in NR to the treatment cows compared to the R animals. According to the results, LE activity can be used as an alternative approach for the evaluation of the NR cows. The protein concentration of uterine discharge increases in cows with endometritis (Cheong et al. 2012). In the current study, the results showed the protein concentration was significantly higher at first examination compared to second examination in the treated cows but it was not significant between 1$^{ts}$ and 2$^{nd}$ examination in the untreated cows. These results showed that the protein concentration was increased in cows with endometritis. The protein concentration was higher in the NR animals than the R cows at both examinations. This finding showed that the amount of immunoglobulin, inflammatory mediators and severity of infection were higher in the NR cows compared to the R group at the first examination; therefore, due to this severe inflammatory condition, the treatment was unsuccessful in the NR cows.

The nitrite strip test is a rapid screening method for possible infections caused by nitrate-reducing bacteria (Graff 1987) such as *E. coli* and *Trueperella pyogenes* (Quinn et al. 2011). These bacteria are the most important pathogens involved in the endometritis of cows (Griffin et al. 1974; Sheldon et al. 2009).

Nitrate test was significantly higher *(P<0.05)* at the first examination in the unresponsive cows than the responsive cows at 5 and 15 minutes but had no significant differences at the second examination. The *E. coli* and *T. pyogenes* can play an important role in the response rate to treatment. Furthermore, the nitrate test can rapidly screen possible infections of uterus by *E. coli* and *T. pyogenes*.

Cervical and uterine horns diameter had no significant difference between the two groups in this study at both examinations *(P > 0.05)*. Mateus et al. (2002) evaluated the effect of puerperal uterine infection on uterine involution from parturition until the sixth week of postpartum in dairy cows and found that cows with mild and severe puerperal endometritis had similar uterine horn diameter to those of normal cows.

Ovarian activity is an important element in the alleviation of endometritis in dairy cows. The increase in estrogen (associated with luteolysis and follicular growth) reinforces immune function and boosts up the uterus protection against bacterial infection and an increase in progesterone suppresses immune function (Lewis 1997; Sheldon et al. 2006). The subsequent estrus results in the expulsion of bacterial contaminants, inflammatory products and improvement of endometritis (Kasimanickam et al. 2005). In the present study, ovarian activity had no significant difference between the two groups at both examinations. Therefore, ovarian activity had no role in the curing of endometritis in this study.

Based on our findings, calving to the first service interval of the recovered cows was significantly lower but FSCR, SPC and DO were not significantly different between the two groups. Intrauterine antibiotics, including procaine penicillin G and oxytetracycline (Thurmond et al. 1993), ceftiofur hydrochloride (Galvão et al. 2009) and ampicillin (Feldmann and Hoedemaker 2005) were not effective in improving the reproductive performance of responsive cows compared to unresponsive cows with clinical endometritis. Since clinical endometritis is a multifactorial disease, several factors can potentially influence the treatment and the reproductive performance of dairy cows; therefore, the cure rate of clinical endometritis and improvement of reproductive performance vary enormously (Lefebvre and Stock 2012). Based on our findings and other studies (Feldmann and Hoedemaker 2005; Galvão et al. 2009; Lefebvre and Stock 2012; Thurmond et al. 1993) the reproductive performance, especially calving to conception interval, is not improved in cows that were responsive to treatment of endometritis compared to the unresponsive animals. However, the cure rate of endometritis and the improvement of reproductive performance vary after the treatment.

## CONCLUSION

In conclusion, results of the present study demonstrated that the percentage of lymphocyte cells, protein concentration and nitrate test were higher in the unresponsive cows at the first examination. According to the results, increasing of percentage of lymphocyte cells, protein concentration, and nitrate test at the first stage of examination in the unresponsive cows showed more sever inflammatory conditions and uterine infection in the unresponsive to treatment cows. These factors can be used to predict the clinical cure of endometritis and the successfulness of the treatment before applying the treatment.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

Ahmadi M, Tafti AK, Nazifi S, Ghaisari H (2005) The comparative evaluation of uterine and cervical mucosa cytology with endometrial histopathology in cows. Comp Clin Path 14 (2): 90-94.

Ahmadi MR, Makki M, Mirzaei A, Gheisari HR (2019) Effects of hypertonic dextrose and paraffin solution as non-antibiotic treatments of clinical endometritis on reproductive performance of high producing dairy cows. Reprod Domest Anim 54 (5): 762-771.

Ahmadi MR, Mogheiseh A, Mirzaei A, Nazifi S, Fallah E (2018) Treatment of cows with clinical endometritis III as cows affected by pyometra-Non antibiotic treatment of severe clinical endometritis. Asian Pac J Reprod 7 (4): 185.

Arango-Sabogal JC, Dubuc J, Krug C, Denis-Robichaud J, Dufour S (2019) Accuracy of leukocyte esterase test, endometrial cytology and vaginal discharge score for diagnosing postpartum reproductive tract health status in dairy cows at the moment of sampling, using a latent class model fit within a Bayesian framework. Prev Vet Med 162: 1-10.

Barakat T, Shawky G, Absy G, Ragab MAE-R (2020) Effect of Cefazolin on endometrial cytology and reagent test strips parameters in bovine endometritis. J Hell Vet Med Soc 71 (4): 2505-2510.

Barlund C, Carruthers T, Waldner C, Palmer C (2008) A comparison of

diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology 69 (6): 714-723.
Brownlow M (1983) Polymorphonuclear neutrophil leucocytes of peritoneal fluid. Equine Vet J 15 (1): 22-24.
Cheong S, Nydam D, Galvão K, Crosier B, Gilbert R (2011) Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. J Dairy Sci 94 (2): 762-770.
Cheong S, Nydam D, Galvao K, Crosier B, Ricci A, Caixeta L, Sper R, Fraga M, Gilbert R (2012) Use of reagent test strips for diagnosis of endometritis in dairy cows. Theriogenology 77 (5): 858-864.
Cohen R, Bernstein M, Ziv G (1995) Isolation and antimicrobial susceptibility of Actinomyces pyogenes recovered from the uterus of dairy cows with retained fetal membranes and post parturient endometritis. Theriogenology 43 (8): 1389-1397.
Couto G, Vaillancourt D, Lefebvre R (2013) Comparison of a leukocyte esterase test with endometrial cytology for diagnosis of subclinical endometritis in postpartum dairy cows. Theriogenology 79 (1): 103-107.
Dubuc J, Fauteux V, Villettaz-Robichaud M, Roy J-P, Rousseau M, Buczinski S (2021) Efficacy of a second intrauterine cephapirin infusion for the treatment of purulent vaginal discharge and endometritis in postpartum dairy cows. J Dairy Sci 104 (3): 3559-3563.
Duncan JR, Prasse KW (1986) Veterinary laboratory medicine. Iowa State University Press.
Feldmann M, Hoedemaker M (2005) Treatment of chronic bovine endometritis and factors for treatment success. DTW. Deutsche Tierarztliche Wochenschrift 112 (1): 10-16.
Ferguson JD, Galligan DT, Thomsen N (1994) Principal descriptors of body condition score in Holstein cows. J Dairy Sci 77 (9): 2695-2703
Galvão K, Greco L, Vilela J, Sá Filho M, Santos J (2009) Effect of intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows. J Dairy Sci 92 (4): 1532-1542.
Galvão KN (2018) Association between immune function and development of uterine disease in dairy cows. Anim Reprod 9 (3): 318-322.
Gilbert R, Shin S, Guard C, Erb H (1998) Incidence of endometritis and effects on reproductive performance of dairy cows. Theriogenology 49 (1): 251.
Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M (2005) Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64 (9): 1879-1888.
Graff L (1987) Análisis de orina: atlas color. Ed. Médica Panamericana.
Griffin J, Hartigan P, Nunn W (1974) Non-specific uterine infection and bovine fertility: I. Infection patterns and endometritis during the first seven weeks post-partum. Theriogenology 1 (3): 91-106.
Haimerl P, Heuwieser W, Arlt S (2018) Meta-analysis on therapy of bovine endometritis with prostaglandin F2α—An update. J Dairy Sci 101 (11): 10557-10564.
Hajibemani A, Mirzaei A, Sharifiyazdi H, Ghasrodashti AR (2016) Vaginal Fornix Discharge Cellularity and Its Leukocyte Esterase Activity for Diagnosis of Endometritis in Dairy Cows. J Fac Vet Med 42 (1): 57-64.
Helfrich AL, Reichenbach H-D, Meyerholz MM, Schoon H-A, Arnold GJ, Fröhlich T, Weber F, Zerbe H (2020) Novel sampling procedure to characterize bovine subclinical endometritis by uterine secretions and tissue. Theriogenology 141: 186-196.
Heuwieser W, Tenhagen B, Tischer M, Lühr J, Blum H (2000) Effect of three programmes for the treatment of endometritis on the reproductive performance of a dairy herd. Vet Record 146 (12): 338-341.
Hussain A (1989) Bovine uterine defense mechanisms: a review. Zoonoses and Public Health 36 (1-10): 641-651.
Kasimanickam R, Duffield T, Foster R, Gartley C, Leslie K, Walton J, Johnson W (2004a) Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62 (1): 9-23.
Kasimanickam R, Duffield T, Foster R, Gartley C, Leslie K, Walton J, Johnson W (2004b) Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62 (1-2): 9-23.
Kasimanickam R, Duffield T, Foster R, Gartley C, Leslie K, Walton J, Johnson W (2005) The effect of a single administration of cephapirin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. Theriogenology 63 (3): 818-830.
LeBlanc S, Duffield T, Leslie K, Bateman K, Keefe GP, Walton J, Johnson W (2002a) Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci 85 (9): 2223-2236.
LeBlanc S, Duffield T, Leslie K, Bateman K, Keefe GP, Walton J, Johnson W (2002b) The effect of treatment of clinical endometritis on reproductive performance in dairy cows. J Dairy Sci 85 (9): 2237-2249.
Lefebvre RC, Stock AE (2012) Therapeutic efficiency of antibiotics and prostaglandin F2α in postpartum dairy cows with clinical endometritis: an evidence-based evaluation. Veterinary Clinics: Food Animal Practice 28 (1): 79-96.
Lewis GS (1997) Uterine health and disorders. J Dairy Sci 80 (5): 984-994.
Malinowski E, Lassa H, Markiewicz H, Kaptur M, Nadolny M, Niewitecki W, Ziętara J (2011) Sensitivity to antibiotics of Arcanobacterium pyogenes and Escherichia coli from the uteri of cows with metritis/endometritis. Vet J 187 (2): 234-238.
Mateus L, Lopes da Costa L, Bernardo F, Robalo Silva J (2002) Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reprod Domest Anim 37 (1): 31-35.
McDougall S, Macaulay R, Compton C (2007) Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. Anim Reprod Sci 99 (1): 9-23.
Okawa H, Fujikura A, Wijayagunawardane MM, Vos PL, Taniguchi M, Takagi M (2017) Effect of diagnosis and treatment of clinical endometritis based on vaginal discharge score grading system in postpartum Holstein cows. J Vet Med Sci 16-0593.
Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, FitzPatrick E (2011) Veterinary microbiology and microbial disease. John Wiley & Sons.
Ricci A, Bonizzi G, Sarasso G, Gallo S, Dondo A, Zoppi S, Vincenti L (2017) Subclinical endometritis in beef cattle in early and late postpartum: cytology, bacteriology, haptoglobin and test strip efficiency to evaluate the evolution of the disease. Theriogenology 94: 86-93.
Runciman D, Anderson G, Malmo J, Davis G (2008) Effect of intrauterine treatment with cephapirin on the reproductive performance of seasonally calving dairy cows at risk of endometritis following periparturient disease. Aust Vet J 86 (7): 250-258.
Santos N (2006) The use of leukocyte esterase reagent strips for diagnosis of subclinical endometritis in dairy cows. Theriogenology 66 666-667.
Sheldon I, Noakes D (1998) Comparison of three treatments for bovine endometritis. Vet Record 142: 575-579.
Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth H-J (2009) Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 81 (6): 1025-1032.
Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006) Defining postpartum uterine disease in cattle. Theriogenology 65 (8): 1516-1530.
Thurmond M, Jameson C, Picanso J (1993) Effect of intrauterine antimicrobial treatment in reducing calving-to-conception interval in cows with endometritis. J Am Vet Med Assoc 203 (11): 1576-1578.
Tison N, Bouchard E, DesCôteaux L, Lefebvre R (2017) Effectiveness of intrauterine treatment with cephapirin in dairy cows with purulent vaginal discharge. Theriogenology 89: 305-317.
Wang H, Yan Z, Wu X, Zhang Y, Wei Y, Zhao X (2021) Using vaginal discharge score (VDS) grading system to evaluate the effect of clinical endometritis on reproductive performance of dairy cows in China. Anim Reprod 18 (1): 1-12
Yavari M, Haghkhah M, Ahmadi M, Gheisari H, Nazifi S (2009) Comparison of cervical and uterine cytology between different classification of postpartum endometritis and bacterial isolates in Holstein dairy cows. International J Dairy Sci 4 (1): 19-26.
Youngquist RS, Threlfall WR (2006) Current Therapy in Large Animal Theriogenology-E-Book. Elsevier Health Sciences